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Research Article

Effect of *Hibiscus sabdariffa* Supplementation on Renal Function and Lipidic Profile in Obese Rats

[1]Alessandra Melchert, [2]Andressa Canuto Rosa, [2]Vanessa Genari, [2]Marina Salvador Gonzales Frontana, [2]Rodrigo Prandini da Costa Reis, [1]Priscylla Tatiana Chalfun Guimarães Okamoto, [3]João Luiz Amaro and [4]Sheila Canevese Rahal

[1]Department of Veterinary Clinic, School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (UNESP), Botucatu, 18618-970 São Paulo, Brazil
[2]School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (UNESP), Botucatu, 18618-970 São Paulo, Brazil
[3]Department of Urology, School of Medicine, Universidade Estadual Paulista (UNESP), Botucatu, 18618-970 São Paulo, Brazil
[4]Department of Veterinary Surgery and Anesthesiology, School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (UNESP), Botucatu, 18618-970 São Paulo, Brazil

Abstract

Background and Objective: Obesity has emerged as a major public health problem and may be associated with chronic kidney diseases. Thus, this study aimed to evaluate the *Hibiscus sabdariffa* effects in the renal function and lipid profile in rats with obesity induced by high fat diet. **Materials and Methods:** Fifty two Wistar male rats were allocated into four groups (n = 13): Control: Fed with commercial rat chow and water intake, *Hibiscus*: Commercial rat chow and *Hibiscus* in aqueous solution intake, obese: High fat diet and water intake, obese+*Hibiscus*: High fat diet and *Hibiscus* in aqueous solution. The rats were weighted at 1st, 4th, 8th, 12th and 16th weeks. Urinary protein:creatinin ratio and creatinin clearance were measured at 8th and 16th weeks. After 16 weeks the rats were euthanized and serum urea, creatinine, triglyceride, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, sodium, potassium, chloride and calcium were evaluated. **Results:** *Hibiscus* significantly reduced weight gain in high fat diet rats. Obese and obese+*Hibiscus* groups showed lower serum urea than control and hibiscus groups. Creatinin clearance was lower in obese than in control and *Hibiscus* groups at 16th week. Urinary protein:creatinin ratio in obese tended to be higher than in control, *Hibiscus* and obese+*Hibiscus* groups at 8th and 16th weeks. *Hibiscus* and obese+*Hibiscus* groups showed increased levels of serum sodium and chloride. Serum LDL increased and HDL decreased were observed in obese group, while obese+*Hibiscus* showed no HDL reduction. Decrease in LDL levels was observed in *Hibiscus* and obese+*Hibiscus* groups. *Hibiscus* reduced triglycerides levels in *Hibiscus* group. **Conclusion:** The nephropathy associated with obesity in the obese Wistar rat model can be reduced by *Hibiscus sabdariffa* supplementation. The protective effect of *Hibiscus* was associated with lipidic profile improvement, as well as correction of a marked reduction in glomerular filtration rate.

Key words: Obesity, *Hibiscus sabdariffa* L., roselle, high fat diet, renal parameters, creatinine clearance, chronic kidney disease, dyslipidemia, lipidic profile, rodents, rats

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Corresponding Author: Alessandra Melchert, Department of Veterinary Clinic, Universidade Estadual Paulista (UNESP), Botucatu, 18618-970 São Paulo, Brazil Tel: +55 14 38802194

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity has emerged as a major public health problem and may be associated with various disorders, such as cardiovascular[1] and kidney diseases[2], among others. Metabolic Syndrome (MS) is a typical metabolic disorder in obese patients associated with insulin resistance, central obesity, dyslipidemia and hypertension[1]. Adipose tissue is an active endocrine organ and adipocyte hypertrophy may result in endocrine dysfunction, which promotes metabolic disease[3]. Thus, an increase in the secretion of pro-inflammatory adipokines may occur with worsening of dyslipidemia and atherosclerosis process[4].

Patients with MS are at higher risk of Chronic Kidney Disease (CKD), predisposing to kidney end-stage disease and cardiovascular mortality[2]. Obesity comorbidities, such as diabetes mellitus and hypertension are risk factors for kidney disease. However, more direct effect has been observed, showing that obesity is an independent risk factor for CKD[5].

