5 – ORIGINAL ARTICLE
MODELS, BIOLOGICAL

Effect of aqueous extract of the *Vigna angularis* in rats subjected to an experimental model of moderate chronic kidney disease[1]

Nilo César do Vale Baracho[I], Natália Fechus Monteiro[II], Manuela Guedes Borges[III], Raíssa Robles de Moura Arguelho[III]

DOI: http://dx.doi.org/10.1590/S0102-865020160080000005

[I]PhD, Full Professor, Pharmacology and Biochemistry, Medical School of Itajubá (FMIt), Brazil. Conception and design of the study, critical revision, final approval.
[II]Graduate student, FMIt, Itajuba-MG, Brazil. Acquisition, analysis and interpretation of data; technical procedures; statitics analysis; manuscript preparation.
[III]Graduate student, FMIt, Itajuba-MG, Brazil. Technical procedures.

ABSTRACT

PURPOSE: To evaluate the effect of the aqueous extract of the *Vigna angularis*, popularly known as "Azuki beans", in rats subjected to an experimental model of moderate chronic kidney disease.

METHODS: Thirty rats underwent two surgeries – Ormrod and Miller (1980) – to obtain Moderate Chronic Kidney Disease (CKD-M). The animals were randomized into 3 groups. Group 1 (Control): distilled water. Group 2 (Azuki): *Vigna angularis* 5% aqueous extract. Group 3 (Treatment): 10mg/kg of enalapril maleate. The rats received their respective treatments for 14 days.

RESULTS: The treatment with azuki beans produced an increase in urine output from the second day until the end of the experiment compared to the Control groups ($p<0.01$) and Treatment ($p<0.05$). The treatment with azuki also produced significant reductions in the levels of glucose, triglycerides, VLDL, uric acid, Alanine aminotransferase ($p<0.05$), urea and serum creatinine ($p<0.01$), besides having produced a significant increase in the levels of HDL when compared to the Control group.

CONCLUSION: Treatment with Azuki beans produced improvements in the parameters of renal function and significantly reduced glucose levels, triglycerides, VLDL, alanine aminostransferase, uric acid and creatinine, besides having produced a significant increase in the levels of HDL in rats submitted to a model of moderate chronic kidney disease.

Key words: Renal Insufficiency, Chronic. Kidney. Rats.

Introduction

The chronic kidney disease (CKD) is defined as a clinical syndrome characterized by a progressive loss of the renal function, with a decline in glomerular filtration rate and an increase in the nitrogenous compounds, resultant of irreversible evolutionary morphological alterations of the renal parenchyma[1].

According to the Brazilian Society of Nephrology (SBN), in 2013, the number of patients with CKD in dialytic treatment in Brazil was of 100.397[2]. SBN also indicates that the number of available spots for patients in hemodialysis treatment in the public healthcare system (SUS) is insufficient and that, between years 2000 and 2014, the number of institutions offering hemodialysis increased 42%, while the number of patients seeking this treatment increased 134%. According to a cross-sectional analysis of the National Health and Nutrition Examination Survey (NHANES), conducted between 1999 and 2004, approximately 13% of the adult population of the United States of America has CKD at different stages[3].

Several clinical and epidemiological evidences suggest an association of multiple factors responsible for the settlement and progression of the disease. CKD might be related to a genetic predisposition or to other diseases, namely as hypertension, diabetes, hyperlipidemia. Additionally, factors such as obesity, smoking, chronical use of certain drugs (paracetamol, non-steroidal anti-inflammatories, caffeine) and low weight at birth, are also related to the increase in the chances of developing CKD.

The progression and the establishment of CKD rely on several risk factors and biomarkers. Hypertension is considered one of the most important variable factors. However, diabetes mellitus, of both types – especially if poorly controlled – accelerates the progression of the diabetic nephropathy, leading to end stage renal disease[4]. Non-variable factors include genetics, race, age and sex, and it known that there are more evidences of CKD progression on elder, male and black patients.

