Cardioprotective and Lipid Lowering Effects Tabebuia Impetiginosa (Lapacho Tea) on Male Rats Fed A High Fat and Fructose Diet

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Abstract

Obesity is a major health issue in the developed countries with a similar trend in the developing countries too. High energy diets, notably from fats and sugars (high-fat/high-sugar diet: HF/HSD) is linked to the development of obesity which causes insulin resistance a hallmark of type 2 diabetes and an important factor in cardiovascular disease. In earlier studies Tabebuia impetiginosa extract inhibited lipase and slowed the increase of postprandial triglycerides in rats given a fat load. Therefore, we investigated its triglyceride lowering and cardio protective effects in Wistar rats fed a High Fat and Fructose Diet (HFFD). In a dose-effect trial three groups of 21 rats each were fed for 74 days only HFFD (controls), or HFFD, to which either 0.3 (HFFD+lowL) or 0.6 mg dry Tabebuia impetiginosa extract per kg food (HFFD+highL) was added. Fasting blood samples were drawn before and at the end of intervention. Tabebuia impetiginosa extract lowered dose-dependently and significantly (p<0.05) plasma Triglycerides (TG), Total Cholesterol (TC), Atherogenic Index (AI), Cardiovascular Risk Index (CRI) and liver TG, as well as Fasting Blood Glucose (FBG) and Glycated hemoglobin (HbA1c), with correlation coefficients (R) between±0.288 and ±0.519 (General Linear Model (GLM) procedure). Fat malassimilation was not observed. In conclusion, Tabebuia impetiginosa extract might be a promising adjunct in the management of hypertriglyceridemia and other risk factors of cardiovascular disease, common in obesity and diabetes.

Keywords: Cardiovascular Disease; Diabetic Obese Rats; High Fat and Fructose Diet; Lipase Inhibitor; Triglycerides; Tabebuia impetiginosa Extract (Lapacho Tea)

Introduction

Background

Obesity is a major health issue in the developed countries with a similar trend being now in the developing countries too. The consumption of high energy diets, notably from fats and sugars (High-Fat/High-Sugar Diet: HF/HSD) is linked to the development of obesity because it causes overconsumption of calories [1]. In fact, obesity is associated with insulin resistance which is the hallmark of type 2 diabetes and a major risk factor in cardiovascular disease. The prevalence of obesity and type 2 diabetes has escalated globally in the recent past due to a
combination of caloric overconsumption and physical inactivity. An increased use of sugar in the form of high fructose corn syrup and fat in modern diets is associated with the rise of the obesity and diabetic epidemics [2,3]. Fructose has unique metabolic properties, triggering de novo lipogenesis and causing overproduction of fatty acids which are deposited in organs. This may culminate in insulin deficiency and increase cardiovascular disease risk [4]. Combining fructose with a high fat diet in rodents causes insulin resistance, leptin resistance, type 2 diabetes and dyslipidemia which resemble the human metabolic syndrome [5].

Dyslipidemia including elevated triglycerides as part of the metabolic syndrome is considered an independent risk factor for cardiovascular diseases and atherosclerosis [6,7]. In order to prevent cardiovascular diseases, one needs to address dyslipidemia, and in particular elevated plasma triglycerides and cholesterol [7]. Lifestyle changes through diet and exercise can lower triglycerides and all other factors of the metabolic syndrome and therefore, currently remain the cornerstones of treatment [7]. However, many patients require drugs since they may fail to meet the required targets and due to progressing development of disease. Drugs such as fibrates, niacin/nicotinic acid and statins are used for treating elevated triglycerides [8]. In some instances, there may be need to use a combination of these drugs [9], which may trigger serious side-effects like myopathy and rhabdomyolysis, especially when statins and fibrates are combined [10]. Moreover, Kolovou and colleagues [7] note that these drugs may fail to address hypertriglyceridemia sufficiently and hence alternative approaches are needed.

