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ABSTRACT

The aim of the present study was to demonstrate vascular changes and the effect of Bidens pilosa extract in fructose induced hypertension. Interactive computerized microscopy was used to study the morphometric alterations in the mesenteric arterial bed of Wistar rats treated with fructose (10%) in drinking water during 3 weeks and for 3 subsequent weeks with fructose, fructose plus Bidens pilosa (75 mg/kg) or fructose plus nifedipine (10 mg/kg). Fructose feeding brought about structural changes in the blood vessels associated with a slight increase in the systolic pressure. The minimal and maximal diameters and lumen areas of the vessels were reduced. The total areas and lumen areas were as well as the number of nuclei in the media were reduced following 6 weeks of fructose feeding. The media/lumen ratios were reduced by 28%. The media area and the density of the nuclei in the media were significantly reduced after 6 weeks of fructose treatment compared with the 3 week fructose treatment. The morphometric study showed that the small arteries underwent structural alterations, which were reversed by Bidens pilosa treatment. Nifedipine, decreased like the plant extract the blood pressure but, fall to attenuated changes in morphological parameters in mesenteric arteries.

Keywords: Mesenteric arteries, Bidens pilosa, morphometric changes, rats, fructose.

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INTRODUCTION

High fructose diets have been known to increase blood pressure in rats [1,2,3,4]. Hypertension develops in Wistar rats as early as 2 weeks after initiation of the diet. In normal rats, as in human, the hypertension is accompanied by insulin resistance, hyperinsulinemia and hypertriglyceridemia [5,6]. Abnormalities of both small and large arteries are implicated in the mechanisms of increased systolic pressure [7]. For a given input flow, increased systolic blood pressure (SBP) in hypertension is due to an increase in vascular resistance, which is produced by a reduction in the calibre of small arteries. In most of the previous morphological studies on blood vessels, the observations were qualitative rather than quantitative, and these studies described a range of changes within the vessel wall that appeared to vary according to site and type of vessel examined. The changes include cross-sectional area and diameter of mesenteric arteries [4], morphologic and functional alterations of the endothelium [8]. *Bidens pilosa* (*B. pilosa*) is a medicinal plant widely used in Cameroon traditional medicine for the management of hypertension. We recently reported that the leaf methanol extract of *B. pilosa* was capable of preventing and attenuating the hypertension induced by high-fructose diet in rats [9]. Our previous results demonstrated that starting at the lower concentration of 75 mg/kg, the leaf methanol extract of *B. pilosa* was capable to prevent and attenuate the establishment of elevated blood pressure in fructose fed rats.

The purpose of this study was to quantify vascular changes and characterize the morphometric changes of the mesenteric vasculature in fructose-induced hypertensive rats using the Highly Optimised Microscope Environment (HOME) system of interactive computerized microscopy. The effects of the leaf methanol extract of *B. pilosa* were also examined. The results of this study constitute novel information on the morphometric changes of the mesenteric arteries studied in an animal model of fructose-induced hypertension.

MATERIALS AND METHODS

Animals

Male Wistar rats from Iffa-Credo (France), initially weighing between 180 and 200 g, were used for the experiments. The animals were housed in temperature- and humidity-controlled conditions and were fed standard rat chow and tap water. The rats were trained for 1 week to become used to the procedure of indirect blood pressure measurement. Rats were then randomly divided into groups of six rats each. Fructose-treated rats were given 10% fructose to drink *ad libitum* for a total of 3 or 6 weeks. Ordinary tap water was given to control animals to drink throughout the entire experimental period. All the procedures and protocols involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies.
Extraction of Plant material

Fresh leaves of *Bidens pilosa* L (Asteraceae) were collected in November around the campus of the University of Yaounde I. The plant material was identified at the national herbarium, in Yaounde where voucher specimen N° HNC/158742 has been deposited. The leaves were sun-dried and ground into powder. Dried material (650 g) was ground and extracted with a 1:1 mixture of methanol and dichloromethane as previously described (Dimo et al., 2002). The CH$_3$OH/CH$_2$Cl$_2$ extract was fractionated with CH$_2$Cl$_2$ to give 20 g of the methanol extract. Four grams of this extract were dissolved in 100 ml of distilled water to give a final concentration of 40 mg/ml.

Blood pressure measurement

The tail cuff method was used to measure the SBP and pulse rate [10]. The equipment used included an electrosphygmomanometer (Le 5002 Storage Pressure Meter) connected to a Le-5650/6 Heater & Scanner. Temperature in the heater chamber was maintained at 36°C. The SBP was measured in an un-anaesthetized state. The mean of five consecutive readings was used as the measurement of the SBP of each rat.