One of the proven methods to slow down the progressive loss of the renal function is the reduction of blood pressure and glomerular hydrostatic pressure, which can be obtained through the use of drugs, such as the angiotensin-converting enzyme inhibitors (ACEI). Another functional method is the use of diuretics, substances that increase urine flux. Clinical use of diuretics also increases Na^+ and Cl^- excretion rates. Since NaCl is the main determinant of extracellular fluid volume, the use of diuretics can lead to a prolonged imbalance between the receipt and the loss of Na^+, a phenomenon that is incompatible with life.

An alternate – and complementary – way of medical treatment is phytotherapy: a common and ancient practice that, in Brazil, holds a cultural aspect[5]. According to the Brazilian health surveillance department, a phytotherapic drug is any technically obtained and elaborated using exclusively plants as raw materials for either prophylactic[6], healing or diagnosis purposes that would led to a personal benefit[7].

Vigna angularis is presented as a phytotherapic new treatment option. Known as Azuki bean, it is a small and red bean originated from China. This legume is rich in dietary fibers, showing an elevated percentage of proteins and minerals such as calcium, phosphorus and magnesium, and containing vitamin A, vitamin B9 and folate. Due to its biochemical components, it has been commonly used to treat renal dysfunctions, hypertension and diabetes[8]. Being a potential phytotherapeutical representative, and the fact that its benefits and possible side effects have yet to be scientifically researched, studies that investigate its pharmacological properties in the treatment of CKD can be considered relevant[9].

The objective of this study is to evaluate the effect of the aqueous extract of the *Vigna angularis* legume in rats subjected to an experimental model of moderate chronic kidney disease.

Methods

The research was approved by the Ethical Committee for Animal Utilization (ECAU) of Medical School of Itajubá (FMIt) under the protocol number 07/13. The study obeyed the Federal Law 11.794 and the guidelines of the Brazilian Code of Animal Experimentation (BCAE).

Collection and preparation of the Vigna angularis aqueous extract

The *Vigna angularis* exsiccatum – whose flowers were collected in city of Virgínia, Minas Gerais state, Brazil – can be found in the herbarium of the Faculdade de Ensino e Pesquisa de Itajubá (FEPI) under the register F.214. The flowers were identified and catalogued by Professor Liliana Auxiliadora Avelar Pereira Pasin (PhD in Agronomy by the Federal University of Lavras - UFLA) through comparison of authentication methods.

One hundred grams of the *Vigna angularis* legume were toasted and, afterwards, the grains were placed in 1L of previously heated distilled water. The mixture was boiled on a Bunsen burner until the volume was reduced to half its original volume.

In the experiment, 30 young, adult and male rats of the *Wistar* lineage were utilized[10], weighing between 200 and 250g and between 60 and 90 days of age, coming from the vivarium of the Medical School of Itajubá. The animals went through an adaptation period consisting of a minimal permanence of 3 days in each environment – before initiating the experimental procedures – and were kept in a bright-dark cycle of 12 hours during all of the experimental.

Development of the experimental model of moderate chronic kidney disease

The experimental model of moderate chronic kidney disease developed in this study is based on the methodology described by Ormrod and Miller[1].

The surgical procedures were performed after anesthesia by intraperitoneal (IP) with a Ketamine (50mg/Kg) / Xylazine (25mg/Kg) solution. Analgesia was performed orally (VO) with a Tramadol (2mg/Kg) solution, once a day, during 3 days.

After the cited anesthetic procedure, the surgical procedure described below was performed:

Surgery to obtain Moderate Chronic Kidney Disease (CKD-M)

After adequate trichotomy and asepsis, the following procedures were performed, all on the left side: incision on the flank and exposure of the kidney – with dissection of the adherent fat, the renal capsule and the adrenal gland. Next, the superior and inferior poles of the kidney were removed, along with 1/3 of the cortical tissue remaining on the external lateral portion of this same kidney. This technique preserved 40% of the left renal tissue and, once the procedure had ended, the incision was sutured.