The discovery of products derived from botanical extracts can be an interesting option in the obesity management[6]. Although the herbal treatment is traditionally safer than synthetic drugs, there are few studies proving its effectiveness in the treatment of obesity[6,7]. *Hibiscus sabdariffa* (Roselle) is an herbal compound that is emerging as a treatment option for dyslipidemias. The compound protects against cardiovascular, kidney and liver diseases. It contains polyphenolic compounds with antioxidant and hypolipidemic activities[8]. However, the diuretic effects of *Hibiscus* are extensively documented, but the results are controversial[9,10].

Therefore, this study aimed to evaluate the *Hibiscus sabdariffa* effects in the renal function and lipid profile changes promoted by obesity induced by High Fat Diet (HFD) in rats, through laboratory analysis. The hypothesis was that the use of high fat diet promotes obesity, renal dysfunction and dyslipidemia in rats and that the use of *Hibiscus sabdariffa* can mitigate these changes. To the best of our knowledge, studies about the effects of *Hibiscus* on renal dysfunction in experimental obesity conditions were not reported.

MATERIALS AND METHODS

Animal selection: This study was approved by the Ethical Committee (Protocol 235/2012) of School of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP. Fifty two Wistar male rats, 52 days old, weighing from 180-220 g were randomly allocated into four groups with thirteen animals:

- **Control group:** Rats were fed with commercial rat chow (Presence®, Purina) and water *ad libitum*
- ***Hibiscus* group:** Animals received commercial rat chow and *Hibiscus* in aqueous solution, in a dose of approximately 60 mg kg^{-1} day^{-1} of polyphenols
- **Obese group:** Rats were fed with High Fat Diet (HFD) and water *ad libitum*
- **Obese+*Hibiscus* group:** animals received HFD and *Hibiscus* in aqueous solution in a dose of approximately 60 mg kg^{-1} day^{-1} of polyphenols

Soluble *Hibiscus* from suitable commercial laboratory with 2.68% of polyphenol content was used. The rats were placed in individual cages and maintained under controlled lighting and temperature. All animals fed diet conform described in the respective groups and were euthanized after 16 weeks. Food intake (g), calorie intake (kcal) and fluid intake (mL) were individually measured every week for 16 weeks. The food and fluid intake for each rat were measured by subtracting the measured amount provided to the remaining amounts in the cage.

High fat diet contained commercial rat chow (Presence®) plus peanuts, milk chocolate and sweet biscuit in a proportion of 3:2:2:1. All components of the hyperlipidic diet were ground and blended and consisted of lipids 24.0%, protein 20.0%, mineral residues 5.0%, carbohydrates 41% and fiber 5.9%. The total energy value of the high fat diet was 480.3 kcal/100 g, with 35% of calories as fats[11]. Commercial rat chow (Presence®) consisted of lipids 4.0%, protein 23.0%, mineral residues 10.0%, carbohydrates 55% and fiber 7.0%. The total energy value was 366 kcal/100 g.

Procedures: Initialy, all animals were weighted using a digital scale and after at 4th, 8th, 12th and 16th weeks until the end of the study. All rats were individually placed for 24 h in metabolic cages at 8th and 16th weeks of the study. Animals were fasted overnight and blood was collected from the dorsal tail vein of all rats to measure serum creatinine (Cr) levels. Urine was collected, centrifuged and stored at -20°C until analysis. Urinary protein and Cr were measured in 24 h urine samples harvested in the metabolic cage.

After 16 weeks, the animals were submitted to general anesthesia with tiletamine/zolazepan (Zoletil®), 30 mg kg^{-1} intramuscularly and sodium thiopental (thiopentax®), 60 mg kg^{-1} intraperitoneally[12]. Euthanasia was performed by exsanguination under anesthesia.

Blood collection by cardiac puncture was performed. The blood was collected in vacuum tubes without anticoagulant

and centrifuged to obtain serum. Serum biochemical (urea, creatinine, triglyceride, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol) and urinary tests (protein and creatinine) were evaluated. The concentration of protein in the urine was measured with the Sensiprot Kit (Labtest®, Lagoa Santa, Brazil) using the colorimetric method of pyrogallol red. The assays were performed using commercially available kits (Labtest®, Lagoa Santa, Brazil) in a Labmax progress automatic analyzer (Labtest®, Lagoa Santa, Brazil).

