SEVEN
publicações acadêmicas

# Effect of aqueous and hydrometallic leaf extracts of *Smallanthus sonchifolius* (Popp.) H. Rob. in renal, hepatic and pancreatic metabolism of type 2 diabetic rats

https://doi.org/10.56238/sevened2023.006-047

**Bruna Souza Moreira**
Graduation - Federal Institute of Paraná - Palmas Campus

**Adrielly Jantara**
Graduation - IFPR - Federal Institute of Paraná - Palmas Campus

**Tiago Oliveira**
Graduation - IFPR - Palmas Campus

**Carolina Ribeiro Noronha de Souza**
PhD - Federal University of Jataí

**Rafael Pires Oliveira**
Doctor - Curitiba Military College

**Débora Raquel Mergen Lima Reis**
Master - Federal Institute of Paraná - Palmas Campus
E-mail: debora.reis@ifpr.edu.br

**ABSTRACT**
Diabetes mellitus affects 415 million people worldwide, 81 to 91% of whom are type 2. The concern with the treatment of this disease leads to a growing search for complementary therapies, such as the use of medicinal plants. *Smallanthus sonchifolius* (Popp.) H. Rob., belonging to the Asteraceae family, is a tuber native to the Andes Mountains popularly known as Yacon, which has therapeutic properties. The objective of this research was to evaluate the effect of the use of Yacon's aqueous and hydrometallic extracts on the metabolism of normal and diabetic animals. Male Wistar rats (14 weeks) were divided into control and diabetic groups. The diabetes was caused by intraperitoneal injection of streptozotocin. Once the diabetic status was confirmed, the treatment was started with an infusion of 5.5g/kg/day of dry leaves and an extract of 7.5mL/kg/day, distributed in bottles according to the weight of the animal, and animals that received only water were controlled. Fifteen days after the beginning of therapy, the rats were anesthetized and blood samples were collected for analysis. After euthanasia, the tissues were removed for analysis of cytotoxic effect. The alkaline phosphatase enzyme in the diabetic groups was elevated, indicating a possible injury to the bile ducts. The urea and albumin tests also showed variation, suggesting renal injury due to diabetes, which was confirmed by histological analyses. The normal group treated with aqueous extract also presented renal injury, proving the toxicity of the extract.

**Keywords:** *Smallanthus sonchifolius* (Popp.) H. Rob., Diabetes, Yacon, Estreptozotocina.

## 1 INTRODUCTION

Diabetes mellitus (DM) is a disease that affects 415 million people worldwide, mainly adults between 20 and 79 years of age, is more common in men than in women, and is responsible for the death of 15 million people worldwide. Brazil is in fourth place in the world *ranking*, with an estimated 14.3 million diabetics (Atlas, 2015). Of these, 87% to 91% have type 2 diabetes, characterized by increased blood glucose resulting from insulin resistance of target tissues (Volpato et al., 2007; Atlas, 2015).

The relationship between diabetes mellitus and diet often leads people to look for functional foods that they can include in their diet, which, in addition to the nutritional value, can contribute to the attenuation of the pathologies caused by the disease, thus making it essential to carry out research aimed at testing the efficacy and possible side effects caused by them (Ribeiro, 2008). Some plants studied have already shown beneficial results in controlling blood glucose and/or inhibiting symptoms and complications characteristic of diabetes, including *Arctium minus* Bernh. (Cavalli et al., 2006), *Allium sativum* L. (KIss et al., 2006), *Smallantus sonchiffolius* (Popp.) H. Rob. (Martins et al., 2011), *Bauhinia candicans* Benth, *Syzygium jambolanum* (Lam.) DC. (Soares et al, 2000), *Passiflora nita* kunth (Lima et al., 2012) and several others. The active ingredients of the species can act by increasing the release of insulin, modifying glucose metabolism, sensitizing cells to the action of insulin, inhibiting hyperglycemic factors, and inhibiting or stimulating the synthesis of enzymes (Dornas et al., 2009).

*Smallanthus sonchifolius* (Popp.) H. Rob. belonging to the Asteraceae family, it is a tuber native to the Andes Mountains popularly known as Yacon (Oliveira, 2011). The root has been standing out as a functional food because it is composed of caffeic, chlorogenic and ferulic acid, which have antioxidant activity in the human body, and mainly by fructans of the inulin type and fructo-oligosaccharides, which are not metabolized by the human digestive tract. Because it consists of a percentage of water of 83 to 90%, the root is a hypocaloric food source with a sweet taste that can be used by diabetics as a substitute for conventional sugar. The stem and leaves are made up of proteins, phenolic compounds, including caffeine, hydrochloric acid, ferulic acid, and flavonoids (Albuquerque & Rolim, 2011). The work of Reis et al. (2006) proved the hypoglycemic effect of Yacon leaves in the treatment of DM, as well as Oliveira et al. (2009) and Rosa (2011) the hypoglycemic action of Yacon's tuberous root.

