Short Communication

Renoprotective and antioxidant effects of *Saururus chinensis* Baill in rats fed a high-fructose diet

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Abstract

This study investigated the preventive effect of *Saururus chinensis* Baill against renal damage induced by a high-fructose diet in rats. The rats (n = 30) were fed either a cornstarch-based (65%), high-fructose (65%), or high-fructose (64.5%) diet with 0.5% *S. chinensis* Baill extract for 10 weeks. Twenty-four hour urine collections were obtained and the animals were sacrificed after an overnight fast. Serum urea and creatinine and urine albumin were measured using colorimetric methods, and creatinine clearance was determined. In addition, thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and the activity of superoxide dismutase (SOD) in the kidney were determined. Kidney samples were also examined histologically. The fructose-fed rats showed renal dysfunction, indicated by decreased creatinine clearance, increased albumin in the urine, and increased urea and creatinine in the serum. These renal function parameters were comparable to control levels in rats that consumed *S. chinensis* Baill. Fructose consumption increased renal TBARS and reduced GSH and SOD activity, whereas these levels were near-normal in the rats consuming *S. chinensis* Baill. The kidneys of fructose-fed rats showed glomerular basement membrane thickening, mesangial matrix expansion, and tubule dilation. These pathological changes were not seen in the rats that consumed *S. chinensis* Baill. Therefore, *S. chinensis* Baill effectively alleviated fructose-induced renal damage in these rats, at least partially due to antioxidant activity.

Key Words: *Saururus chinensis* Baill, fructose, kidney, creatinine, antioxidant

Introduction

Fructose is a naturally occurring monosaccharide found in honey, fruits, and vegetables. Fructose intake has increased several-fold in the last three decades, mainly due to the use of high-fructose syrup and crystalline fructose in the food industry [1,2]. High-fructose consumption has been reported to facilitate renal damage in normal rats [3-5]. In such rats, a high-fructose diet has adverse effects on renal morphology and biochemical parameters of renal function, such as creatinine clearance and levels of urea and creatinine in the serum. High-fructose intake also increases oxidative stress in the kidneys [4-6]. However, the consumption of genistein improves renal morphology and function in fructose-fed rats [4], and this isoflavone may exert its effects by acting as an antioxidant.

Saururus chinensis Baill is a perennial herbaceous plant found in China, Japan, and Korea. As a Chinese medicine, *S. chinensis* Baill has been used to treat beriberi, pneumonia, edema, urination, jaundice, and gonorrhea [7]. *S. chinensis* Baill shows strong antioxidant activity [8,9], and its extract has been shown to reduce lipid peroxide levels in rats fed a high-fat diet [10] and in rats with carbon tetrachloride-induced hepatic fibrosis [11]. Therefore, *S. chinensis* Baill might protect against renal damage related to fructose consumption. However, this has not been studied. Therefore, we investigated the effects of *S. chinensis* Baill on renal function and histology as well as antioxidant status in rats fed a high-fructose diet.

Materials and Methods

Chemical

Fructose was obtained from Daejung Chemicals & Metals (Siheung, Gyonggi-do, Korea). Assay kits for creatinine and urea were purchased from BioAssay Systems (Hayward, CA, USA) and kits for albumin were obtained from AsanPharm (Seoul, Korea). Cornstarch was purchased from Daesang (Seoul, Korea).

Casein, a mineral mixture, and a vitamin mixture were purchased from ICN Pharmaceuticals (Costa Mesa, CA, USA), and soybean oil was purchased from Cheiljedang (Seoul, Korea). Alpha-cellulose, choline bitartrate, and all other reagent-grade chemicals were obtained from Sigma Chemical (St. Louis, MO, USA).

Preparation of S. chinensis extract

The aerial parts of *S. chinensis* Baill including the leaves, stems, and flowers was obtained from a local market in Busan, Korea, in December 2009, and were freeze-dried, powdered, and extracted with ten volumes of 95% ethanol for 12 h twice at room temperature. The solvent was removed by rotary evaporation at 40℃. The extraction yield was 8.6%.

