UNIVERSIDADE ESTADUAL DE MONTES CLAROS

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Avaliação do perfil fitoquímico e do efeito do extrato hidroetanólico da folha de *Lafoensia pacari* (Lythraceae)ASt.-Hill no metabolismo de camundongos com obesidade induzida por dieta

Montes Claros
Fevereiro – 2019

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Orientador: Prof. Dr. Sérgio Henrique Sousa Santos

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MESTRANDO(A): NATÁLIA GONÇALVES RIBEIRO

TÍTULO DO TRABALHO: "Efeito do extrato da folha de Lafoensia pacari(Lythraceae) Ast.-Hill no metabolismo de camundongos com obesidade induzida por dieta"

ÁREA DE CONCENTRAÇÃO: Mecanismos e Aspectos Clínicos das Doenças

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RESUMO

A flora brasileira é rica em plantas com propriedades medicinais, que através do uso popular e de pesquisas específicas, tem contribuído para o desenvolvimento de uma gama de produtos homeopáticos que utilizam as plantas para tratamento e cura de doenças. Entretanto, estudos que utilizam plantas do cerrado brasileiro no tratamento de desordens metabólicas ainda são escassos na literatura. O objetivo deste trabalho foi analisar como a *Lafoensia pacari*, conhecida popularmente como mangava-brava, atua no metabolismo de camundongos com obesidade induzida por dieta hiperlipídica. O material vegetal foi coletado no município de Bonito de Minas, MG, Brasil respeitando todos os preceitos legais. Os testes qualitativos para a presença de classes de metabólitos secundários foram realizados. Quanto a experimentação animal, 36 camundongos machos da linhagem *swiss* e com 4 semanas de idade participaram dos experimentos divididos em dois grupos de número igual (n=18), o da dieta padrão e o da dieta hiperlipídica para indução da obesidade. Após a indução da obesidade por 4 semanas, os animais foram distribuídos em 6 grupos onde foi realizado um tratamento por gavagem durante 4 semanas utilizando o extrato hidroetanólico das folhas da planta, a planta suspensa em água bidestilada e um grupo controle recebendo apenas água e etanol. Realizamos testes bioquímicos do soro, análise histológica do tecido hepático. A fitoquímica comparativa da planta não demonstrou alteração entre as partes da planta que foram avaliadas. Após o tratamento, foram observadas maiores alterações nos animais que receberam a dieta rica em gordura e o extrato hidroetanólico, como nos níveis de transaminase glutâmico oxalacética(TGO), transaminase glutâmica pirúvica (TGP), colesterol total, albumina e creatina aumentados nos animais que receberam a dieta padrão. Não foram verificadas diferenças significativas no peso corporal, triglicérides e colesterol total nos animais que receberam a dieta rica em gordura e tratados com o extrato. Na análise histológica, os animais que receberam quaisquer tratamentos com a planta, obtiveram maior número de células necrosadas, demonstrando assim uma possível toxicidade. Concluímos que a *Lafoensia pacari* deve ser melhor avaliada para consumo oral, podendo a mesma causar danos hepáticos.

Palavras-chave: Metabolismo. Obesidade. Plantas medicinais. *Lafoensia pacari*. Mangava-brava.