The Yacon leaf is mainly made up of phenolic compounds and sesquiterpene lactones, which have anti-inflammatory, antifungal and antibacterial properties, but on the other hand have known toxic effects (Oliveira, 2011). Thus, the aim of this study was to evaluate the toxicity of the infusion and polar extract of Yacon leaves on the metabolism of the main organs involved in the processes of diabetes mellitus, the liver, kidneys and pancreas, and especially the cytotoxicity on the same tissues in a treatment period of 15 days.

## 2 MATERIAL AND METHODS

### 2.1 EXPERIMENTAL ANIMALS

Thirty-six male rats of the species *Rattus norvegicus albinus,* Wistar lineage, approximately 14 weeks old and weighing about 500 grams, were used. The animals obtained from the USP Vivarium in Ribeirão Preto were kept in a room air-conditioned at 25ºC, distributed in cages under a

12-hour light cycle, receiving standard rodent feed and water *ad libitum* in an environment of the IFPR (Federal Institute of Paraná) – *Palmas Campus* throughout the experiment.

The use of experimental animals was approved by CEUA (Ethics Committee on the Use of Animals) of the Federal Technological University of Paraná – *Two* Neighbors Campus, according to protocol No. 2015-003 (ANNEX II).

## 2.2 DIABETES INDUCTION

After a period of acclimatization, the animals were weighed and the initial blood glucose was evaluated from a blood sample from the tip of the tail and test strips with a glucometer. The animals were then divided into six groups of six animals. Three of these groups received three doses of 32mg/kg of streptozotocin (diluted in 0.01M sodium citrate buffer), one every 24 hours, to induce type 2 diabetes mellitus. And the other three groups were kept as non-diabetic control and received only i.p. injection of sodium citrate. Twenty-four hours after diabetes induction, blood glucose was assessed, and only rats with a level greater than 200 mg⁄dL were considered diabetic. From then on, the animals received the respective treatments and blood glucose was monitored every seven days.

## 2.3 PREPARATION OF EXTRACTS

For the treatment of the experimental animals, two extracts were prepared from the dried leaves of *S. Sonchifolius*: an aqueous extract (AE), rich in chlorogenic acids and sesquiterpene lactones, and a hydrometallic extract (EP), rich only in chlorogenic acids, according to Oliveira's (2011) methodology. The AE was prepared from the infusion for 20 min. of 32g of leaves, previously dried in a drying oven, in 2L of boiling water. The product of this extraction was then filtered for use. For the preparation of the EP, the leaves, previously dried in a drying oven, were first washed quickly in acetone to extract the sesquiterpene lactones present in the glandular trichomes and then dried again in an oven. The leaves were then macerated in 70% methanol (3 x 24h) and the product was filtered, routeevaporated and subjected to liquid-liquid partitioning with n-hexane. From this partition, the hydromethanolic fraction was used for the treatment.

## 2.4 EXPERIMENTAL GROUPS

In order to verify the effect of treatment with Yacon leaf extracts on diabetes, non-diabetic animals, and those considered diabetic according to the description above, were separated into 6 experimental groups: 1) animals with untreated type 2 diabetes (AD); 2) untreated non-diabetic animals (NA); 3) animals with type 2 diabetes treated with AE (DEA); 4) non-diabetic animals treated with AE (NEA); 5) animals with type 2 diabetes treated with PE (DEP); 6) non-diabetic animals treated with PE (NEP).

## 2.5 TREATMENT

For 15 days, the animals received the extracts (EA or EP) diluted in the water offered in bottles. EA was administered at a dose of 5.5g/kg/day of leaves, in 2L of boiling water. On the other hand, the PE was diluted daily at a dose of 7.5mL/kg/day in 2L of water. During the treatment, body weight was checked every 2 days and blood glucose every 7 days, and after 15 days of treatment, the animals were anesthetized and sacrificed by cervical dislocation.

## 2.6 DATA COLLECTION AND ANALYSIS

At the end of the treatment, the animals were anesthetized with an injectable anesthetic composed of ketamine (70 mg/kg) and xylazine (10 mg/kg) administeredintraperitoneally, and blood was collected through an incision in the axillary plexus, in order to obtain a larger volume of blood, necessary for the analyses.

Among the parameters analyzed are the contraction of the enzymes alanine aminotransferase, aspartate aminotransferase and phosphates and alkaline, which, when altered, may indicate damage to the hepatocyte; changes in serum creatinine, albumin and urea concentration, and high creatinine and urea values and hypoalbuminemia may be indicators of changes in renal function; and alterations in pancreatic amylase, since pancreatitis can occur at high levels (Motta, 2009). The serum collected from the animals at the end of the experiment was analyzed using commercial kits for biochemical analysis Labtest, by the Aldes Laboratory of Clinical Analysis of Palmas – Paraná.