Animals and diets

This study was approved by the Animal Resource Center at Inje University, Korea. Male Sprague-Dawley rats (n = 30) weighing 110-130 g were purchased from Bio Genomics (Seoul, Korea) and housed individually in stainless steel wire-bottomed cages in an environmentally controlled room at 24 ± 5℃ and 55 ± 5% relative humidity with a regular 12-h light:12-h dark cycle. After 1 week of adaptation during which the animals had free access to commercial chow, they were divided randomly into three groups. The control group was fed a diet based on the AIN-76 diet containing 65% cornstarch, whereas the fructose group was offered a diet containing 65% fructose (Table 1). The *S. chinensis* Baill group was fed a diet containing 64.5% fructose and 0.5% *S. chinensis* Baill extract. A previous study reported that ethanol extract of *S. chinensis* Baill at 0.5% of the diet significantly reduced hepatic lipid peroxidation in mice fed a high fat diet [10]. Thus, the renoprotective and antioxidant effects of *S. chinensis* Baill extract consumed at 0.5% of the total diet were investigated in this study. The assigned diets were offered *ad libitum* for 10 weeks. Body weight was monitored weekly and food intake was documented three times per week.

Measuring creatinine clearance

At the end of the 10-week experimental period, the animals were placed in individual metabolic cages and urine samples were collected for 24 h. Urine volume was measured and the urine was stored at -70℃ until further analysis. Approximately 50 µL of blood was collected from the tail vein. The blood samples were centrifuged at 1,500 g for 15 min to isolate the serum. Serum and urine creatinine were measured by calorimetric methods using commercial kits (BioAssay Systems, Hayward, CA, USA). The glomerular filtration rate (GFR) was calculated as the creatinine clearance, which was calculated as [Ucr × V]/Pcr, where Ucr is the creatinine concentration in the collected urine, V is the urine flow rate (mL/min), and Pcr is the serum creatinine concentration. Creatinine clearance was expressed in mL/min/100 g body weight.

Biochemical analysis

The animals were sacrificed by lethal CO_2 inhalation and exsanguinated by cardiac puncture after an overnight fast. Kidney samples were collected. The blood samples were centrifuged and the serum was stored at -70℃. One kidney was stored at -70℃ until further analysis. The other kidney was fixed in 10% phosphate-buffered formalin solution for histopathological examination.

Serum urea and creatinine were measured by calorimetric methods using commercial kits (BioAssay Systems, Hayward, CA, USA). Urinary albumin was measured colorimetrically using an assay kit (AsanPharm, Seoul, Korea). The antioxidant effect of *S. chinensis* Baill was determined by measuring lipid peroxides and reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity in the kidney. Lipid peroxides were assessed as thiobarbituric acid reactive substances (TBARS) according to the method developed by Ohkawa *et al.* [12]. Briefly, a kidney homogenate was prepared in five volumes of 10 mM sodium phosphate buffer (pH 7.4). The homogenate was mixed with a solution containing 15% TCA, 0.4% thiobarbituric acid (TBA), and 2.5% HCl. The reaction mixtures were boiled at 100℃ for 45 min, cooled, and centrifuged at 1,700 g for 10 min. The absorbance of the supernatant was measured at 534 nm. The protein content was measured using the Bradford method [13] with bovine serum albumin as the standard. The level of lipid peroxides was expressed as nmol malondialdehyde (MDA)/mg of protein. GSH was determined by Ellman's method [14] modified by Nagi *et al.* [15]. The kidney homogenate was reacted with Ellman's reagent (5,5-dithio-2-nitrobenzoic acid) in phosphate buffer (pH 8.0) and the absorbance was measured at 412 nm. The GSH concentration was calculated using a standard