Treatment of the animals

Thirty rats were divided into five groups of six rats each and given either fructose in drinking water (n = 24) or given tap water (n = 6). After 3 weeks of chronic fructose feeding, group I (n = 6) of fructose-fed rats was sacrificed by decapitation, and the mesenteric vascular system was dissected for histomorphometric studies. The other three groups of fructose-fed rats were treated, respectively with fructose only, fructose plus *B. pilosa* extract (75 mg/kg per day), or fructose plus nifedipine (10 mg/kg per day) for a further 3 weeks. Body weight, cardiac mass, SBP and pulse rate of all the rats were recorded at the end of the experiment.

Histomorphometric study of arterial mesenteric system

The mesenteric vascular tree was collected by dissecting the superior mesenteric artery and its branches up to their penetration into the jejunum. The vessels were immersed for 6 h in Bouin solution and kept in 70% ethanol as previously described by Mechaly et al. [11].

Cross-sections of tissue samples were cut in the general direction of the vessels, at the distal portion of the jejunum arteries resulting from the division of the cranial mesenteric artery, and embedded in paraplast (Histomed Standard, Labo. Moderne, France). Three mm thick transverse sections, obtained 5 mm from the distal end of the arteries, were stained with Periodic Acid Schiff (PAS) for histomorphometric analysis in order to visualize elastic laminae.

Morphometric measurements were performed using the axioHOME system (Carl Zeiss, Oberkochen, Germany). Briefly, the system consists of an IBM-PC compatible computer using the 2.04 version of the Zeiss-Alcatel TITN Answare software (Meylan,
France) and an in-built light microscope (a high resolution image is superimposed on the optical image of the specimen). Six to eight arteries (vessels showing several elastic laminae) per animal were quantified, given a total of 36 – 41 vessels per group. For each artery examined, the following parameters were measured; maximal and minimal diameters (D and d), total vessel area (T), media + lumen area (ML), lumen area (L), number of nuclei in media (nM) and number of nuclei in adventitia (nADV). Wall area (W), adventitia area (ADV), and media area (M) were calculated as (T – L), (T – ML), and (ML – L), respectively, assuming that the intima area was negligible in the conditions of quantification. Coefficients of variation were lower than 1% for diameters or nuclei, and lower than 3% for areas.

Statistical analysis

Statistical comparisons were performed using the Statgraphics software (Uniware, Paris, France). A multiple range test was performed after ANOVA. When a significant difference was obtained (p < 0.05), a least significance difference (LSD) test was used to compare each pair of means.

All the five groups of animals were considered independently for comparisons. For morphometric data, areas and nuclei numbers were transformed into their logarithmic values, so that normal distribution and homogeneity of variance between groups were obtained for each measured or calculated parameter. Nested (hierarchical) analysis of variance was performed on the 308 vessels examined (20 to 45 vessels per group) for comparison of measured and quantified parameters between treated groups, the “rat” factor being nested within the “group” factor. The ANOVA for each parameter tested the significance of each of the two factors.

RESULTS

General features of the animals

Table 1 illustrates the effects of fructose-feeding and B. pilosa extract administration on the body weight, cardiac mass, systolic blood pressure and heart rate of the rats. These results demonstrate that weight gain and heart rate were similar in all the five groups. Fructose feeding alone was associated with a small but significant increase in tail systolic blood pressure at 3 and 6 weeks of treatment as compared with the control and fructose with nifedipine or B. pilosa groups. Fructose alone or fructose with B. pilosa extract treatment had no significant effect on the cardiac mass of the rats at the end of the 6th week of observation.

Morphometric analysis of the mesenteric arterial system

Results from histomorphometric measurements of the arterial system are given in Table 2. Chronic fructose feeding in drinking water during three weeks resulted in a significant reduction of the minimal and maximal diameters and of the lumen areas. As such, the media/lumen ratio increased by 54% compared to the untreated control animals. Fructose treatment for 6 weeks was associated with a significant reduction of total and lumen areas, as well as the number of nuclei in the media of the arterial mesenteric system.
compared to the control group. In the animals receiving fructose alone, the total vessel area (area geometric means) reduced from 37 881 µm² to 32 575 µm² after 3 weeks, and to 31 914 µm² at the end of the 6th week. Artery wall areas (31 768 µm² in the control) decreased by 9% for fructose-fed rats at week 3 and by 13% at week 6.