Plants have been man’s companion for a long time and are a basis for the development of many conventional drugs [11]. The WHO encourages the use of plants in form of herbal medicine in the treatment of various illnesses, acknowledging that up to 80% of the population in the developing countries use traditional medicine in primary healthcare [11,12]. However, it emphasizes the need for studies to authenticate the use and safety of herbal medicine in the population through rigorous scientific scrutiny [13].

Tabebuia impetiginosa (Lapacho tea tree) is a tree from the Bignoniaceae family, native to South America from Brazil to northern Argentina and the extract of the inner bark was traditionally used to treat diabetes, ulcers, cancer, malaria, stomach and bladder disorders [14]. Lapachol and β-lapachone are components of methanolic extracts of Tabebuia impetiginosa which show bioactivity against various ailments [14]. In vitro studies indicated that an ethanolic extract of Lapacho tea inhibits pancreatic lipase [15]. Accordingly, in an acute experiment with Wistar rats treated with Triton-WR-1339 and fed on a high lipid load, ethanolic extracts of Tabebuia impetiginosa reduced the rate of increase of post-prandial triglycerides in the plasma of the rats probably by inhibiting pancreatic lipase [16].

Trials with Orlistat demonstrated that long-term lipase inhibition may reduce fasting and postprandial triglyceridemia, fasting LDL cholesterol (LDL-C) and diabetes incidence [17,18]. To date, no study has been done on the effect of Lapacho tea extract on plasma lipids, glycemia and insulin resistance in a long-term dose effect experiment.

Objectives

Thus, the primary objective of the study was to examine triglyceride lowering and cardioprotective effects of Lapacho tea extract in rats with diabetes and obesity, which were induced by feeding a fat- and fructose-rich diet. This model was chosen in order to investigate the effects of overconsumption of fat and fructose in the human diet since it mimicks the dietary habits in humans.

Materials and Methods

All aspects of animal care and experimentation performed in this study conformed to the Guide for Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and were in accordance with the European Economic Community (EEC) directive of 1986 (86/609/EEC) and were approved by the ethical committee of the Ministry for Agriculture, the Environment and Rural Areas of Schleswig-Holstein, Germany.

Study Design

63 six weeks old Wistar rats, weighing 196 ± 11 g, were randomly divided into three even-numbered groups: a control group (HFFD only) and two experimental groups receiving HFFD plus either 0.3 mg (HFFD+lowL) or 0.6 mg/kg Lapacho tea dry plant extract (HFFD+highL). Diets were fed for 74 days to the individually housed rats, experimental unit was the single animal.

Experimental Animals, Housing and Interventions

Three-week-old male Wistar rats (Wistar Han IGS; strain code: 273 Charles Rivers, 97633 Sulzfeld, Germany), weighing 50 g at the time of arrival, were individually housed in metal cages at ambient temperature and humidity with a 12 h light-dark cycle (lights on at 07:00). Cages were lined with carton material and sawdust for bedding material, which was changed weekly. Water and food were available ad libitum and were changed daily, uneaten food was weighed again early in the morning. Until the trials started, animals were maintained for two weeks on Ssniff® NR pellets (Ssniff®, Germany, containing 17.5, 34.8 and 47.7% of energy fat, protein and carbohydrates), and in the third week on the HFFD diet (Table 1).
Blood was collected via retro bulbar bleeding into EDTA tubes before intervention, in order to measure baseline parameters (day 0), and at the end of the intervention (day 74) via the abdominal aorta. Urine and faeces were collected before and at the end of the intervention, during 4-day balance periods, in metabolic cages. Body weight was measured weekly, while food consumption was recorded thrice weekly. At the end of the intervention period, rats were sacrificed after a 12h fast between 7.30 and 11.30h. Before termination, the rats were anaesthetized by intraperitoneal ketamine-xylazine anaesthesia (mixed in the ratio 4:1) with 0.25 mL/100 g body weight, and then terminated by cardiac puncture injection. Blood was collected, plasma immediately separated by centrifugation (4000 × g for 10 min), and stored at -20°C, while organ aliquots (liver, visceral, subcutaneous and muscle tissues) were excised immediately using dissection method from the carcass, weighed and snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

The technical staff involved in sample analysis and the scientist responsible for the statistical data analysis were not involved in the animal handling and sample collection during experiments and had no access to the animal facility.