Table 1. Composition of experimental diets (%)

Ingredients	Group		
	Control	Fructose	*S. chinensis* Baill
Corn starch[1]	65.0	0.0	0.0
Fructose[2]	0.0	65.0	64.5
Casein[3]	20.0	20.0	20.0
Corn oil[4]	5.0	5.0	5.0
Alpha-Cellulose[5]	5.0	5.0	5.0
Mineral mixture[6]	3.5	3.5	3.5
Vitamin mixture[7]	1.0	1.0	1.0
D,L-methionine[5]	0.3	0.3	0.3
Choline bitartrate[5]	0.2	0.2	0.2
S. chinensis Baill extract	-	-	0.5

[1] Daesang Co., Korea
[2] Daejung Co., Korea
[3] ICN Pharmaceuticals Inc., U.S.A.
[4] Cheiljedang Co., Korea
[5] Sigma Co., U.S.A.
[6] AIN-76 Mineral mixture, ICN Pharmaceuticals, U.S.A.
[7] AIN-76 Vitamin mixture, ICN Pharmaceuticals, U.S.A.

solution of GSH. The SOD activity was assayed according to the method developed by Marklund and Marklund [16]. One unit of SOD was defined as the amount of enzyme that reduced the rate of autoxidation of pyrogallol by 50%. The enzyme activity was expressed as U/mg of protein.

Histological examination

Kidney samples from each group were prepared for histopathological assessment and placed in 10% neutral buffered formalin. Each specimen fixed in 10% formalin solution was embedded in paraffin wax. Sections of 4 μm in thickness were stained with hematoxylin and eosin, and examined under a light microscope. A single urologist and pathologist blinded to the treatment group independently evaluated the staining patterns.

Statistical analysis

All data are expressed as the mean ± standard deviation (SD). The data were evaluated statistically using one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was defined as $P < 0.05$.

Results

Effects on renal function and antioxidant status

Body weight and weight gain significantly increased in the rats fed the high-fructose diet compared to the control rats ($P < 0.05$, Table 2). The body weight and weight gain of the *S. chinensis* Baill group were not significantly different from those of the control group. Food intake was not significantly different among the three groups.

Fructose consumption significantly increased albumin in the urine as well as urea and creatinine levels in the serum (Table 3). However, the consumption of *S. chinensis* Baill resulted in the recovery of urine and serum parameters of renal function that were comparable to control levels. Creatinine clearance levels were significantly reduced by the high-fructose diet compared to the control rats, whereas *S. chinensis* Baill recovered

Table 2. Body weight and food intake of the rats

Group	Control	Fructose	*S. chinensis* Baill
Initial body weight (g)	149.4 ± 14.4	148.7 ± 13.9	149.8 ± 13.2
Final body weight (g)	421.7 ± 30.2[a]	463.1 ± 34.3[b]	429.9 ± 24.0[a]
Weight gain (g/d)	3.89 ± 0.41[a]	4.49 ± 0.49[b]	4.00 ± 0.36[a]
Food intake (g/d)	24.2 ± 3.1	22.1 ± 3.2	24.0 ± 2.6

The rats were fed diets containing either 65% cornstarch (Control gorup), 65% fructose (Fructose group), or 64.5% fructose and 0.5% *S. chinensis* Baill extract (*S. chinensis* Baill group) for 10 weeks. Values represent means ± SD (n = 10). Means within a row not sharing a common letter are significantly different from each other (*P* < 0.05).