After seven days, the animals were once again submitted to the same protocol so that the following procedures, all on the right side, could be made: incision on the flank and removal of the kidney – after ligature of the right renal artery, right renal vein and right ureter. The right adrenal gland was preserved. Once this procedure ended, the incision was sutured.

Experimental design

After the aforementioned experimental surgical procedures, the animals were allocated in metabolic cages and randomized in three distinct experimental groups, each containing 10 animals (n=10). The rats received water or *Vigna angularis* aqueous extract and animal food *"ad libitum"* for 14 days.

Group 1 – Control: CKD-M rats treated with distilled water – VO;

Group 2 – Azuki: CKD-M rats treated with 5% *Vigna angularis* aqueous extract – VO[10];

Group 3 – Treatment: CKD-M rats treated with 10mg/Kg of Enalapril Maleate – VO (Gavage)[11].

The Enalapril Maleate utilized was from Royton Química Farmacêutica and the studied doses were determined after proper revision of the literature[12].

Final weight, water and meal feedings

The animals were weighed on a scaled provided by the vivarium of the Medical School of Itajubá at the beginning of the experiment – that is, after the surgical procedures – and at the end of the experiment – after being subjected to the treatments. Regarding the liquid feeding, the animals received either distilled water or *Vigna angularis* aqueous extract, as well as animal food "*ad libitum*".

Urine sampling

Urine samples were collected and measured during all 14 days of the experiment.

Blood sampling and dosage

At the end of the experimental period, the animals were submitted to anesthesia by intraperitoneal (IP) with Ketamine (50mg/Kg) / Xylazine (25mg/Kg) and aspiration of the left ventricle for blood collection. The collected blood was centrifuged in a clinical centrifuge (Excelsa, Fanen) at 2000 rpm during 10 minutes and the serum obtained (2 ml/rat) was kept in closed tubes, frozen to -20ºC, for the following laboratorial dosages: urea, creatinine, uric acid, AST, ALT, glucose, total cholesterol, triglycerides, HDL, LDL and VLDL (Kits Labtest). The concentration of the referred parameters was measured using a Digital Visible Spectrophotometer (Quimis Q-108 Drd).

Statistical analysis

The statistical analysis of the data was conducting using the Bioestat 5.0 software. The data between two independent groups were compared by the ANOVA Test, and the data analysis between the three studied groups studied was obtained through a Tukey

Test. The differences were considered statistically significant – when the p-value is less than 0.05 – or highly significant – when the value is less than 0.01.

Results

The data obtained, under the experimental conditions utilized in this study, show that, regarding renal function, the Azuki bean treatment produced an increase on the urinary debit, from the second day to the end of the experiment, when compared to the Control ($p<0.01$) and Treatment ($p<0.05$) groups (Figure 1).

The treatment also produced reductions on the serum levels of uric acid ($p<0.05$), creatinine and urea ($p<0.01$) – important markers of the glomerular function – when compared to the Control group (Figure 2). The Azuki bean and Enalapril Maleate treatments did not generate significant alterations on the water and meal feedings ($p>0.05$).

In the analysis of hepatic function, a reduction on the serum levels of ALT were observed, when compared to the Control group ($p<0.05$). However, regarding the serum dosage of AST, no significant results were found (Figure 3).

In the study of the lipid profile, there were no alterations on the serum levels of the total cholesterol and the LDL ($p>0.05$), however there was an important increase on the HDL levels ($p<0.05$) and a significant reduction on the triglycerides and VLDL levels when compared to the Control group (<0.05) (Figure 4). Regarding glucose – important metabolic marker – a reduction on its serum levels was also found ($p<0.05$) (Figure 5). The Azuki bean treatment did not make significant alterations on the animal weight (before and after the experimental period) and on the water and meal feedings ($p>0.05$).