ABSTRACT

The Brazilian flora is rich in plants with medicinal properties, which through popular use and specific research, has contributed to the development of a range of homeopathic products that use plants to treat and cure diseases. However, studies that use Brazilian plants in the treatment of metabolic disorders are still scarce in the literature. The objective of the present study was to analyze how *Lafoensia pacari* acts on the metabolism of mice with obesity induced by high-fat diet. The plant material was collected in the municipality of Bonito de Minas, MG, Brazil respecting all legal precepts. Qualitative tests for the presence of classes of secondary metabolites were performed. As for animal experimentation, Swissmale mice (4 weeks old) participated in the experiments and were divided into two groups (n = 18 each), fed standard and the high-fat diet to induce obesity. After the obesity induction (4 weeks), the animals were distributed into 6 groups where a gavage treatment was carried out for 4 weeks using the *L. pacari* leaves hydroethanolic extract, the plant suspended in doubly distilled water and a control group receiving only water and ethanol. After the treatment period, the animals were killed and samples of blood and liver tissue were collected. Biochemical and histological analysis were performed. The comparative phytochemical analysis of the plant did not show alteration between the different plant parts. After treatment, greater changes were observed in the animals that received the high-fat diet and the hydroethanolic extract, as in the levels of GOT, GPT, total cholesterol, albumin and creatine that were increased in the standard-fed animals. There were no significant differences in body weight, triglycerides and total cholesterol in the animals that received the high-fat diet. In the histological analysis, the animals that received any treatments with the plant, displayed anincreased number of necrotic cells, thus demonstrating a possible toxicity. We conclude that *Lafoensia pacari* should be better evaluated for oral consumption and may cause liver damage.

Keywords: Metabolism. Obesity. Medicinal plants. *Lafoensia pacari*. Mangava-brava.

LISTA DE ABREVIATURAS E SIGLAS

SM	Síndrome Metabólica
ANVISA	Agencia Nacional de Vigilância Sanitária
HDL	Lipoproteína de alta densidade
LDL	Lipoproteína de baixa densidade
DM2	*Diabetes Mellitus* tipo 2
DH	Dieta hiperlipídica
DP	Dieta padrão
SISBIO	Sistema de Autorização e Informação em Biodiversidade
IEF	Instituto estadual de florestas
SisGen	Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado
HE	Extrato Hidroetanólico
PL	Folhas da planta
TGO	Transaminase glutâmico-oxalacética
TGP	Transaminase glutâmico-pirúvica

SUMÁRIO

1INTRODUÇÃO

1.1 CERRADO

O Cerrado é o segundo maior bioma da América do Sul, composto por cerca de 2 milhões/km² e dividido em 22 microrregiões. Tais regiões são classificadas segundo clima, geomorfologia, solo e vegetação (1). Atualmente este bioma passa por uma transformação de solo e vegetação devido as atividades agrícolas e agropecuárias. Estima-se que 80% do seu território já tenha sido modificado devido a essas atividades (2).

Figura 1. Área de Cerrado da Área de Preservação Ambiental da Bacia do rio Pandeiros. (Fonte: Acervo pessoal)

O Cerrado está localizado próximo ao Pantanal e este influencia de forma positiva na formação do seu diversificado bioma (3). Em 1986, Cole definiu esta vegetação como sendo parte da Savana Brasileira, devido as suas características ecológicas e fisionômicas (4). Para a definição de savana foi considerado o solo, composição química, disposição de água, nutrientes e composição física, a latitude, morfologia, localização geográfica, ocorrência de queimadas, profundidade do lençol freático, dentre outras características(5).

Segundo Collinson, o Cerrado é “uma formação tropical com domínio de gramíneas, contendo uma proporção maior ou menor de vegetação lenhosa aberta e árvores associadas.” Além de uma vasta diversidade vegetal, o Cerrado também conta com uma gama de plantas medicinais de uso popular (Figura 1).

1.2 PLANTAS MEDICINAIS

As plantas medicinais podem ser definidas como “toda planta ou parte vegetativa que contenha substâncias ou classes de substâncias responsáveis pela ação terapêutica” (ANVISA) – Brasil, 2010. O uso de plantas medicinais tem aumentado gradativamente, porém, uma planta só apresenta valor medicinal se for utilizada de maneira correta. Algumas plantas possuem grande toxicidade, como a Babosa, que possui amplas propriedades terapêuticas mas é considerada tóxica em outras utilizações, devido a presença de aloina, que leva a inflamações renais e hepáticas (8).