After collection of individual urine (24 h) samples at weeks 8 and 16 of the study, creatinine clearance (CCr) was calculated from the urinary Cr, serum Cr, 24 h urine volume and body weight by using the following equation[13]:

$$\mathrm{CCr\ (mL\,min^{-1}\,kg^{-1})} = \frac{\mathrm{Urine\,Cr\ (mg\,dL^{-1})} \times \mathrm{Urine\,volume\ (mL)}}{\mathrm{Serum\,Cr\ (mg\,dL^{-1})} \times \{1000/\mathrm{body\,weight\ (g)}\} \times 1440\,\mathrm{(min)}}$$

The urine protein:creatinine ratio (UPCr) was obtained by dividing urine protein by urine creatinine.

Statistical analysis: Data are expressed as Means±SE. The study of quantitative variables (body weight, serum urea, creatinine and electrolytes, CCr, UPCr and lipid profile), considered the model of repeated measurements in a scheme of two factors (diet and *Hibiscus*) and complemented by Bonferroni multiple-comparison test. Comparison among the body weight measurements in each group was performed using ANOVA repeated measures, followed by the Tukey *post hoc* test. The statistical analysis was performed using SigmaStat, version 2.0. Results were considered significant at $p<0.05$.

RESULTS

Weight changes, food and fluid intake: In the 1st week, there was no significant difference in body weight evaluation among the four groups, demonstrating experimental homogeneity. At the 4th, 8th, 12th and 16th weeks, the weight was significantly higher in obese and obese+*Hibiscus* groups, compared to control and *Hibiscus* groups. However, at the 12th and 16th weeks, the magnitude of statistical significance proved to be stronger for obese group than obese+*Hibiscus* group, when compared to control and hibiscus groups (Table 1).

The changes in body weight of each group during the 16 weeks showed that control, *Hibiscus* and obese+*Hibiscus* groups gained weight over evaluated weeks and from 12-16th week the weight gain was not significant. However, in obese group the weight gain was statistically significant in all evaluated weeks (Table 1).

In the food intake (g), control and *Hibiscus* groups showed higher intake than obese and obese+*Hibiscus* groups in most weeks of the study ($p<0.05$), due to lower caloric density of the commercial food. Although the food intake was significantly low with High Fat Diet (HFD), the total calorie intake (kcal) was not statistically significantly among the four groups. In the assessment of fluid intake, there were no statistical differences among the four groups.

On average, rats feed with commercial rat chow consumed 27.6±1.8 g and 101.0±6.8 kcal in the control group and 25.8±2.1 g and 96.0±7.7 kcal day^{-1} in the *Hibiscus* group, during the 16 week diet period. The rats feed with HFD consumed 21.4±2.4 g and 102.8±11.9 kcal in the obese group and 21.3±3.2 g and 102.5±12.7 kcal day^{-1} in the obese+*Hibiscus* group, during the 16 week diet period.

Renal function: In the renal function evaluation at 8th and 16th weeks, the urinary protein:creatinine ratio (UPCr) was higher in obese group than control, *Hibiscus* and obese+*Hibiscus* groups, but but there was no statistically significant difference. Table 2 shows UPCr in obese rats tended to be higher than control animals, but this difference disappeared in obese rats treated with *Hibiscus* in aqueous solution.

Creatinine clearance (CCr) was statistically lower in obese than in control and *Hibiscus* groups, at 16th week. At

Table 1: Body weight (g) of the rats in the control, obese, *Hibiscus* and obese+*Hibiscus* groups at the 1st, 4th, 8th, 12th and 16th weeks

	Groups			
Weeks of evaluation	Control (Mean±SD)	Obese (Mean±SD)	*Hibiscus* (Mean±SD)	Obese+*Hibiscus* (Mean±SD)
1st	200.5±13.4^{Aa}	215.9±22.0^{Aa}	211.5±18.7^{Aa}	212.2±22.0^{Aa}
4th	359.0±35.0^{Ba}	412.7±61.8^{Bb}	371.2±27.1^{Ba}	420.5±48.7^{Bb}
8th	422.6±43.3^{Ca}	496.4±83.0^{Cb}	410.6±38.4^{Ca}	494.9±67.5^{Cb}
12th	464.8±54.1^{Da}	562.2±93.5^{Db*}	441.4±41.2^{Da}	538.8±68.7^{Db**}
16th	493.2±52.7^{Da}	616.2±98.9^{Eb*}	470.4±42.6^{Da}	591.8±78.9^{Db**}