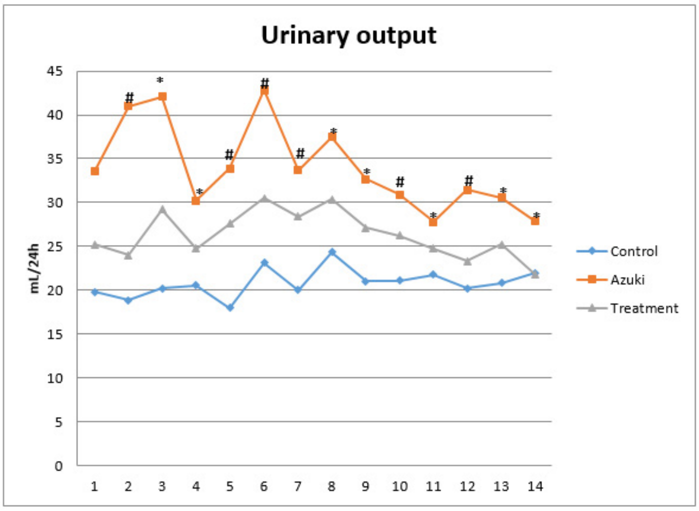

FIGURE 1 - Urinary output of the Control, Azuki and Treatment groups during the 14 days of the experiment. *p<0.05; #p<0.01.

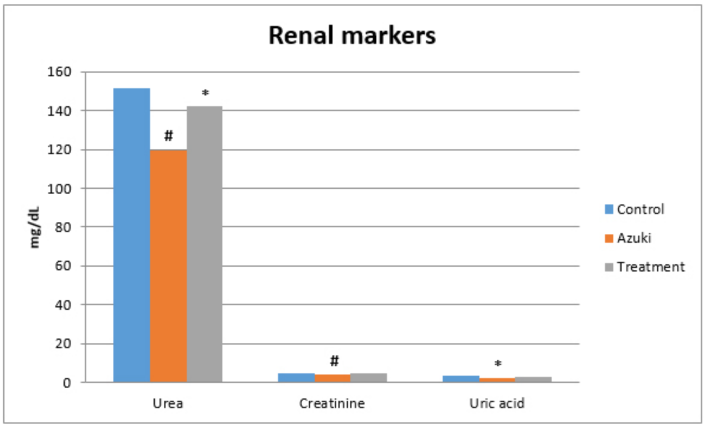

FIGURE 2 - Serum concentrations of urea, creatinine and uric acid from the Control, Azuki and Treatment groups. *p<0.05; #p<0.01.

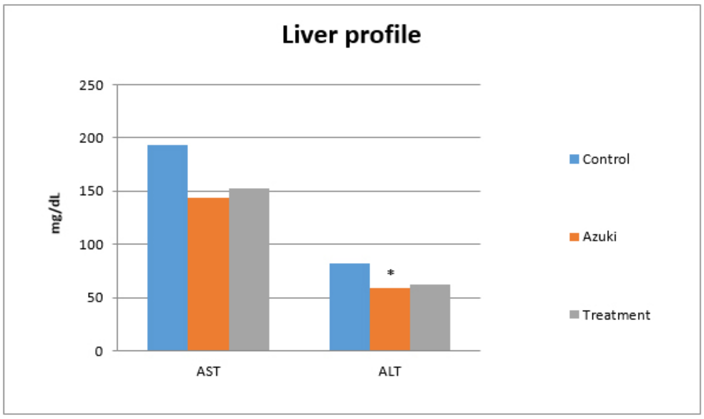

FIGURE 3 - Serum activity of AST and ALT of Control, Azuki and Treatment groups. *p<0.05.

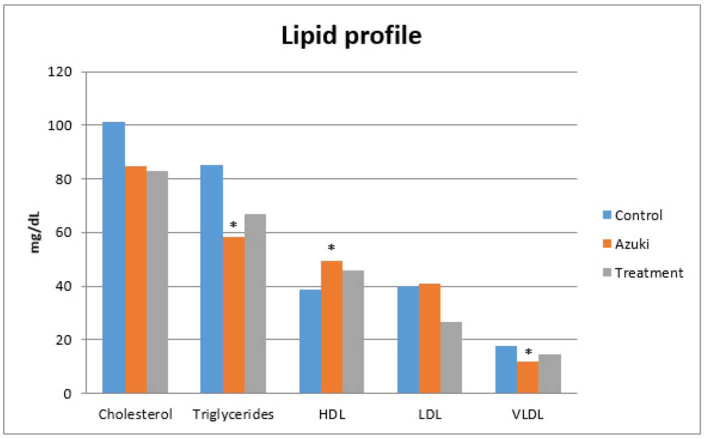

FIGURE 4 - Serum concentrations of total cholesterol, triglycerides, HDL, LDL and VLDL of Control, Azuki and Treatment groups. *p<0.05.