Table 3. Biomarkers to measure renal functions and antioxidant status

| Group	Control	Fructose	*S. chinensis* Baill
Serum			
Urea (mg/dL)*	10.1 ± 2.1[a]	14.1 ± 2.3[b]	11.1 ± 2.0[a]
Creatinine (mg/dL)*	0.54 ± 0.10[a]	0.74 ± 0.13[b]	0.62 ± 0.09[a]
Urine			
Albumin (g/dL)**	0.13 ± 0.02[a]	0.39 ± 0.07[b]	0.18 ± 0.04[a]
Kidney			
TBARS (nmol/mg protein)*	1.79 ± 0.30[a]	2.24 ± 0.46[b]	1.71 ± 0.33[a]
GSH (μmol/mg protein)*	16.1 ± 2.4[a]	12.6 ± 1.8[b]	15.1 ± 2.0[a]
SOD (U/mg protein)*	4.14 ± 0.50[a]	3.46 ± 0.67[b]	4.30 ± 0.62[a]
Creatinine clearance (mL/min/100 g BW)*	0.24 ± 0.04[a]	0.17 ± 0.04[b]	0.22 ± 0.03[a]

The rats were fed diets containing either 65% cornstarch (Control gorup), 65% fructose (Fructose group), or 64.5% fructose and 0.5% *S. chinensis* Baill extract (*S. chinensis* Baill group) for 10 weeks. Values represent means ± SD (n = 10). Means within a row not sharing a common letter are significantly different from each other (**P* < 0.05, ***P* < 0.01).

these values which were similar to those of the control group.

Renal TBARS levels were increased and GSH levels and SOD activity were significantly decreased by the high-fructose diet compared to the controls, while these parameters were not significantly different between the *S. chinensis* Baill group and controls.

Renal histology

Results from the histological analyses are shown in Fig. 1. The histological section of the control group contained normal glomeruli and tubules. Here there are clearly delineated basement membranes and good definition of capillary loops versus mesangial structures. On the other hand, the glomerulus of the kidney in the fructose-fed rats shows thickening of the glomerular basement membrane and expansion of the mesangial matrix. A striking abnormality was severe tubular dilatation filled with hyaline materials and atrophy with interstitial fibrosis. These pathological changes were prevented by the consumption of *S. chinensis* Baill.
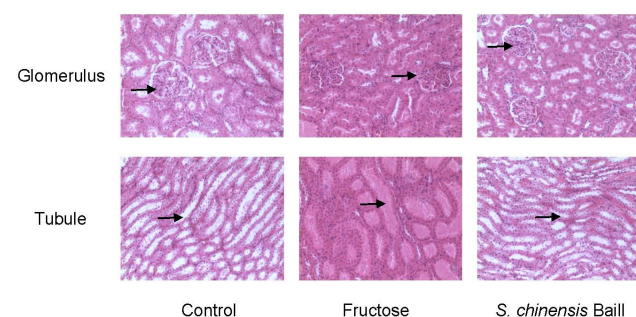
Fig. 1. Renal histopathology of rats. Results of hematoxylin and eosin staining to characterize the morphological changes in rats fed diets containing 65% cornstarch (Control gorup), 65% fructose (Fructose group), or 64.5% fructose and 0.5% *S. chinensis* Baill extract (*S. chinensis* Baill group) for 10 weeks. (original magnification x 200)

Discussion

Chronic consumption of a diet rich in fructose has been reported to accelerate chronic renal disease in rats [4-6,17]. Fructose feeding has been demonstrated to induce renal hypertrophy with tubular cell proliferation and tubulointerstial injury, which may consequently impair renal function. This study investigated the effects of *S. chinensis* Baill on renal damage in rats fed a high-fructose diet.

The rats fed fructose weighed more than those of the control group. Several studies have demonstrated that the consumption of a high-fructose diet (60%) results in higher body weight than a starch-based diet [4,17]. The consumption of *S. chinensis* Baill resulted in significantly lower body weight and weight gain compared to the fructose group, without significantly influencing food intake. A previous study showed that consumption of an aqueous extract of *S. chinensis* Baill decreased body weight in rats fed a high-fat diet [18]. The benefits and mechanism for the weight-controlling effect of *S. chinensis* Baill must be determined in further studies.