**Experimental Outcomes**

According to our hypothesis that the expected health effects of Lapacho tea are based primarily on its lipase inhibitory activity, plasma triglycerides concentration was chosen as the primary parameter of the study. Secondary parameters were the effects of Lapacho tea on Total Cholesterol (TC), HDL-Cholesterol (HDL-C) and LDL-C, on liver metabolism (liver fat, liver Triglycerides (TG), C-Reactive Protein (CRP), Gamma-Glutamyl Transferase (GGT), Alanine Aminotransferase (ALT)) and glycemia (Fasting Blood Glucose (FBG), glycated haemoglobin (HbA1c)).

**Sample Size and Randomisation**

The number of required animals was estimated on the basis of earlier trials and verified by a pilot trial (not published). Thus, the difference between the intervention-induced changes of the primary parameter (plasma TG) in the control and experimental group was used for calculation of sample size in the main experiment: From an expected mean difference of 34.5 mg/dL, standard deviations of 57.9 or 19.7 mg/dL, α = 0.05, and β = 0.10, and applying one-sided statistical tests (because Lapacho tea as a lipase inhibitor [15] will decrease plasma TG - if there is any effect at all), this required at least 17 rats per group. Group size was increased to 21 rats, so that altogether 63 six-week-old rats were distributed randomly to the control and experimental groups using computer-generated random numbers.

**Statistical Analysis**

Since some of the parameters required killing of the animals, the primary evaluation focused on values measured at the end of the experiment, in order to treat all data in the same manner.

For statistical analysis of a dose effect of *Tabebuia impetiginosa* extract on HFFD fed rats the General Linear Models (GLM) procedure was applied using the software package “Statgraphics Plus for Windows” (version 4.5, Manugistics, Rockville, MD, USA). This corresponded largely with a simple linear regression model based on 3 Lapacho tea concentrations (0.0, 0.3 and 0.6 mg dry extract/kg diet) as the X and the values for each tested parameter of all rats as the Y values. This provided correlation coefficients (values between -0.5 and +0.5 mean a weak correlation), coefficients of variation (CV, the proportion of the variance (or the dose-effect) predictable from the independent variable) and the p-values of the Lapacho tea effects on the parameters considered at the 95% confidence level (as determined by ANOVA).

**Diets**

The *Tabebuia impetiginosa* bark, which is marketed as Lapacho tea, was purchased from Libertee, Kiel, Germany. Dried
and ground material was mixed with a fivefold volume of ethanol (abs., v/v) and extracted for 2 h at 37°C. The mixture was then centrifuged at 6100 × g for 10 min. The resulting extract contained 0.13 mg dry plant residue/mL. All experimental and control diets were based on Ssniff® NR powder, a complete (breeding and maintenance) feed for nude rats with enhanced energy density (GE = 17.4 MJ/kg; Ssniff GmbH, 59494 Soest, Germany) as shown in table 1.

From this the fat- and fructose-enriched control diet (HFFD) was prepared by adding 40 g of lard and 20 g of fructose to 100 g Ssniff®-NR powder. The total energy was provided by 56% fat, 14% protein and 30% carbohydrates (Table 1). Both experimental diets (HFFD+-lowL and HFFD+-highL) were prepared by carefully mixing 0.3 or 0.6 mg dry Lapacho tea plant extract with one kg of HFFD powder each (Table 1).

**Extraction of Lipids from Faeces**

In both experiments, lipids were extracted from faeces according to Dole [21]. Briefly, hydrochloric acid (4 mol/L, 2.5 mL) was added to 500 mg of dried and powdered faeces, mixed well and heated at 100°C for 15 minutes then cooled on ice for 5 minutes. 7.5 mL of a 40:10:1, isopropanol, heptane and sulphuric acid solution was then added to the mixture, mixed well and incubated for 1 hour at room temperature. Heptane (5 mL) and distilled water (7.5 mL) were then added causing the mixture to separate into two phases with the heptane phase, containing the lipids, on the top. The heptane phase (2.5 mL) was recovered by siphoning, evaporated to dryness and then used for lipid quantification. Total lipids content was quantified by subtracting the empty weight of the glass from the weight of the glass plus dry lipid extract.