The results were analyzed using the ANOVA (*analysis of variance*) statistical test, with Tukey's post-test for a significance level of $p< 0.05$. Thevariable treatment was used, including animals that received aqueous extract, hydrometallic extract and water, the disease variable, including normal diabetic animals.

Subsequently, the rats were euthanized by cervical dislocation. Then, with the aid of a scalpel, the kidneys of the animals of the six groups were removed for the preparation of histological slides for analysis and comparison of a possible cytotoxic effect of Yacon. The tissues were fixed in formaldehyde for 72 hours. They were then washed to eliminate excess fixative and stored in 70% alcohol. The alcohol was replaced by xylol and, finally, by paraffin fused at 60º, obtaining small blocks that were sectioned with the use of a microtome to obtain thin and uniform cuts (Timm, 2005). The carcasses were frozen for further incineration. The processes mentioned were carried out in the Physiology Laboratory of IFPR - *Palmas Campus* . The sections were submitted to hematoxylin-eosin staining, examined and photographed by a light microscope, in a qualitative way, to show the foci of injury in the tissues.

# 3 RESULTS AND DISCUSSION

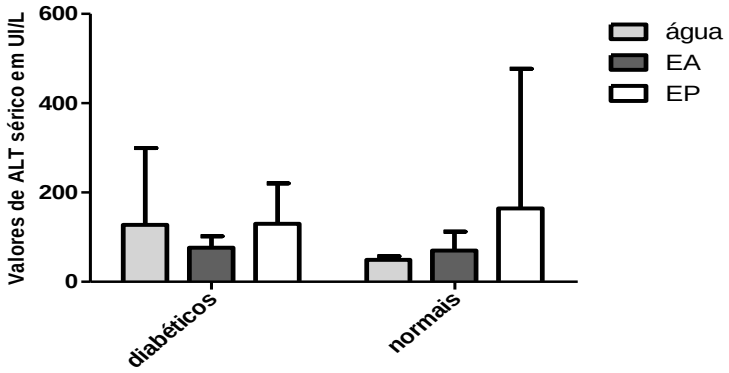
## 3.1 HEPATIC METABOLISM

To verify the action of the experimental treatments on hepatic metabolism, the enzymes alanine aminotransferase (ALT), aspartate aminotranferase (ALT) and alkaline phosphatase (ALP), which are enzymes present in large quantities in liver cells, were analyzed. Thus, the measurement of the concentration of these enzymes in the plasma is useful to help determine the effect of the disease and the tested extracts on the liver cells (Motta, 2009).

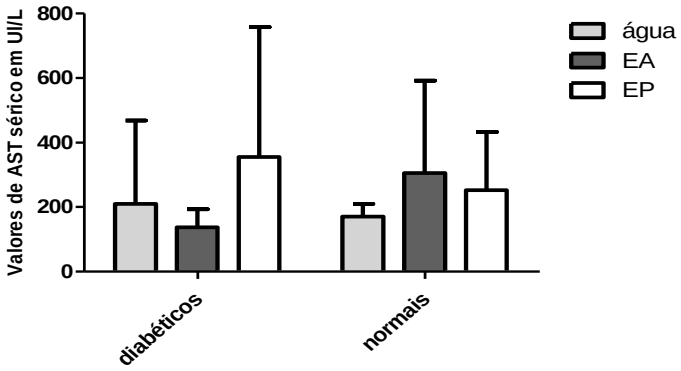
AST and ALT are intracellular enzymes present in large quantities in the cytoplasm of hepatocytes that are released into the circulation when liver cells are injured or destroyed, for this reason they are useful parameters in the evaluation of liver lesions (Jesus et al, 2014). These transaminases are enzymes that indicate liver damage and are common in diabetic animals due to disease-induced fatty liver disease (Pöppl & Gonzalez, 2005).

The analyses of ALT and AST did not indicate a significant difference, i.e., plasma levels remained within the parameters, which indicates that there was no liver damage caused by diabetes or treatment (Graph I and Graph II).

GRAPH I: Serum ALT (alanine aminotransferase) values of all treated groups, with mean and standard deviation. There was no significant difference for the disease factor ($p = 0.7433$) and for the treatment factor ($p = 0.4633$). Considering $p<0.05$.