In this study, we found that fructose feeding caused deterioration in both glomerular and tubular structures. Histologically, Palanisamy *et al.* [4] and Kizhner and Werman [17] demonstrated that fructose feeding led to the accumulation of tubular hyaline casts and mesangial thickening of the kidney due to collagen deposits. Furthermore, Nakayama *et al.* [19] reported tubular hyperplasia and proliferation of proximal tubules in fructose-fed rats.

We also found that the fructose-treated rats showed renal dysfunctions such as greater urinary excretion of albumin, lower creatinine clearance, and higher serum urea and creatinine. These findings are in line with previous studies demonstrating that high fructose resulted in proteinuria and decreased creatinine clearance [3,4]. Creatinine clearance is used to estimate the GFR which is a fundamental measurement of renal function [20]. However, the parameters of kidney function in the rats fed *S. chinensis* Baill were comparable to those of the control rats, and the kidneys of the rats fed *S. chinensis* Baill showed no obvious morphological changes compared to the controls. In addition, the creatinine clearance values of the control and *S. chinensis* Baill groups were similar to those found in normal rats as reported by Aybar *et al.* [21]. These results demonstrate that *S. chinensis* Baill slowed the progression of functional and structural damage to the kidney in fructose-fed rats.

Although the exact mechanisms for renal damage caused by fructose treatment have not been established, oxidative stress, the lipogenic effect, release of inflammatory cytokines, and endothelial dysfunction may be underlying mechanisms [22]. It has been reported that *S. chinensis* Baill exert hypolipidemic effect in rats fed high-fat diet [18] and streptozotocin-induced diabetic rats [23]. Several studies have demonstrated that high-fructose feeding increases oxidative stress in rats [4,5,24]. In fructose-fed rats, the kidney is vulnerable to oxidative attack

[5]. Fructose consumption increases levels of lipid peroxides and decreases activities of antioxidant enzymes in the kidney [4-6]. High fructose produces reactive oxygen species (ROS) *in vitro* and *in vivo* [25]. In addition, high fructose causes downregulation of hexose monophosphate pathway enzymes such as glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase leading to decreased generation of reducing equivalents such as NADPH and NADH [26]. Increased catabolism of fructose can result in cellular energy depletion which can make cells more susceptible to peroxidation.

There is considerable evidence demonstrating the importance of ROS in renal injury [27]. ROS have been shown to be involved in renal tubular apoptosis associated with renal failure induced by toxins or drugs [28]. It has been reported that ROS leads to a reduction in GFR as well as proximal tubular damage in puromycin-induced nephropathy [29]. It has also been suggested that ROS contribute to the progression of renal damage in fructose-fed rats [4,30]. In addition, genistein, an antioxidant flavonoid, exerts a renoprotective effect in rats fed a high-fructose diet [4], and l-carnitine reduces antioxidant status and attenuates pathological changes in the kidney that are induced by fructose [5].

In the present study, a high-fructose diet resulted in increased renal TBARS levels, a marker for enhanced lipid peroxidation, whereas *S. chinensis* Baill resulted in decreased TBARS levels comparable to those of control rats. Also, fructose-induced reductions in GSH and SOD activity were alleviated by *S. chinensis* Baill. GSH, a tripeptide with a free thiol group, protects cells from excess oxidant stress, both directly and as a cofactor of glutathione peroxidases [31]. SOD converts superoxide anions into hydrogen peroxide, which is then further degraded into water or oxidized glutathione disulfide (GSSG) [32]. Flavonol glycosides with free radical scavenging activities [33] and lignins with antioxidant activity [34] have been identified in *S. chinensis*. Therefore, *S. chinensis* Baill could help make the kidney less susceptible to oxidative damage.

In conclusion, *S. chinensis* Baill effectively ameliorated fructose-induced renal damage in rats, at least partially due to antioxidant activity. Further studies are necessary to elucidate the underlying mechanisms of the renoprotective effects of *S. chinensis* Baill.

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