**Extraction of Lipids from the Liver**

In the experiments, liver triglycerides were extracted according to the method by Folch [22]. Briefly, this is a biphasic solvent system procedure for lipid extraction, whereby 1 g of liver was homogenised with a 20-fold its volume with chloroform / methanol (2:1(v/v)) using a Potter-Elvehjem glass homogeniser. The homogenate was then mixed with 0.2 times its volume with an aqueous 0.58% NaCl-solution, and centrifuged, thus obtaining a biphasic system containing lipids in the lower phase. This lower phase was siphoned, evaporated to dryness by nitrogen gas then reconstituted using isopropanol with 10% Triton X-100, and the levels of triglycerides assayed enzymatically using commercially available kits (Thermo Fisher Scientific, Passau, Germany) as described by the manufacturers in a spectrophotometer (Uvikon 860, Kontron, Burladingen, Germany) at 510 nm.

**Results and Discussion**

High fat and sugar diets induce gut microbiota dysbiosis, causes gut inflammation, triggers remodelling of gut-brain axis leading to an increase in body fat mass [1,23]. From our study, we postulate that lapacho tea, which is a lipase inhibitor [16], may have favourably improved the gut microbiome by reversing dysbiosis hence the hypolipidemic and cardioprotective effects observed in the rats fed a high fat and fructose diet.

Morphological and metabolic data at the start of the experiment and the effects of 0.3 and 0.6 mg dry Lapacho-tea extract / kg food on various body and metabolic parameters compared with controls are shown in table 2. The average energy intake, body weight, liver weight and percentage of liver fat content were similar in the Lapacho groups and the control rats, and liver enzymes (ALT, GGT) were not affected significantly, whereas liver TG were decreased with increasing administration of Lapacho tea extract (p = 0.054).
The metabolic parameters were favourably affected by Lapacho tea. With increasing doses of Lapacho tea extract, plasma TG and TG/HDL-C, TC and TC/HDL-C, FBG and HbA1c significantly decreased (p<0.05). The CRP increased significantly (p<0.05). LDL-C also decreased, and HDL-C increased, however, not significantly. For the “Significant” parameters, between 8 and 27% of the positive effect could be explained by the administration of Lapacho tea extract (corresponding to a CV = 8.3 to 27.0%), with the corresponding correlation coefficients ranging between ±0.288 and ±0.519. This implies that Tabebuia impetiginosa extract may possess hypotriglyceridemic and cardioprotective effects, since atherogenic and coronary risk indices are powerful predictors for cardiovascular disease.