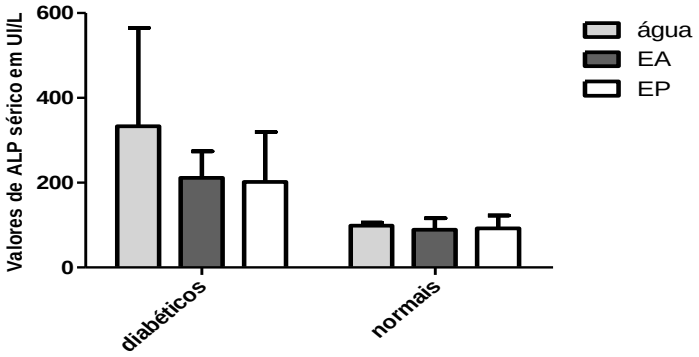


GRAPH II: Serum AST (aspartate aminotransferase) values of all treated groups, with mean and standard deviation. There was no significant difference for the disease factor (p = 0.9140) and for the treatment factor (p = 0.5016). Considering p<0.05.




Alkaline phosphatase (ALP) levels showed a significant variation in relation to the non-diabetic and diabetic groups, with the latter having elevated ALP values in relation to the normal groups. The reference value of the control group was 98.8 ± 9.3 Ul/L, while the diabetic groups had mean values of 332.53 Ul/L (AD), 211.7 Ul/L (DEA) and 201.5 Ul/L (DEP) (Graph III).

GRAPH III: Serum ALP (alkaline phosphatase) values of all treated groups, with mean and standard deviation. There was a significant difference for the disease factor (p = 0.0002) and no difference for the treatment factor (p = 0.2442). Considering p<0.05.




According to Motta (2009), the increase in alkaline phosphatase levels is caused by the retention of bile acids in the liver and bone problems caused by osteoblastic hyperactivity, which occur as a result of diabetes.

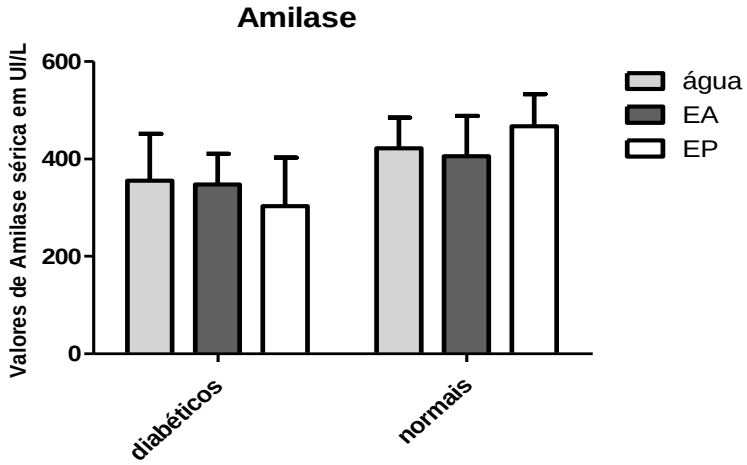
## 3.2 PANCREATIC METABOLISM

Amylase is an enzyme of the hydrolase class that catalyzes the processing of starch and glycogen ingested in the diet. Serum amylase is secreted primarily by the salivary glands and acinar

cells of the pancreas. Pancreatic amylase is released into the intestinal tract through the pancreatic duct (Motta, 2009).

The results of the serum amylase analyses showed a difference between the normal groups and those induced to DM, with the latter having decreased values in relation to the control group, while the normal groups receiving AE and PE remained in the same parameters as the control group (Graph IV). In other words, the decrease in serum amylase concentration is a result of the induction of DM and the possible damage caused to pancreatic cells.

GRAPH IV: Mean and standard deviation pancreatic amylase serum values for all treated groups, mean and standard deviation. There was a significant difference for the disease factor ($p = 0.0011$) and no difference for the treatment factor ($p = 0.9343$). Considering $p<0.05$.



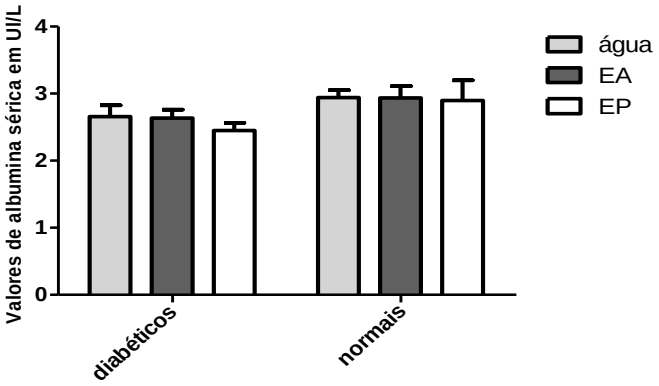

## 3.3 RENAL METABOLISM

In addition to liver metabolism, diabetes also affects the functioning of the kidneys. To study possible alterations in renal metabolism, plasma creatinine, albumin and urea levels were measured, which, when altered, can lead to kidney damage.