Table 1: Effects of *B. pilosa* (Bp) extract and nifedipine (Ni) on the body weight, cardiac mass, systolic blood pressure and heart rate of fructose hypertensive rats.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control</th>
<th>Fructose only</th>
<th>Fructose plus Bp (75 mg/kg)</th>
<th>Fructose plus Bp (75 mg/kg) Ni (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>0</td>
<td>306 ± 9</td>
<td>297 ± 12</td>
<td>280 ± 9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>348 ± 11</td>
<td>335 ± 17</td>
<td>292 ± 6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>379 ± 15</td>
<td>374 ± 19</td>
<td>348 ± 14</td>
</tr>
<tr>
<td>Cardiac mass (mg/100 g B.W)</td>
<td>3</td>
<td>282 ± 7</td>
<td>268 ± 9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>267 ± 5</td>
<td>277 ± 6</td>
<td>282 ± 11</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>0</td>
<td>105 ± 4</td>
<td>108 ± 3</td>
<td>118 ± 4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>104 ± 1</td>
<td>132 ± 1*</td>
<td>115 ± 3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>118 ± 1</td>
<td>146 ± 4*</td>
<td>109 ± 7</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>6</td>
<td>80 ± 7</td>
<td>89 ± 8</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>0</td>
<td>334 ± 4</td>
<td>369 ± 13</td>
<td>388 ± 17</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>311 ± 6</td>
<td>365 ± 17</td>
<td>400 ± 8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>335 ± 8</td>
<td>369 ± 13</td>
<td>392 ± 11</td>
</tr>
</tbody>
</table>

Data are given as mean ± SEM, n = 6 per group.
* p < 0.5, significantly different compared to normal control rats.
†p < 0.5, significantly different compared to 6 weeks fructose-fed control rats.
SBP, systolic blood pressure; DBP, diastolic blood pressure.

In order to examine the effect of the leaf methanol extract of *B. pilosa* on the morphometric changes of the mesenteric vessels, the animals that had received fructose for 3 weeks were treated by gavage with the plant extract for 3 subsequent weeks. Our results indicate that *B. pilosa* treatment (75 mg/ kg per day) restored the histomorphometric changes toward the normal values. The total and lumen areas were similar between rats treated with *B. pilosa* extract and the control rats that were not subjected to fructose treatment. The total vessel wall area of *B. pilosa*-treated rats increased by 23% compared to the rats receiving fructose only for 6 weeks. The morphometric study also revealed a significant reduction of the adventitia and media areas of the vessels of rats treated with fructose only as compared to the fructose plus *B. pilosa*-treated group. The thickness of the nuclei in the media was significantly increased in animals treated with the plant extract and with nifedipine (10 mg/kg) as compared with the controls and the rats treated with fructose only.