Different uppercase letters in columns indicate statistically significant differences ($p<0.05$), different lowercase letters in row wise indicate statistically significant differences ($p<0.05$), *$p<0.01$ × control and $p<0.001$ × *Hibiscus*, **$p<0.05$ × control and $p<0.01$ × *Hibiscus*

Table 2: Urinary protein:creatinine ratio (UPCr) and creatinine clearance (CCr) in the control, obese, *Hibiscus* and obese+*Hibiscus* groups, analyzed at 8th and 16th weeks

Groups	Control (Mean±SD)	Obese (Mean±SD)	*Hibiscus* (Mean±SD)	Obese+*Hibiscus* (Mean±SD)
8th week				
UPCr	0.84±0.37	1.06±0.64	0.80±0.22	0.84±0.62
CCr	2.76±1.66	1.65±0.76	2.86±1.35	2.00±0.84
16th week				
UPCr	0.79±0.46	1.06±0.59	0.69±0.34	0.68±0.21
CCr	2.69±1.3	1.34±0.83*	2.66±1.5	1.89±0.89

*p<0.05×control and *Hibiscus*

Table 3: Serum levels of urea, creatinine and electrolytes in the control, obese, *Hibiscus* and obese+*Hibiscus* groups, analyzed at the end of the 16 weeks

Parameters	Control (Mean±SD)	Obese (Mean±SD)	*Hibiscus* (Mean±SD)	Obese+*Hibiscus* (Mean±SD)
Urea	44.00±6.03*	31.55±6.33	57.45±8.98**	33.81±3.75
Creatinine	0.40±0.08	0.42±0.11	0.40±0.11	0.42±0.07
Calcium	8.35±0.71	8.42±0.37	8.38±0.73	8.32±0.33
Chloride	93.54±2.18#	95.31±2.50##	98.00±2.80	99.46±2.99
Sodium	138.23±2.55#	137.92±2.25##	139.77±3.90	142.15±1.91
Potassium	5.22±1.82	4.65±0.95	4.35±0.63	4.45±0.40

*p<0.05×obese and obese+*Hibiscus*, **p<0.05×control, obese and obese+*Hibiscus*, #p<0.05×*Hibiscus* and obese+*Hibiscus*, ##p<0.05×obese+*Hibiscus*

Table 4: Serum lipid profile parameters in the control, obese, *Hibiscus* and obese+*Hibiscus* groups, analyzed at the end of 16 weeks

Parameters	Control (Mean±SD)	Obese (Mean±SD)	*Hibiscus* (Mean±SD)	Obese+*Hibiscus* (Mean±SD)
Cholesterol	52.08±28.89	42.00±7.82	44.69±7.32	44.77±8.44
Triglycerides	83.23±34.11	86.77±36.55	46.31±15.35*	84.08±32.44
HDL	45.92±1.44	44.54±0.78**	46.31±1.03	45.46±0.88
LDL	7.54±5.14	7.31±2.29	5.00±1.23#	5.62±1.19##

*p<0.05×control, obese and obese+*Hibiscus*, **p<0.05×control and *Hibiscus*, #p<0.05×control and obese, ##p<0.05×obese

8th week, CCr in obese rats tended to be lower than control animals (control and *Hibiscus* groups), but there was no statistically significant difference. Although CCr in obese+*Hibiscus* group was not completely normalized at 8th and 16th weeks, a *Hibiscus* protective effect was clearly observed in this group, when it was compared with obese group (Table 2). Because at 8th and 16th weeks obese rats had higher body weight than control animals, CCr was corrected by considering body weight of each animal.

Urea values of obese and obese+*Hibiscus* groups were statistically lower than those of control and *Hibiscus* groups. In addition, *Hibiscus* group showed higher values than control group (Table 3). On the other hand, serum creatinine measurements of the four groups showed no statistically significant difference (Table 3).

Regarding the serum electrolytes, calcium dosages did not show any statistically significant among the four groups. Sodium and chloride serum levels in *Hibiscus* group were significantly higher than control group, while the obese+*Hibiscus* group had higher values of these electrolytes than control and obese groups. Serum potassium levels were not significantly different among the four groups. Serum levels of calcium, sodium, chloride and potassium are shown in Table 3.