Albumins are produced by the liver and comprise about 60% of the proteins present in human plasma. They contribute to the osmotic effect of the plasma, producing pressure, which prevents the loss of plasma through the capillaries, and transport and store various compounds, many of which are poorly soluble in water (Guyton & Hall, 2011; Motta, 2009).

The results of the albumin analyses showed a mean of 2.94 Ul/L for the control group, with a reference value of 2.94 ± 0.16 Ul/L, while the means of the other groups were: 2.94 Ul/L (NEA), 2.9 Ul/L (NEP), 2.66 Ul/L (DA), 2.64 Ul/L (DEA) and 2.45 Ul/L (DEP) (Graph V). The data found that none of the diabetic groups fit the reference values of the control group, i.e., diabetes induced a decrease in plasma albumin levels, regardless of treatment.

GRAPH V: Serum albumin values of all treated groups, with mean and standard deviation. There was a significant difference for the disease factor ($p < 0.0001$) and no difference for the treatment factor ($p = 0.1892$). Considering $p<0.05$.



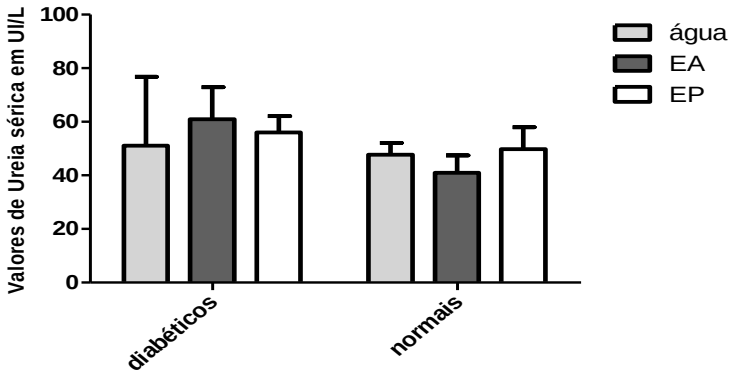

Hypoalbuminemia is a significant effect of diabetes, resulting from the loss of proteins through the urine, which, according to Motta (2009), can be classified as glomerular proteinuria, caused by the loss of integrity of the glomerulus membrane, which becomes progressively permeable to proteins, particularly albumin (Moreira et al., 2008).

According to Motta (2009), another factor indicative of kidney injury is the alteration in the levels of urea, a nitrogenous compound synthesized by the liver from compounds of important uremic toxicity such as ammonia. Urea is the main excretion product of excess nitrogen from the catabolism of amino acids in humans. Approximately 90% of the nitrogen released as a byproduct of protein catabolism is converted to urea, which is released into the bloodstream and must be excreted by the kidney.

Regarding plasma urea levels, the data showed a significant difference in relation to the induced and non-induced groups of diabetes mellitus. The control group (NA) had a reference value of 47.7 ± 7.8 Ul/L and the means of the non-diabetic treated groups remained within the reference value (41 Ul/L - NEA and 49.9 Ul/L - NEP). On the other hand, the means of the induced diabetic groups were 51.1 Ul/L (DA), 61 Ul/L (DEA) and 56 Ul/L (DEP), indicating a significant increase in plasma serum urea levels (Graph VI).

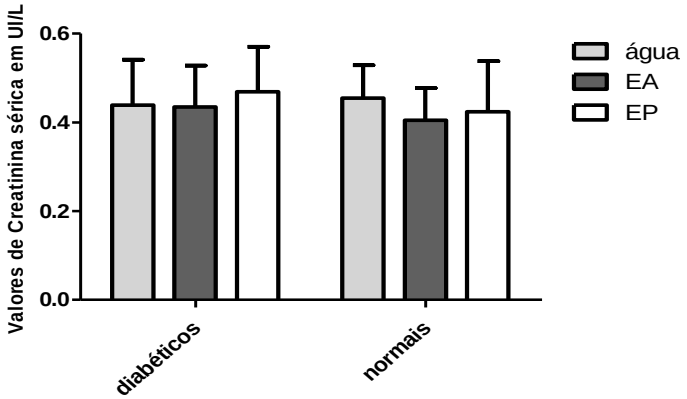
This allows us to affirm that hyperuremia is the result of the diabetic condition, in which the lack of insulin causes a decrease in protein synthesis and promotes the breakdown of protein and lipid reserves to the detriment of carbohydrates, generating enormous amounts of plasma amino acids, which reach the liver in large quantities and are used for gluconeogenesis and for the production of ATP. increasing serum levels of nitrogenous compounds such as urea (Meneghetti, 2010).

GRAPH VI: Serum urea values of all treated groups, with mean and standard deviation. There was a significant difference for the disease factor (p = 0.0275) and no difference for the treatment factor (p = 0.7918). Considering p<0.05.