As plantas medicinais são distribuídas por categorias mediante a sua ação no organismo, tais quais: calmantes, energizantes, diuréticas, hipotensoras, depurativa, dentre outras. (9). Elas são utilizadas para auxiliar e/ou substituir a utilização de medicação industrializada. A utilização de tais plantas ocorre como uma alternativa ás populações de baixa renda e rurais que necessitam de outras opções para tratamento de algumas enfermidades. (10) Dentre as plantas com potenciais terapêuticos com poucos estudos, temos a *Lafoensia pacari* que pode surgir como uma alternativa a tratamentos de obesidade e síndrome metabólica.

1.3 Lafoensia pacari

A *Lafoensia pacari* é uma planta arbórea popular do Cerrado, pertencente à família Lythraceae, conhecida por diversos nomes de acordo com a região. No estado de Goiás é chamada de “mangava-brava”, de “louro da serra” em Santa Catarina e de “dedaleiro” na região de São Paulo. No gênero *Lafoensia*, são encontrados compostos químicos da classe dos taninos, quinonas e, principalmente, alcalóides. Na espécie *L. pacari*, foram evidenciados como princípios ativos os taninos, flavonóides, saponinas, esteróides, triterpenoides e alcalóides. Como a casca é muito utilizada para feridas e úlceras, em estudos fitoquímicos do extrato hidroalcoolico da casca do caule, houve a presença de ácido gálico e elágico,

catequinas, taninos, esteróides, triterpenos, saponinas, chalcolonas, auronas, flavonóides, leucoantocianidinas, antraquinonas e fenóis (11). A *L. pacari* é altamente recomendada para arborização urbana e recomposição de áreas degradadas (12).
A floração ocorre de Outubro a Dezembro e os frutos de Abril à Junho. É utilizada na medicina popular como cicatrizante (casca), diaforética (folhas) e no tratamento da pneumonia (frutos) (13-15).

Nas avaliações das propriedades biológicas da *L. pacari*, foram listadas as atividades antibacterianas, antifungicas, antivirais, antidepressiva, antiinflamatória, antieosinófilica (atuou sobre a interleucina-5, que apresenta papel importante nos processos alérgicos),antiedermatogênica, antipirética, antioxidante, antisecretória gástrica (utilizada no tratamento das úlceras estomacais), ansiolítica, analgésica e antimoluscicida. Também foi avaliada a eficácia como larvicida, mas não houve benefício e na avaliação da toxicidade*in vitro* e a planta não apresentou efeitos tóxicos (15).

O ácido elágico é um polifenol abundante que se destaca nessa espécie. Ele pode estar presente em outras espécies, principalmente em flores e frutos. Tem grandes benefícios para a saúde humana, sendo descrito pela literatura como antiviral, antibacteriano, anti-inflamatório, cardioprotetor e hepatoprotetor (16). Estudo realizado na Universidade Federal de Goiás (17) demonstrou que o ácido elágico quando usado em camundongos *knowout* para hipertensão, resultou em diminuição da espessura de parede aórtica e menor calcificação, o que favorece a biodisponibilidade de óxido nítrico. A partir desses dados, ficou claro que o ácido elágico atenuou a hipertensão nesses camundongos. Em camundongos com modelo de asma, este contribuiu para a diminuição da inflamação eosinófila, sugerindo uma possibilidade nos tratamentos de alergias (18).

1.4 SINDROME METABÓLICA

A síndrome metabólica (SM) é caracterizada por diversas anormalidades interligadas, o que aumenta o risco de doenças cardiovasculares e resistência à insulina. Em 1998, a Organização Mundial da Saúde estabeleceu o termo unificado síndrome metabólica, pois os estudos não identificaram a presença de resistência à insulina como único fator causal de todos os componentes da síndrome. A patologia da doença ainda está sendo avaliada, não havendo

explicações precisas sobre o seu desenvolvimento(4).O diagnóstico da SM leva em consideração o fato desta não poder ser tratada separadamente como outras síndromes, mas considerar uma combinação de fenótipos relacionados (11).