Table 2: Effects of different doses of Lapacho tea ethanolic extract on male Wistar rats fed on a HFFD diet in the main (dose-effect) experiment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HFFD</th>
<th>HFFD+lowL</th>
<th>HFFD+highL</th>
<th>p</th>
<th>R^2(%)</th>
<th>Corr. Coeff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>122 ± 11.5</td>
<td>412 ± 33.5</td>
<td>122 ± 14.7</td>
<td>421 ± 44.0</td>
<td>0.563</td>
<td>0.6</td>
</tr>
<tr>
<td>Energy intake (KJ/day)</td>
<td>182 ± 13.5</td>
<td>207 ± 24.3</td>
<td>179 ± 17.5</td>
<td>215 ± 24.8</td>
<td>0.503</td>
<td>0.7</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>9.87 ± 1.23</td>
<td>10.3 ± 1.26</td>
<td>10.0 ± 0.78</td>
<td>6.685</td>
<td>0.3</td>
<td>0.052</td>
</tr>
<tr>
<td>Liver fat (%)</td>
<td>2.08 ± 1.53</td>
<td>2.38 ± 0.83</td>
<td>2.39 ± 0.68</td>
<td>0.389</td>
<td>1.2</td>
<td>0.110</td>
</tr>
<tr>
<td>Liver TG (mg/g)</td>
<td>364 ± 390</td>
<td>384 ± 252</td>
<td>194 ± 125</td>
<td>0.054</td>
<td>6.0</td>
<td>-0.244</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>4.21 ± 3.81</td>
<td>6.67 ± 5.12</td>
<td>9.38 ± 18.94</td>
<td>0.563</td>
<td>6.59 ± 7.21</td>
<td>0.955</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>30.8 ± 7.26</td>
<td>45.1 ± 31.3</td>
<td>66.3 ± 89.7</td>
<td>0.389</td>
<td>79.1 ± 149</td>
<td>0.352</td>
</tr>
<tr>
<td>CRP (U/L)</td>
<td>5.07 ± 0.63</td>
<td>4.96 ± 0.33</td>
<td>4.43 ± 0.49</td>
<td>0.389</td>
<td>4.42 ± 0.51</td>
<td>0.015</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>42.5 ± 12.1</td>
<td>69.6 ± 18.6</td>
<td>60.9 ± 15.5</td>
<td>0.022</td>
<td>58.4 ± 11.5</td>
<td>0.022</td>
</tr>
<tr>
<td>TG/HDL-C</td>
<td>0.81 ± 0.27</td>
<td>1.70 ± 0.60</td>
<td>1.44 ± 0.40</td>
<td>0.097</td>
<td>1.33 ± 0.34</td>
<td>0.014</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>75.9 ± 11.9</td>
<td>74.0 ± 12.2</td>
<td>73.8 ± 11.2</td>
<td>0.004</td>
<td>55.8 ± 5.86</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>53.7 ± 8.47</td>
<td>53.1 ± 7.56</td>
<td>42.8 ± 6.00</td>
<td>0.337</td>
<td>44.4 ± 4.81</td>
<td>0.337</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>12.8 ± 4.91</td>
<td>12.7 ± 3.92</td>
<td>12.5 ± 3.02</td>
<td>0.166</td>
<td>5.69 ± 1.99</td>
<td>0.166</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>1.42 ± 0.15</td>
<td>1.39 ± 0.11</td>
<td>1.35 ± 0.12</td>
<td>0.139</td>
<td>1.26 ± 0.07</td>
<td>0.139</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>0.24 ± 0.10</td>
<td>0.24 ± 0.06</td>
<td>0.24 ± 0.06</td>
<td>0.24 ± 0.06</td>
<td>0.13 ± 0.04</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>146 ± 30.7</td>
<td>251 ± 56.4</td>
<td>220 ± 35.3</td>
<td>0.009</td>
<td>215 ± 32.0</td>
<td>0.009</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>3.37 ± 0.14</td>
<td>3.94 ± 0.18</td>
<td>3.03 ± 0.16</td>
<td>0.393 ± 0.23</td>
<td>3.28 ± 0.19</td>
<td>0.380 ± 0.15</td>
</tr>
</tbody>
</table>

121 male Wistar rats per group were given either a High Fat and Fructose Diet (HFFD) only or this diet plus either 0.3 mg/g (HFFD+lowL) or 0.6 mg/g (HFFD+highL) of Lapacho tea extract for 74 days and various parameters evaluated at the start and end (or only end) of the experiment. Values are expressed as mean ± SD. *p < 0.05, §p < 0.1 (GLM).

Table 2: Effects of different doses of Lapacho tea ethanolic extract on male Wistar rats fed on a HFFD diet in the main (dose-effect) experiment.
disease risk [24,25], and elevated plasma lipids and blood glucose are common scenarios in diabetes and the metabolic syndrome.