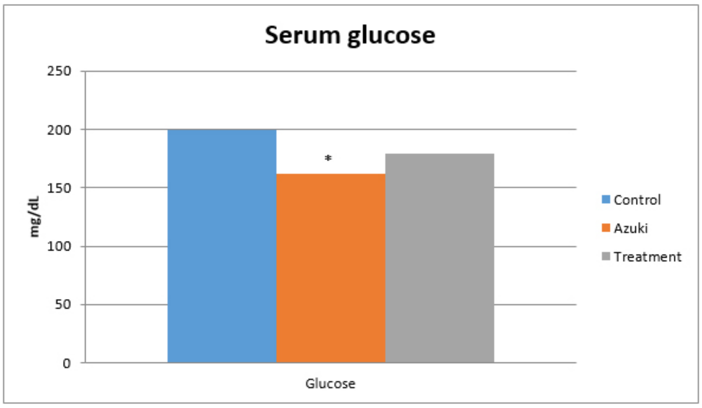

FIGURE 5 - Serum glucose of Control, Azuki and Treatment groups. *p<0.05.

Discussion

The *Vigna angularis* legume, common in Asian cuisine and popular medicine, has been used to treat renal dysfunctions, hypertension and diabetes – not only as a treatment option, but also as supporting therapy. Countless therapeutic methods to the aforementioned pathologies have been studied, and given that the number of diagnostics – especially the late ones – is exorbitantly growing every year, the range of effective treatment options has not accompanied this growth.

Relevant and satisfactory results have been observed in this research, starting from the rectification of the renal function, observed by a reduction of the serum levels of uric acid, urea and creatinine – important glomerular function markers. Although no guidelines were found in the literature regarding those renal dosages, it is known that the analyzed parameters are elevated with the progression of CKD.

Maruyama and collaborators obtained similar results to the ones found on this research regarding the lipid profile – on the triglycerides, total cholesterol and LDL dosages. On the HDL analysis, however, their work did not present significant results, unlike this study, that demonstrated an important increase on its serum levels – a result that can be explained by the soluble fibers found in the legume, that have the function of stabilizing total cholesterol levels and toxins[13].

In two other researches, whose focuses are the effects of macrobiotic diets – in which the Azuki bean is utilized – the authors found similar results to the triglycerides, LDL and glucose dosages. Along with the soluble fibers, the Azuki bean contains – according to recent studies – antidiabetic properties. This characteristic is given by the inhibition of the α-glucosidase enzyme, that is involved in the process of carbohydrate digestion, which would explain the reduction of serum glucose levels[14].

In contrast, a disagreeing result was found regarding total cholesterol – parameter in which a significant reduction was found – and regarding the HDL dosage – in which no significant difference was found between the groups studied by Maury e collaborators. In the cited research, the studied sample took another approach: the authors analyzed the effect of the legume in patients with type 2 diabetes – main cause of CKD –, and concluded that, beyond the decrease in insulin consumption, the legume was capable of decreasing the global cardiovascular risk in that particular group[15].

For the results regarding hepatic function, it is known that ALT (Alanine Transaminase) is found almost exclusively in liver cells, unlike AST (Aspartate Transaminase), that can be found inside many different cell of the body. In this way, ALT – a more specific enzyme – is responsible for metabolization of proteins, and the reduced levels obtained in this study point to infections of the urinary tract and renal insufficiency.