Embora o papel genético ainda não esteja completamente elucidado, este ainda é o fator que mais predispõe a SM. Esta suposição se deve ao fato da SM ser encontrada em 10-30% dos casos em que foi realizada uma triagem familiar e foi demonstrado que esta seria hereditária (19). Existem alguns fatores que predispõe a SM como: circunferência abdominal acima de 94cm em homens e 80cm em mulheres, dislipidemia com aumento do triglicérides e diminuição do HDL, pressão arterial sistólica acima de 130mmHg e diastólica acima de 85mmHg e *diabetes mellitus* do tipo II (20).

Para a saúde pública, os gastos que são aplicados em tratamentos para melhora do quadro da SM poderiam ser aplicados em estratégias que visam prevenir o surgimento da mesma. Programas que incentivam a atividade física, consumo de dieta saudável rica em verduras, legumes e folhas, campanhas de abandono ao tabaco e acompanhamento contínuo dos pacientes que já possuem a doença, podem melhorar o desenvolvimento da mesma.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito do extrato da folha de *Lafoensia pacari* sobre o metabolismo de camundongos com obesidade induzida por dieta.

2.2 Objetivos específicos

2.2.1 Avaliar o perfil fitoquímico entre as folhas, a casca e das partes aéreas da *Lafoensia pacari.*

2.2.2 Avaliar o efeito do extrato hidroetanólico das folhas da *Lafoensia pacari* nos níveis séricos lipídico e de transaminases em modelo animal com obesidade induzida.

2.2.3 Analisar as diferenças histológicas no tecido hepático na utilização do extrato hidroetanólico da folha e da suspensão do pó da folha em água em camundongos com obesidade induzida por dieta.

3 PRODUTO

Produto1:

Effect of Lafoensia pacari (Lythraceae) ASt.-Hill leaf extract on the metabolism of diet-induced obese mice formatado segundo as normas para publicação do periódico Life Sciences.

3. 1 PRODUTO 1:

Effect of Lafoensiapacari (Lythraceae) ASt.-Hill leaf extract on the metabolism of diet-induced obese mice

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Abstract

The Brazilian flora is rich in plants with medicinal properties, which through popular use and specific research, has contributed to the development of a range of homeopathic products that use plants to treat and cure diseases. However, studies that use Brazilian plants in the treatment of metabolic disorders are still scarce in the literature. The objective of the present study was to analyze how *Lafoensia pacari* acts on the metabolism of mice with obesity induced by high-fat diet, and to verify the phytochemical difference between the *L. pacari* bark of the trunk, leaves, and the branches. The plant material was collected from April to May in the municipality of Bonito de Minas, MG, Brazil respecting all legal precepts. Qualitative tests for the presence of classes of secondary metabolites were performed for leaves, branches and bark of the trunk. Through histological analysis, we evaluated hepatocytes and cell lesions in the liver. The comparative phytochemical analysis of the plant did not show alteration between the different plant parts. It is preferable to use the leaves to make the extract to be applied, aiming to reduce the plant aggression. After treatment, greater changes were observed in the animals that received the high-fat diet and the hydroethanolic extract, as in the levels of GOT, GPT, total cholesterol, albumin and creatininethat were increased in the standard-fed animals. There were no significant differences in body weight, triglycerides and total cholesterol in the animals that received the high-fat diet. In the histological analysis, the animals that received any treatments with the plant, displayed anincreased number of necrotic cells, thus demonstrating a possible toxicity. We conclude that *Lafoensiapacari* should be better evaluated for oral consumption and may cause liver damage.

Keywords: Metabolism. Obesity. Medicinal plants.