Lipid profile: In the lipid profile analysis, there was no statistically significant difference in total cholesterol levels among four groups. On the other hand, there was a significant difference in LDL cholesterol, whose measurements were higher in control and obese groups compared to *Hibiscus* group and in obese group compared to obese+*Hibiscus* group. The total cholesterol and LDL values of the four groups are expressed in Table 4.

In relation to HDL, the obese group showed significantly lower dosage than in control and *Hibiscus* groups. The same did not occur in obese+*Hibiscus* group (Table 4). On triglycerides analysis, the *Hibiscus* group presented values statistically lower than those of the control, obese and obese+*Hibiscus* groups. It should be emphasized that obese+*Hibiscus* group did not show significant difference compared to obese group (Table 4).

DISCUSSION

Hibiscus sabdariffa has been considered effective in reducing serum lipids and the possibility of acting as an anti-obesity agent has been suggested[14]. In the present study, *Hibiscus* consumption in High Fat Diet (HFD) significantly reduced weight gain of the rats, especially from 12-16 weeks. A significant reduction in body weight was also detected in rats supplemented with *H. sabdariffa* and evaluated by 8 weeks[15]. This differed from the current study, since the modulation of *Hibiscus* on body weight gain was observed only from the 12th week.

Obesity is often associated with diabetes and hypertension, two of the most common risk factors for the end-stage renal disease (ESRD)[16]. However, obesity has also emerged as an independent risk factor for chronic kidney disease (CKD)[17]. Mediators involved in obesity-induced renal injury are poorly understood, since the most of the available information are from studies in rats and dogs fed with HFD and from a model of genetic obesity[16].

Kidney is the primary organ for removing nitrogenous waste products, such as creatinine and urea and regulating electrolytes, thereby helping in maintaining optimal composition of body fluids[17]. In this study, researchers indicated that HFD leads to kidney dysfunction, characterized by an increase in the urinary protein concentration with a decline in creatinine clearance[17,18], in agreement with the results of the current study.

In the present study, besides a progressive weight gain and renal disease, characterized by proteinuria and creatinine clearance reduction, that were observed in rats fed with HFD, the alterations were ameliorated when the obese rats were fed with HFD and supplemented with *Hibiscus* in aqueous solution. These findings were consistent with a previous report[19], but little is known about the mechanism of renal protective effect of the *Hibiscus*[14].

Creatinine clearance (CCr) is a direct measure of glomerular filtration rate (GFR) and GFR is an index of renal function[20]. Physiological studies in human patients have shown greater GFRs in overweight and obese adults compared with lean adults, but no differences in GFRs were noted after correction for body size[21].

Since nephron number did not increase with gain weight, the increased GFR and effective renal plasma flow before correction for body size suggested an increased glomerular intracapillary pressure due increased of single-nephron perfusion, which may lead to glomerulosclerosis and loss of GFR over time[21]. This is in agreement with the present study and the results showed that rats presented an important impairment renal function, since the results showed CCr reduction in obese rats group, indicating reduced GFR.

Furthermore, the absence of reduction in Ccr observed in the obese+*Hibiscus* group in the present study indicated improvement of renal function, demonstrating nephroprotective effects of the *Hibiscus*. The effect of aqueous extract of *Hibiscus sabdariffa* on biochemical indices of renal function was evaluated in rats and the CCr in treated group was slightly increased, but not significantly different from control group[22]. Thus, the authors concluded that the *Hibiscus* did not impair kidney function.

The nephroprotective effect of *Hibiscus* can be attributed to the action of anthocyanins in increasing the GFR via inhibition of angiotensin II production[20]. Angiotensin II adversely changes the renal perfusion and increases oxidative stress through inflammatory and thrombotic effects and it was considered to play a pivotal role in CKD progression[23]. The vasodilatory actions of *Hibiscus* constituents, such as eugenol and quercetin, probably contribute to the increase in CCr by increasing renal blood flow and GFR[20]. This hypothesis may explain the absence of clearance reduction in the obese rats supplemented with *Hibiscus* in the present study.