**DISCUSSION**

We earlier reported that chronic fructose feeding in drinking water induced hypertension, hyperinsulinaemia and hypertriglycerideremia in wistar rats [9,11]. It has been documented that insulin is a growth factor promoting vascular smooth muscle growth, thereby, resulting in a narrowing of the lumen of resistance vessels, consequently raising vascular resistance [12]. The results of the present study demonstrate that 3 to 6 weeks of high fructose feeding in rats resulted in a reduction of the lumen area of the vessels and
Table 2: Histomorphometric parameters of the mesenteric arteries of fructose hypertensive rats treated with *B. pilosa* extract (Bp) or nifedipine (Ni).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Fructose only- 3 weeks</th>
<th>Fructose only- 6 weeks</th>
<th>Fructose + Bp (75 mg/kg)</th>
<th>Fructose + Ni (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d (µm)</td>
<td>188.42 ± 5.57</td>
<td>175.71 ± 4.03*</td>
<td>184.59 ± 4.47</td>
<td>190.67 ± 4.09b</td>
<td>180.12 ± 4.50</td>
</tr>
<tr>
<td>D (µm)</td>
<td>263.57 ±13.11</td>
<td>248.48 ± 9.61*</td>
<td>222.11 ± 6.85*</td>
<td>278.33 ± 10.94††b</td>
<td>248.50 ± 10.22</td>
</tr>
<tr>
<td>W ln (µm²)</td>
<td>10.36 ± 0.06</td>
<td>10.26 ± 0.05</td>
<td>10.23 ± 0.04</td>
<td>10.44 ± 0.04††b</td>
<td>10.27 ± 0.05</td>
</tr>
<tr>
<td>T ln (µm²)</td>
<td>10.54 ± 0.06</td>
<td>10.39 ± 0.05</td>
<td>10.37 ± 0.04*</td>
<td>10.60 ± 0.05††b</td>
<td>10.41 ± 0.05</td>
</tr>
<tr>
<td>ML ln (µm²)</td>
<td>9.92 ± 0.07</td>
<td>9.77 ± 0.06</td>
<td>9.75 ± 0.05</td>
<td>10.01 ± 0.05† b</td>
<td>9.84 ± 0.06</td>
</tr>
<tr>
<td>ADV ln (µm²)</td>
<td>9.74 ± 0.06</td>
<td>9.60 ± 0.05</td>
<td>9.58 ± 0.04</td>
<td>9.79 ± 0.05††b</td>
<td>9.57 ± 0.05*</td>
</tr>
<tr>
<td>M ln (µm²)</td>
<td>9.57 ± 0.07</td>
<td>9.53 ± 0.06</td>
<td>9.47 ± 0.04</td>
<td>9.70 ± 0.05 † b</td>
<td>9.57 ± 0.05</td>
</tr>
<tr>
<td>L ln (µm²)</td>
<td>8.65 ± 0.09</td>
<td>8.18 ± 0.08*</td>
<td>8.29 ± 0.07*</td>
<td>8.66 ± 0.08† † b</td>
<td>8.28 ± 0.11*</td>
</tr>
<tr>
<td>ADV ln (n)</td>
<td>3.08 ± 0.06</td>
<td>3.10 ± 0.05</td>
<td>3.02 ± 0.05</td>
<td>3.20 ± 0.04†</td>
<td>3.15 ± 0.05</td>
</tr>
<tr>
<td>M ln (n)</td>
<td>3.96 ± 0.11</td>
<td>4.00 ± 0.06</td>
<td>3.78 ± 0.06* † b</td>
<td>4.22 ± 0.06* † b</td>
<td>4.16 ± 0.07†</td>
</tr>
<tr>
<td>M/L</td>
<td>2.68 ± 0.15</td>
<td>4.13 ± 0.21*</td>
<td>3.43 ± 0.23*</td>
<td>2.96 ± 0.14†</td>
<td>4.18 ± 0.37*</td>
</tr>
<tr>
<td>dNADV(10⁻³ µm²)</td>
<td>1.35 ± 0.09</td>
<td>1.57 ± 0.08*</td>
<td>1.48 ± 0.09</td>
<td>1.41 ± 0.05†</td>
<td>1.68 ± 0.07*</td>
</tr>
<tr>
<td>dNM (10⁻³ µm²)</td>
<td>3.83 ± 0.24</td>
<td>4.12 ± 0.15</td>
<td>3.46 ± 0.17 † b</td>
<td>4.26 ± 0.12† †</td>
<td>4.66 ± 0.18‡ † b</td>
</tr>
</tbody>
</table>

*d*, minimal diameter; *D*, maximal diameter; *W*, wall area; *T*, total vessel area; *ML*, media plus lumen area; *ADV*, adventitia area; *M*, media area; *L*, lumen area; *nADV*, number of nuclei in adventitia; *nM*, number of nuclei in media; *M/L*, Media/Lumen ratio; *dNADV*, density of nuclei in adventitia; *dNM*, density of nuclei in the media.

Data are given as mean ± SEM of a total of 20 to 45 arteries per group (4 to 8 arteries per rat, 6 rats per group). (*) Corresponds to a significant difference (p < 0.05) as compared to control rats; (b) significantly different (p < 0.05) compared to 3 weeks fructose only group; (‡) significantly different (p < 0.05) compared to 6 weeks fructose only group; (†) significantly different compared to fructose plus Nifedipine- treated rats.
significant increase in the media/lumen ratio as compared to the control untreated group, indicating a vasoconstriction of the mesenteric vessels. The structural changes leading to decreased luminal diameter of resistance vessels are central to the increased peripheral resistance common to most forms of chronic hypertension [13,14]. It is well known that increase pressure results in a decrease diameter, acutely by an increase of myogenic tone, and chronically by hypertrophy of smaller mesenteric arteries and by remodelling or concentric growth [15,16]. In contrast to previous studies using other models of hypertensive rats which showed significant increases in media cross-section wall area and thickness of mesenteric resistance vessels [17,18], our study showed as previously reported by Parker et al. [19] no significant changes of the total vessel, media and adventitia areas. The present results in fructose fed rats are in contrast with those observed by Puyo et al. [20] showing an increased thickness and area of the media in 22 weeks fructose treated rats when compared with the controls. The differences between those results can be explained by the duration of the treatment (6 vs 22 weeks). Wall/lumen ratios (geometric means) were 5.33 in normal control rats; corresponding values for fructose-treated rats at weeks 3 and 6 were significantly higher (8.04 ± 0.58 and 6.93 ± 0.82, respectively). The thickness of media nuclei following 6 weeks of fructose treatment was reduced by 16% compared with values obtained after 3 weeks, indicating a reduction of hyperplasia [13] during the last three weeks of chronic fructose feeding (Table 2). It has been demonstrated that in hypertension, changes in small artery structure are basically of 2 kinds: (1) inward eutrophic remodeling, in which outer and lumen diameter are decreased, media/lumen ratio is increased, and cross-sectional area of the media is unaltered; and (2) hypertrophic remodeling, in which the thickens to encroach on the lumen, resulting in increased media-cross sectional area and media/lumen ratio [21]. In our study, we observed no difference between the media cross-sectional area of the animal receiving fructose alone and the control group, thus suggesting the presence of remodeling instead of hypertrophy or hyperplasia [22].