In addition to albumin and urea, serum creatinine values were also measured, which is the most commonly used marker for assessing renal function (NUNES et al., 2010). This molecule derives from the breakdown of phosphocreatine in the muscles and brain mainly, and when phosphocreatine is metabolized part of it is transformed into creatinine, which is released into the bloodstream to be eliminated by the kidney. According to Guyton (2011), creatinine is a larger molecule than urea, and cannot be reabsorbed in the tubular membranes of the renal nephron. Therefore, only a tiny part of the filtered creatinine is reabsorbed, that is, a large part of the creatinine filtered by the glomerulus is excreted in the urine, so the increase of this in the plasma means that there may be glomerular insufficiency.

GRAPH VII: Serum creatinine values of all treated groups, with mean and standard deviation. There was no significant difference for the disease factor (p = 0.5405) and for the treatment factor (p = 0.7353). Considering p<0.05.




As can be seen in the graph (Graph VII), there was no significant variation in serum creatinine values, since the values of the control group were 0.46 ± 0.13 Ul/L and all the other groups remained

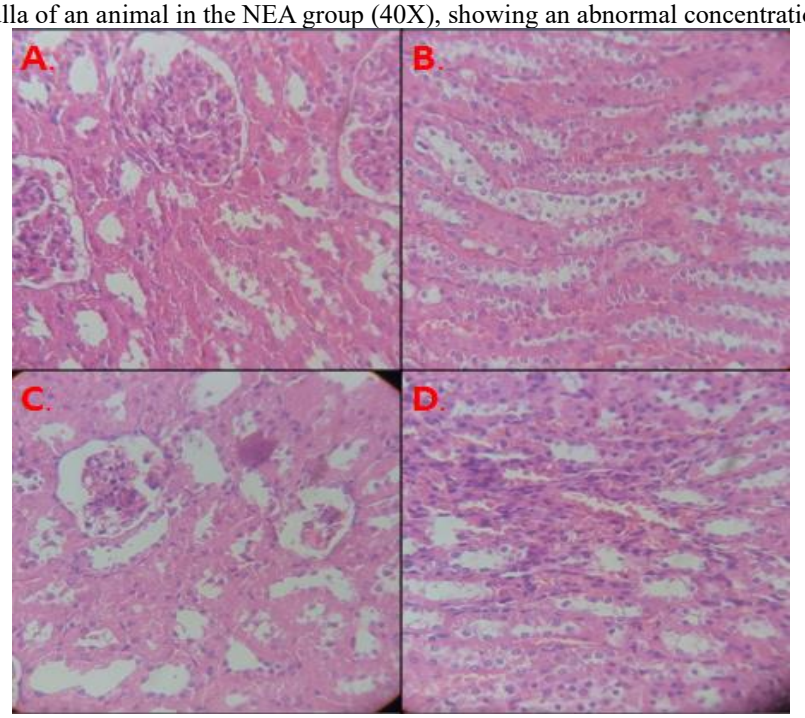
within normal parameters, with values of 0.41 Ul/L (NEA), 0.42 Ul/L (NEP), 0.44 Ul/L (DA), 0.44 Ul/L (DEA). 0.47 ul/l (DEP).

In the results of this study, we observed that the statistical differences are evident in urea and not in creatinine, which may be related to the experimental period, and urea is more sensitive in primary changes in renal conditions (Meneghetti, 2010).

To verify the potential renal injury indicated by the results of the biochemical analyses of serum albumin and urea, histological slides of the kidneys were analyzed.

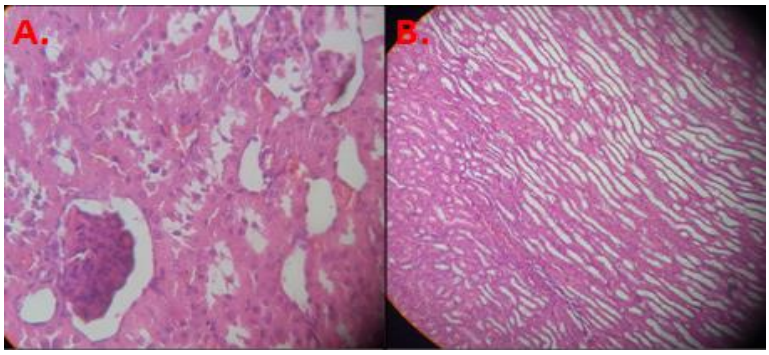
The structural characteristics of the renal tissue of the control group were normal (Figure I.A and I.B). Nor was there any damage to the kidneys of normal animals that received polar extract, with all structures having a normal appearance, corroborating the results of the work of Oliveira (2011) who justifies the toxicity of Yacon with the presence of sesquiterpene lactones, considered toxic substances of Yacon. However, glomerular and tubular alterations were observed in normal animals treated with AS (Figure I, C and I D).