INTRODUCTION

Lafoensiapacari is a tree plant belonging to the family Lythiraceae. It is a popular plant of the Brazilian Cerrado biome and is known as “mangava-brava”, “Louro da serra” and “dedaleiro”, among other names. *L. pacari* flowering occurs from October to December and fruits, from April to June. It is used in the popular alternative medicine as healing (bark), diaphoretic (leaves) and in the treatment of pneumonia (fruits)[1-3]. In the *Lafoensia* genus,

chemical compounds of the class of tannins, quinones and, mainly, alkaloids are found. In the *L. pacari* species, tannins, flavonoids, saponins, steroids, triterpenoids and alkaloids were shown as active principles. As the bark is widely used for wounds and ulcers, in phytochemical studies of the hydroalcoholic extract of the stem bark, gallic and ellagic acid, catechins, tannins, steroids, triterpenes, saponins, chalcolones, auronas, flavonoids, leucoantocianidines, anthraquinones and phenols were identified[5].

In the evaluations of the biological properties of *L. pacari*, the following activities were listed: antibacterial, antifungal, anti-viral, antidepressive, anti-inflammatory, antieosinophilic (acting on interleukin-5, which are responsible for allergic processes), antidematogenic, antipyretic, antioxidant, gastric (antisecretory in the treatment of stomach ulcers), anxiolytic, analgesic and anti-molluscicidal properties.

In addition to the properties described above, *L. pacari* is rich in ellagic acid, a polyphenol present in some fruits and vegetables. It has great benefits for human health, asdescribed in the literature to present antiviral, antibacterial, anti-inflammatory, cardioprotective and hepatoprotective activities[6]. A study carried out at the Federal University of Goiás[7] has shown that ellagic acid when in hypertensive mice resulted in a smaller aortic wall thickness and less calcification, which favors the nitric oxide bioavailability. From these data, it was clear that ellagic acid attenuated hypertension in these mice. In mice with an asthma model, ellagic acid contributed to the reduction of eosinophilic inflammation, suggesting a possibility in allergy treatments[8].

Based on the presented data, that study aimed to evaluated the phytochemical differences between the branches, leaves and the bark of the *Lafoensia pacari* trunk in order to improve the use of this species and to preserve the species avoiding the trunk extraction of this plant. We also discuss this species effects on the metabolism of mice with obesity induced by high-fat diet.

METHODS

Material collection and plant phytochemical analysis

Plant Material

Samples of *Lafoensia pacari* were collected between April and May, 2018, in the municipality of Bonito de Minas, MG, Brazil (15 ° 13'31.4 "S 44 ° 55'01.5" W) (Fig. 1),

previously authorized by the SISBIO (System of Biodiversity Information and Authorization) under the protocol number 66693-1, approved by the State Forestry Institute (IEF) and registered in SisGen (National System for the Management of Genetic and associated Traditional Knowledge), under the protocol number A6B40FC. The collected material was duly identified by *Lafoensiapacari*, and a specimen was deposited in the herbarium Montes Claros at UniversidadeEstadual de Montes Claros, Minas Gerais, Brazil and identified as number MCMG 3626.

The plant botanical name was verified using the www.theplantlist.org website.Undamaged leaves, free from the attack of insects, or fungi were selected. The leaves, branches and bark of the trunk were dehydrated in an incubator (model 400, ND, New ethics, Vargem Grande Paulista, SP) with forced air circulation at 45 ºC until reaching a constant mass. After drying, the material was milled in a knife mill and stored in a hermetically sealed amber glass, to avoid photo degradation.

Fig.1 Representation of the collection area in the municipality of Bonito de Minas / MG. Image accessed December 2018. Google Earth image.

Phytochemical Characterization

Qualitative tests for the presence of classes of secondary metabolites were performed for leaves, shoot branches and bark of the trunk. For tannins, Pb (C2H3O2) 2 10% (neutral lead acetate) and 2% FeCl3 (iron chloride) were used. The flavonoids were identified

via 2% FeCl3 and Shinoda reagent, while the alkaloids were identified by Mayer, Bouchadart, Betrand and Dragendorf, and thesaponins by the resistant foam test[10-11].