Massive obesity has been shown to produce nephrotic syndrome and proteinuria and segmental glomerulosclerosis can be present in obese patients, even in the absence of diabetes[16]. In the present study, the effects of *Hibiscus* against obesity renal alterations were demonstrated. The use of *Hibiscus* reduced proteinuria and inhibited CCr alteration caused by obesity. In a similar way, *Hibiscus* significantly inhibited albuminuria and CCr elevation caused by early diabetic nephropathy in rats[23].

In the current study, an important oscillation in serum urea levels was observed. The groups in HFD (obese and obese+*Hibiscus*) had serum urea levels statistically lower than those of control and *Hibiscus* groups. These results were in agreement with another study[24] in which rats on high fat diet excreted less nitrogen (and reduction in urea excretion) than in standard commercial diet. The findings were explained by reduction of urea precursors in hepatocytes with HFD, decreasing urea cycle enzyme function and declining in the hepatic uptake of amino acids with this diet. Likewise, Sabater *et al.*[25] reported reduction in serum urea in rats with HFD, caused by blockage in the urea cycle. Nitrogen amino acids are spared when other sources of energy such as glucose or fat are abundant, which can reduce the catabolism of amino acids and urea production[24].

The *Hibiscus* group showed serum urea higher than the control group in the present study. Similarly, Ukoha *et al.*[26] described increased serum urea after the use of aqueous extract of *Hibiscus* in rats at doses of 200, 500 and 800 mg kg^{-1} for 21 days. The literature proves to be controversial. There were no changes in Blood Urea Nitrogen (BUN) in rats with diabetic nephropathy and supplemented with *H. sabdariffa*[8], as well as in obese humans supplemented with the extract for 12 weeks[27].

On the other side, in rats with diabetic nephropathy, supplemented or not with *Hibiscus* was detected elevation of BUN in the untreated[28]. The *Hibiscus* was able to reduce the urea in healthy rats induced to hyperoxaluria by ethylene glycol[29]. According to these reports, *Hibiscus* showed effect in reducing serum urea, opposed to the current study.

Despite the changes on the serum urea levels, *Hibiscus* did not influence serum creatinine in the present study.

Creatinine levels were not altered in human obese patients supplemented with *H. sabdariffa* for 12 weeks[27] as well as in healthy people or with urolithiasis, under therapy with 3 g day^{-1} of *H. sabdariffa* during 15 days[10]. In addition, *Hibiscus* did not influence in creatinine levels in rats with diabetic nephropathy[8]. Conversely, other studies reported creatinine reduction by the *Hibiscus* in healthy rats[26,29] and in rats induced to hyperoxaluria by ethylene glycol[29].

About electrolyte profile, there are reports with no changes in plasmatic[8] and serum sodium levels[10]. However, the aqueous extract of *Hibiscus sabdariffa* is rich in sodium, chloride and potassium and its oral consumption may result in increased plasmatic sodium[30]. This may explain the increase of sodium and chloride observed in the groups that received the compound in the current study (*Hibiscus* and obese+*Hibiscus*).

The roselle extract at a dose of 3.5 mg day^{-1} was able to reduce the deposition of calcium oxalate crystals in the kidneys of mice with induced hyperoxaluria by glycolate, increasing the urinary excretion of calcium while maintaining serum calcium levels unchanged[31]. In the present study, the dosages of calcium were not influenced by diet or *Hibiscus* ingestion.

Reduction of serum potassium was described in healthy rats supplemented with aqueous extract of *Hibiscus*, but the mechanism for this change was not clear[26]. However, serum potassium elevation in healthy mice receiving *Hibiscus* might occurred possibly due low palatability of the compound, causing fluid intake reduction and a state of water deprivation and consequently raising this electrolyte[30]. Such reports differ from the present study, in which no changes in serum potassium was observed. However, there was no reduction of fluid intake in the groups supplemented with *Hibiscus*.

Among lipid abnormalities related to obesity, elevated serum LDL-cholesterol with a reduction in serum HDL-cholesterol may occur[17] as observed in the present study. Alterations in the lipids metabolism are very common in obese populations and the hyperlipidemia may contribute to renal disease progression[16]. Obesity is characterized by a different composition of LDL-cholesterol, resulting in a more atherogenic small dense LDL concentration, which in turn can move through endothelial fenestrations, entering the subendothelial space where transformation into plaque can occur, leading to atherosclerosis and CKD[17].