FIGURE I: Longitudinal sections of the cortex and medulla of the study animals. A. Glomerulus of a control group animal (40X). B. Bone marrow of a control group animal (40X). C. Glomeruli of an animal in the NEA group (40X), showing degeneration. D. Medulla of an animal in the NEA group (40X), showing an abnormal concentration of nuclei.



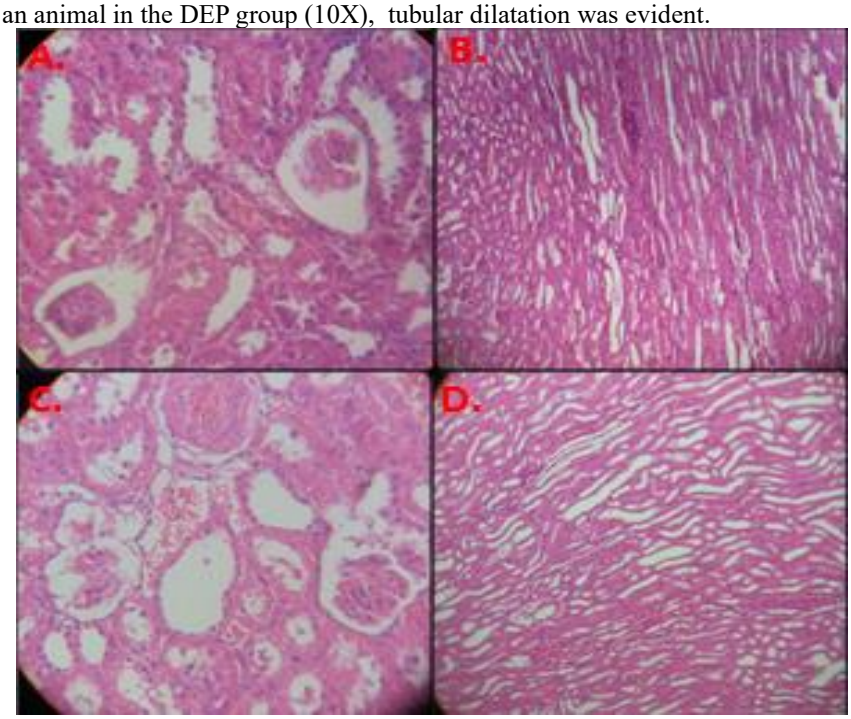

In the kidneys of untreated diabetic animals, some foci of glomerular degeneration and atrophy were observed, as well as minimal medullary areas with abnormal concentration of nuclei (Figure II A and II B).

FIGURE II: Longitudinal sections of the cortex and medulla of the study animals. A. Glomerulus of an untreated diabetic animal (40X) showing degeneration. B. Bone marrow of an untreated diabetic animal (40X) showing abnormal nuclei concentration.




The tissues of diabetic animals treated with AS and PE showed few foci of glomerular degeneration and small dilatation of the tubules in the medullary region, which may be a probable result of the loss of glomeruli (Figure III). The group that received AS also had foci with a slightly higher number of nuclei than normal (Figure III B).

FIGURE III: Longitudinal sections of the cortex and medulla of the study animals. A. Glomerulus of an animal in the DEA group (40X), showing degeneration. B. Medulla of an animal in the DEA group (10X ) showing small tubular dilation and abnormal concentration of nuclei. C. Glomeruli of an animal in the DEP group (40X), showing degeneration. D. Bone marrow of an animal in the DEP group (10X), tubular dilatation was evident.




## 4 CONCLUSION

According to the results obtained in the present study, it can be concluded that the significant differences in serum urea and albumin levels between the diabetic and non-diabetic groups resulted from renal injury in the diabetes-induced animals, confirmed by histological analyses. Through histological analyses, it was also possible to verify the toxicity of AE, since renal lesions were

observed in non-diabetic animals that received AE, which is probably due to the presence of sesquiterpene lactones, as stated by Oliveira (2011). The results for ALP showed a significant variation that may be a consequence of injury to the bile ducts or bone problems caused by osteoblastic hyperactivity in diabetic animals. The extracts have not shown any protective effect. To confirm the effects of Yacon extracts, further studies are needed using different induction protocols, longer treatment time and different dosages for treatment.

## REFERENCES


ALBUQUERQUE, E. N.; ROLIM, P. M. Potencialidades do yacon (Smallanthus sonchiffolius) no diabetes Mellitus. Revista Ciências Médicas, 99 – 108, 2011.

CAVALLI, V. L. L. O. et al. Avaliação *in vivo* do efeito hipoglicemiante de extratos obtidos da raiz e folha de bardana *Arctium minus* (Hill.) Bernh. Revista Brasileira de Farmaognosia, v. 19, p. 64-70, 2006.