Still regarding the several properties of *Vigna angularis*, it has been observed that the administration of the bean has a therapeutic effect on collagen and that this is related to the inhibitor activity of the IL-6/STAT3 signal. These results suggest that the extract or the compounds of the plant could be a useful treatment to illnesses related to IL – 6[16]. Furthermore, in studies with hypertensive and hyperglycemic animal, it has been observed that this kind of treatment plays an important role in suppressing increases of oxidative stress and macrophage infiltration on kidneys[17].

Pharmacologic activities of the legume also point to the inhibition of the development of skin lesions in a dose-dependent way. In animals treated with *Vigna angularis* extract, the number of mast cells in the skin, the ratio of eosinophils to peripheral leukocytes, the expression of mRNA relative to inflammatory cytokines in the spleen, and the levels of IgE in the serum were significantly reduced. The results suggest that the bean extract can be an effective therapeutic alternative to atopic dermatitis[18].

Others studies point even to favorable effects of the legume on Systemic Arterial Hypertension (SAH)[19] and also on cancer prevention and immunotherapy[20] – parameters that have not been targeted by this study. Although there are not many researches that approach the pharmacological activities of the legume, current studies – including this one – point to a great therapeutic potential of the plant.

The Azuki bean, object of this study, arises as a viable alternative for the CKD treatment considering the obtained results, legume's low cost and great availability. The ideal dose of the aqueous extract is not yet consensual and its possible toxic effects are stil unknown, which justifies new scientific studies to obtain such data.

Conclusion

Azuki bean treatment was able to produce a satisfactory improvement on the renal function parameters, significantly decreasing glucose, triglycerides, VLDL, uric acid, ALT, urea, and creatinine levels, as well as significantly increasing HDL levels in rats submitted to a model of CKD-M.