In the current study, the serum LDL increased and HDL decreased was observed in HFD (obese group), which may represent a key event for developing kidney disease associated with obesity. Furthermore, beneficial effects of *Hibiscus* on the lipid profile of in HFD rats were observed. Although there is no reduction in total cholesterol, decrease in LDL levels was observed both in conditions of HFD as conventional diets. Despite the reduction of LDL levels, *Hibiscus* showed no potential to HDL increase, but avoided the significant reduction in animals on HFD (obese+*Hibiscus* group).

Total cholesterol was not changed, but significant decrease in LDL was found in mice on high calorie diet and supplemented with *H. sabdariffa* for 8 weeks[15]. Likewise, different doses of the aqueous extract of *Hibiscus* were able to reduce plasma LDL in mice subjected to high-fat diet[32]. Chang *et al.*[27] did not observe hypocholesterolemic ability (total cholesterol) of *Hibiscus* in obese humans.

In mice with diabetes mellitus type-1, *Hibiscus* was able to reduce cholesterol levels at a dose of 200 mg kg^{-1}, in addition to more intense cholesterol reduction than lovastatin[33]. In the present study, the polyphenol dosage was 60 mg kg^{-1}. Due to the varied nature of the reports, may be higher doses of *Hibiscus* are required to obtain total cholesterol reduction, in condition of obesity induced by HFD.

Several studies have shown the hypolipidemic activity of *Hibiscus*[32,33]. Such effects may arise from: Inhibition of cholesterol intestinal absorption; lipoproteins production interference; upregulation of hepatic LDL receptors with higher blood LDL removal; increased catabolism and degradation of cholesterol. These factors, alone or in combination, can reduce LDL and thus total cholesterol[34].

Despite the reduction of LDL, *Hibiscus* showed no potential to HDL increase, but avoided the significant reduction in animals on HFD. Similar results were reported by Huang *et al.*[32], which reported reduction of LDL, but not the increase of HDL in mice in high-fat diet and treated with *Hibiscus sabdariffa* in different doses (25, 50 and 100 mg rat^{-1}). Chang *et al.*[27] showed no increase in HDL in obese humans supplemented with *H. sabdariffa* for 12 weeks. However, these researchers also reported no reduction in LDL, differing from the data of the present study.

Farombi and Ige[33] did not observe *Hibiscus* ability to increase the HDL levels in mice with diabetes mellitus, which was attributed to the fact that *Hibiscus* may not affect the HDL reverse transport. On the other hand, Wang *et al.*[8] reported that the *Hibiscus* was able to increase the HDL in rats with diabetic nephropathy, but only at a dose of 400 mg kg^{-1}.

In the current study, the *Hibiscus* did not reduce triglycerides in rats in high fat diet, but this reduction occurred in mice on standard commercial diet. In agreement with this, Alarcon-Aguilar *et al.*[14] did not notice triglyceride reductions in obese mice supplemented with *H. sabdariffa*. Under commercial diet for rats, studies have shown a significant

triglycerides reduction in rats with diabetic nephropathy and supplemented with *Hibiscus* extract[8,28] as well as in the present study. These reports reinforce the triglyceride reduction potential by *Hibiscus* in the presence of diets with controlled fat levels.

The underlying pathophysiologic mechanisms for the progression of renal disease caused by alterations in lipid levels are not yet fully understood[35]. Oxidative stress and insulin resistance may mediate the lipid-induced renal damage[16]. High-fat diet causes glomerulosclerosis due macrophage infiltration in rats[35].

It was showed that lipid-lowering agents seem to ameliorate glomerular damage, preventing glomerulosclerosis and intersitial fibrosis in an animal model[35]. Once *Hibiscus sabdariffa* contains a variety of bioactive compounds with antioxidant properties and produces reduction in plasma C-reactive protein, interleukin-6 and other inflammatory cytokines[8] and has hypolipidemic properties[14], the compound shows therapeutic promise in amelioration of hyperlipidemia and prevention of renal disease in obesity patients.

CONCLUSION

In conclusion, the present data demonstrated that 16 weeks feeding rats on HFD causes marked obesity with impairment of the kidney function. The nephropathy associated with obesity in the obese Wistar rat model could be reduced by *Hibiscus sabdariffa* supplementation. The protective effect of *Hibiscus* was associated with reduction in LDL-cholesterol without reduction in HDL-cholesterol levels as well as correction of a marked reduction in glomerular filtration rate.

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