DORNAS, W. C. et al. Efeitos antidiabéticos de plantas medicinais. Revista Brasileira de Farmacognosia, v.19, p. 488-500, 2009.

GONZALEZ,F. A. D.; PÖPPL, A. G. Aspectos epidemiológicos e clínico-laboratoriais da Diabetes Mellitus em cães. Acta scientiae veterinariae. Porto Alegre. Vol. 33, n. 1, p. 33-40, 2005.

GUYTON, A. C.; HALL, J. E. Tratado de Fisiologia Médica. 12 ed. Rio de Janeiro: Guanabara Koogan, 2011.

JESUS, G. C. et al.; Principais patologias e biomarcadores das alterações hepáticas. Revista estudos Vida e Saude. v. 41, n. 3, p. 525-537, 2014.

KISS, A.C.I. et al. Efeito do extrato aquoso de *Allium sativum* L. sobre parâmetros bioquímicos de ratas com diabete induzido por Streptozotocin. Revista Brasileira de Plantas Medicinais, v.8, n.3, p.24-30, 2006.

LIMA, E.S.; SCHWERTZ, M.C.; SOBREIRA, C.R.C., BORRAS, M.R.L. Efeito hipoglicemiante da farinha do fruto de maracujá-do-mato (*Passiflora nítida* Kunth) em ratos normais e diabéticos. Revista Brasileira Plantas Medicinais, v.14, n.2, p.383-388, 2012.

MARTINS, M. L. R.; DELMASCHIO, K. L.; CORDEIRO, A. A. Efeitos da utilização de *Smallanthus sonchifolius* (yacon) no tratamento de indivíduos com diabetes Mellitus. Ceres, v. 6, n. 1, p.35 - 43, 2011.

MENEGHETTI, I. C.; Efeito terapêutico do chá verde na morfologia das glândulas submandibulares de ratos com diabetes induzido pela estreptozotocina. 2010. 157p. Dissertação (Mestrado - Faculdade de Odontologia de Bauru) Universidade de São Paulo, Bauru.

MOREIRA et al. Diabetes mellitus, hipertensão arterial e doença renal crônica: estratégias terapêuticas e suas limitações. Revista Brasileira de Hipertensão. v.15, n.2, p.111-116, 2008.

MOTTA, V. T. Bioquímica Clínica: Princípios e Interpretações. 5 ed. Porto Alegre: Editora médica Missau, 2009.

NUNES T. F. e cols. Insuficiência renal aguda. Revista Medicina. v.43, n.3, p.272- 282, 2010.

. OLIVEIRA, L. A. Yacon (smallanthus sonchifolius): compostos fenólicos totais e efeitossobre a glicemia e estresse oxidativo em ratos diabeticos. 2010.Dissertação ( Mestrado - Ciências Nutricionais) Faculdade de Ciências Farmacêuticas de Araraquara – UNESP.

OLIVEIRA, R., B. Atividade antidiabética, anti-inflamatória e toxicologia de Smallantus sonchiffolius (Poepp. e Endl.) H. Robinson – Asteraceae. 2011. 74p. Tese (Doutorado na Área de Ciências Farmacêuticas). Universidade de São Paulo, Ribeirão Preto.

REIS, D. R. M. L. ; PARDAL, D. P. H. Efeito do uso da infusão de extratos foliares da Smallanthus sonchifolius (Yacon) no tratamento do diabetes mellitus: Estudo experimental em ratos da linhagem Wistar. Revista JOPEF. v.1, n.5, 2006.

RIBEIRO, J. A. Estudos químico e bioquímico do Yacon (*Smallanthus sonchifolius*) in natura e processado e influência do seu consumo sobre índices glicêmicos e lipídeos fecais de ratos. 2008. 166p. Dissertação (Mestrado - Ciências dos Alimentos) Universidade Federal de Lavras, Lavras.

ROSA, L. P. S. Effect of yacon potato flour (*Smallanthus sonchifolius*) on blood glucose levels of rats. 2011. 78p. Dissertação (Mestrado - Ciencias da Saude) - Universidade Federal de Goiás, Goiânia.

SOARES, J. C. M.; COSTA, S. T.; CECIM, M. Níveis glicêmicos e de colesterol em ratos com diabetes *mellitus* aloxano induzido, tratados com infusão de *Bauhinia candicans* ou *Syzygium jambolanum.* Ciência Rural, v. 30, n. 1, p. 113-118, 2000.

TIMM, L. L. . Técnicas rotineiras de preparação e análise de lâminas histológicas. Cadernos La Salle, v. 2, n.1, p. 231-239, 2005.

VOLPATO, G. T. et al. Efeito do extrato aquoso de folhas de *Polymnia sonchifolia* (yacon) em ratas diabéticas. Revista Brasileira de Plantas Medicinais, v. 9, n. 2, p. 88-93, 2007.