In the present study, we found that when the leaf methanol extract of *B. pilosa* (75 mg/kg) was associated with the fructose feeding for 3 weeks, there was no significant difference in wall, lumen, adventitia, media and total areas as compared to the untreated control group. *B. pilosa* extract corrected the structural changes undergone by the small arteries. Thus, the leaf methanol extract of *B. pilosa* was able to reverse the vascular remodeling changes induced by the high fructose diet. The results also show that nifedipine, a dihydropiridine derivative used in the same experimental conditions like the plant extract, did not correct the altered morphometric parameters resulting from mild hypertension observed in this model. The lack of effect of nifedipine in our study suggests that inhibition of Ca$^{2+}$ entry through L-channels is not a decisive factor regulating the vascular remodeling in this model of hypertension. These results are consistent with the previous findings in the muscular arteries in the kidney, heart and mesenteric bed [23,24]. In addition, nifedipine, in association with fructose feeding, provoked a decrease of the adventitia areas while *B. pilosa* extract reversed this effect. Moreover, nifedipine treatment did not reverse in this study the fructose-mediated hypertrophy in cardiac muscle. It is known that high-fructose feeding leads to hypertension by several mechanisms including sodium retention and fluid volume expansion, stimulations of the sympathetic nervous system and small vascular muscle proliferation [25,26]. Lund-Johansen et al. [27] have reported that nifedipine-treated patients have strongly elevated plasma norepinephrine levels which have been reported to
be potent inducers of cardiac hypertrophy. Therefore, cardiac hypertrophy observed in fructose plus nifedipine fed rats may be the additive results of sympathetic stimulation induced by both fructose and nifedipine. Our study has shown that in contract of the plant extract, nifedipine can decrease blood pressure without large changes in morphological parameters in mesenteric arteries. These results indicate that the mechanism by with nifedipine decrease blood pressure is different from that used by the plant extract. In our previous study, we have observed that high fructose-induced insulin resistance induces cardiac hypertrophy, before the occurrence of hypertension, suggesting that cardiac remodeling may not be directly the consequence of hypertension [28] methanol extract of B. pilosa was capable to prevent and attenuate the increases in both plasma insulin and blood pressure suggesting that the extract promotes insulin sensitivity [9]. It therefore seems that the plant extract exerts its antihypertensive effect and beneficial effects on vascular alterations induced by high fructose by improving insulin sensibility and possibly by other mechanisms such as free radical scavenging and lipid antiperoxidation activity attributed to the presence of resveratrols and flavonoids in the plant extract [29,30]. Interestingly, these compounds are known to dilate vascular beds in disease of vascular wall involving inflammation [31] and reduce smooth muscle proliferation [32]. Indeed, we have shown that high fructose diets are associated with oxidative stress characterized by an early increase in superoxide anion production and elevated plasma concentrations of lipid and protein oxidative products [28]. In addition, increase in superoxide anion production could also be linked to the activation of the renin-angiotensin system in fructose-fed rats [33].

In conclusion, we have shown that after drinking a high fructose solution for 3 to 6 weeks, normal rats exhibited significant increase of the systolic blood pressure associated with alterations of the arteries of the mesenteric bed. In particular, fructose-fed rats showed decreases in vascular lumen and total areas as well as the number of nuclei in the media. Wall/lumen ratios were also significantly increased. Treatment with B. pilosa extract reversed some morphometric changes suggesting a cardioprotective effect of the plant extract. Additional studies are required to critically investigate the underlying mechanisms responsible for the fructose-induced alterations in the arterial mesenteric system, as well as the mechanism by which B. pilosa extract reverses these effects.

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