References

1. Costa AFN, Pereira LPM, Ferreira ML, Silva PC, Chagar VLA, Schanaider A. Modelo cirúrgico de insuficiência renal crônica. Rev Col Bras Cir. 2009;36(1):78-84. doi: 10.1590/S0100-69912009000100014.
2. Mendonça AEO, Dantas JG, Andrade DA, Tedeschi C, Torres GV. Perfil sociodemográfico e clínico de idosos submetidos à hemodiálise. Cogitare Enferm. 2015;20(1):60-6.
3. Bastos MG, Kirsztajn GM. Doença renal crônica: importância do diagnóstico precoce, encaminhamento imediato e abordagem interdisciplinar estruturada para melhora do desfecho em pacientes ainda não submetidos à diálise. J Bras Nefrol. 2011;33:1. doi: 10.1590/S0101-28002011000100013.
4. El Nahas M. The global challenge of chronic kidney disease. Kidney Int. 2005 Dec;68(6):2918-29. PMID: 16316385.
5. Cordeiro CHG, Chung MC, Sacramento LVS. Interações medicamentosas de fototerápicos e fármacos: Hipericum perforatum e Piper methysticum. Rev Fitoter Bras Farmacogn. 2005;15(3):273-7. doi: 10.1590/S0102-695X2005000300019.
6. França ISX, Souza JA, Baptista RS, Britto VRS. Popular medicine: benefits and drawbacks of medicinal plants. Rev Bras Enferm. 2008;61(2):201-8. doi: 10.1590/S0034-71672008000200009.
7. Veiga Junior VF, Pinto AC, Maciel MAM. Plantas medicinais: cura segura? Quím Nova. 2005;28(3):519-28. doi: 10.1590/S0100-40422005000300026.
8. Vieira RF, Vieira C, Moura, EM. Comportamento do feijão Azuki em diferentes épocas de plantio em Coimbra e Viçosa, Minas Gerais. Rev Ceres. 2000;47:411-20.
9. Mesquita FR, Corrêa AD, Abreu CMP, Lima RAZ, Abreu AFB. Linhagens de feijão (phaseolus vulgaris L.): composição química e digestibilidade protéica. Ciênc Agrotecnol. 2007;31:1114-21. doi: 10.1590/S1413-70542007000400026.
10. Han KH, Fukushima M, Ohba K, Shimada K, Sekikawa M, Chiji H, Lee CH, Nakano M. Hepatoprotective effects of the water extract from adzuki bean hulls on acetaminophen-induced damage in rat liver. J Nutr Sci Vitaminol (Tokyo). 2004;50(5):380-3. PMID: 15754502.
11. Eriksson U, Danilczyk U, Penninger JM. Just the beginning: novel functions for angiotensin-converting enzymes. Curr Biol. 2002;12(21):R745-52. doi: 10.1016/S0960-9822(02)01255-1.
12. Ferrario CM, Chappell MC. Novel angiotensin peptides. Cell Mol Life Sci. 2004;61(21):2720-7. PMID: 15549173.
13. Maruyama C, Araki R, Kawamura M, Kondo N, Kigawa M, Kawai Y, Takanami Y, Miyashita K, Shimomitsu T. Azuki bean juice lowers serum triglyceride concentrations in healthy young women. J Clin Biochem Nutr. 2008;43(1):19-25. doi: 10.3164/jcbn.2008039.
14. Maury CP, Landín AA, Zayas AM, Calheiros RVD, Triana MH, Hernández JM, Sánchez MED, Sosa LMM, Huergo LCC, Pianesi M. Efecto terapéutico de la dieta macrobiótica Ma-Pi 2 en 25 adultos con diabetes mellitus tipo 2. Rev Cubana Invest Bioméd. 2007;26:2.
15. Porrata-Maury C1, Hernández-Triana M, Rodríguez-Sotero E, Vilá-Dacosta-Calheiros R, Hernández-Hernández H, Mirabal-Sosa M, Campa-Huergo C, Pianesi M. Medium- and short-term interventions with ma-pi 2 macrobiotic diet in type 2 diabetic adults of Bauta, Havana. J Nutr Metab. 2012;2012:856342. doi: 10.1155/2012/856342.
16. Oh HM, Lee SW, Yun BR, Hwang BS, Kim SN, Park CS, Jeoung SH, Kim HK, Lee WS, Rho MC. Vigna angularis inhibits IL-6-induced cellular signalling and ameliorates collagen-induced arthritis. Rheumatology (Oxford). 2013;53(1):56-64. PMID: 24097134.
17. Sato S, Mukai Y, Yamate J, Kato J, Kurasaki M, Hatai A, Sagai M. Effect of polyphenol-containing azuki bean (Vigna angularis) extract on blood pressure elevation and macrophage infiltration in the heart and kidney of spontaneously hypertensive rats. Clin Exp Pharmacol Physiol. 2008;35(1):43-9. PMID: 18047626.
18. Collantes MT, Rho MC, Kwon HJ, Alfajaro. Azuki bean (Vigna angularis) extract inhibits the development of experimentally induced atopic dermatitis-like skin lesions in NC/Nga mice. Food Chemistry. 2012;132(3)1269-75. doi: 10.1016/j.foodchem.2011.11.100.
19. Mukai Y, Sato S. Polyphenol-containing azuki bean (Vigna angularis) seed coats attenuate vascular oxidative stress and inflammation in spontaneously hypertensive rats. J Nutr Biochem. 2010;22(1):16-21. PMID: 20185287.
20. Nakaya K, Nabata Y, Ichiyanagi T, An WW. Stimulation of dendritic cell maturation and induction of apoptosis in leukemia cells by a heat-stable extract fromazuki bean (Vigna angularis), a promising immunopotentiating food and dietary supplement for cancer prevention. Asian Pac J Cancer Prev. 2012;13(2):607-11. PMID: 22524832.

Correspondence:
Nilo César do Vale Baracho
Avenida Renó Júnior, 368
37502-138 Itajubá-MG Brasil
Tel.: (55 35)99115-3471
nafechus@hotmail.com
nilocvbaracho@yahoo.com.br

Received: Apr 18, 2016
Review: Jun 20, 2016
Accepted: July 21, 2016
Conflict of interest: none
Financial sources: PDIC-FMIt and FAPEMIG

[1]Research performed at Biochemistry and Pharmacology Laboratory, Medical School of Itajubá (FMIt), Brasil.