In fact, Tabebuia impetiginosa extract inhibited pancreatic lipase in vitro [15] and delayed the rate of increase of postprandial TG in Triton WR-1339 treated rats fed a fat load, during an acute experiment [16]. In our study long-term administration of Tabebuia impetiginosa extract improved diabetes in the rats as indicated by a less pronounced increase in Urinary Glucose (UG), and a significant reduction of FBG and %HbA1c in comparison to the control, indicating improved glycemic control. Indeed, these effects are not surprising, since Tabebuia impetiginosa extract delayed increase in postprandial TG [16] and postprandial hypertriglyceridemia as well as intrahepatic lipogenesis resulting in hypertriglyceridemia, are associated with gluconeogenesis and insulin resistance [16,26].

Hepatic de novo biosynthesis and VLDL release may result in hypertriglyceridemia, if VLDL clearance by lipoprotein lipase does not increase correspondingly [27]. Loss of insulin activity results in lower expression of lipoprotein lipase and consequently in reduced clearance of TG in VLDL and chylomicrons. This may explain why Orlistat, a potent lipase inhibitor, has effects on glycemia, too [17]. The findings of our studies are also in agreement with reported folkloric use of Tabebuia impetiginosa in the diabetes treatment [14].

Blocking of digestive enzymes is among the current trends in obesity and diabetes treatment. This, however, may result in unfavourable side-effects such as steatorrhea in Orlistat treatment [28,29]. In contrast, Tabebuia impetiginosa extract did not increase fecal fat excretion (Table 2) even though it a) inhibits pancreatic lipase, as indicated by in vitro data [15] and b) delays the increase of postprandial TG after a fatty meal in a rodent model [16]. This may be due to: a) incomplete inhibition of the lipase as a result of a low dosage of the active component in our study and b) reversible inhibition of lipase due to a lower affinity of the active component to lipase as compared to Orlistat. Dissociation during transit through the small bowel and absorption or degradation of the active component and hence, loss of activity during gastrointestinal transit may withdraw the active component from the inhibitory reaction. The lack of steatorrhea when using Lapacho tea extract may be an advantage over Orlistat, which has never been a fully successful anti-obesity drug due to gastrointestinal side effects such as steatorrhea [30]. Whether this holds true has to be clarified by dose-effect studies in humans evaluating effects and side-effects.

In Orlistat, lipase inhibition causes a decrease in body weight [28]. However, we did not find a decrease in body weight by Lapacho tea extract (Table 2). This could be due to the lack of energy losses by steatorrhea or by more complex regulatory effects bound to the transit of fat into lower parts of the intestine. Liver steatosis is a common cause of liver injury in obesity and diabetes [31]. Although the liver weights and liver TG concentrations were slightly higher in Tabebuia impetiginosa extract group (Table 2), these did not seem to cause liver injury, since ALT, CRP and GGT levels did not significantly differ from the control. In fact, liver TG were significantly reduced in the high Lapacho tea diet (Table 2). We may therefore speculate that Tabebuia impetiginosa extract protected the rats against liver steatosis.

Conclusions

We report for the first time that long-term administration of Tabebuia impetiginosa extract to rats fed a high fat high fructose diet, significantly and dose-dependently lowered plasma TG, TC, AI, CRI and liver triglycerides as well as fasting blood glucose and HbA1c. Similar beneficial effects are known for other lipase inhibitors in humans. This, together with the general acceptance of the fat-and fructose-rich fed rat as a model mimicking dietary habits in humans in industrialized countries and the comparability of the underlying metabolic mechanisms in rats and humans, suggests that our results can qualitatively be transferred to humans. Thus, Lapacho tea extract may be useful for treating and preventing, respectively, hypertriglyceridemia and cardiovascular disease which are common traits of the metabolic syndrome, especially in Western societies with its high consumption of fat and sugars. The observed lack of steatorrhea, which might, at least partly, result from a lower lipase inhibition by Lapacho tea extract, raises hopes for fewer side effects compared to Orlistat. This, however, has to be verified in human trials.

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Conflicts of Interest

The authors declare no competing interests, however, J. Schrezenmeir held a patent on lipase inhibitory extracts including that from Lapacho tea (US 2008/0299234 A1, (Schrezenmeir, 2006 ).

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