Preparation of the*Lafoensiapacari*hydroethanolic extract

The hydroethanolic extract (EH) was prepared with 10 mg of *L. pacari* leaves and 30 mL of ethanol. The samples were extracted for 8 days and the extract obtained was filtered and stored in separate amber glasses for daily use (30 glasses) in order to reduce contamination during the experimental period. At the time of the gavage, the extract was dissolved in doubly distilled water. The choice of the hydroethanolic extract was because this method is closer to the form used by rural communities that already use the plant for ingestion. The lyophilization of the extract wasn't performed due to the same reason.

Animals

In order to carry out the experiments, male mice of the SWISS lineage of approximately 4 weeks (n = 36 animals) were obtained from the Department of Biochemistry and Molecular Pharmacology of the Biological Sciences Institute (ICB) of the Federal University of Minas Gerais (UFMG). The mice were kept in the vivarium of the Center for Biological Sciences and Health of Unimontes. The mice were submitted to an initial adaptation phase for a period of 10 days under suitable conditions of temperature [22 ± 2ºC], relative humidity of 60 ± 5%, 12h of light / dark cycles and fed with Purina-Labina® diet[9]. The animals were further divided into 6 experimental groups.

Induction of obesity

At approximately 5 weeks of age, the animals were divided into 2 major groups: 1 – High-fat Diet (HFD) (N = 18) and 2 - Standard Diet (STD) (N = 18). The HFD group will receive a diet rich in fat (36.59% carbohydrates, 12.88% proteins and 50.53% lipids), and the STD group will receive ration (Purina-Labina®) containing 50.3% carbohydrates , 41.9% protein and 7.8% fat. The diets were administered *ad libitum* throughout the experimental period, and after 8 weeks of induction of theinduction of obesity, the animals of each group were divided into 3 new groups, which were treated by gavage daily for 30 days with

hydroethanolic extract of *Lafoensia pacari*, suspended plant in double distilled water and a standard group with vehicle (water + ethanol) for control.

Euthanasia and collection of materials

The animals were killed by guillotine decapitation, where samples of blood, and liver were collected, weighed and immediately frozen in liquid nitrogen and then transferred to freezer at -80ºC.

Biochemical parameters

The serum was obtained after blood centrifugation (3000 rpm for 10 minutes at 4ºC). The following parameters were assessed: total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein cholesterol (LDL-cholesterol) and very-low density lipoprotein cholesterol (VLDL-cholesterol), triglycerides (TG), albumin, creatinine and the alanine transaminases (AST/GOT) and aspartate (ALT/GPT) transferase using enzymatic kits (Wiener Laboratories, Rosario, Argentina). The measurements were performed on a Wiener BT-3000 plus Chemistry Analyzer (Wiener Laboratories, Rosario, Argentina).

Histology

Liver samples were fixed in 10% formaldehyde solution. Subsequently, the samples were dehydrated in ethyl alcohol, diaphanized in xylol and included in paraffin. The tissues sections (5 μm) were examined using an optical microscope where images were captured with the Evolution LC color light camera (Media Cybernetics®, USA) after staining with hematoxylin and eosin (H&E).

Data analysis

The statistical analysis was performed in the GraphPad Prism software (version 5.0®, San Diego, California, USA), with 95% ($p<0.05$) confidence. Data was given as mean ± Standard error (SE). The normality was verified by Shapiro Wilk test. The statistical significance of the values for the different groups were estimated by one-way ANOVA and

one-way ANOVA (plasma glucose and body weight), followed by the post-Turkey multiple-comparison test.

RESULTS

Phytochemical profile

The qualitative evaluation of metabolites for different vegetative parts is summarized in table 1. A lighter amount of phenolic compounds and flavonoids distributed in the stem, branches and leaves were found, especially in the branches. The presence of alkaloids was confirmed in the different vegetative parts with moderate level degree. Regarding the saponins, its presence was also verified between the vegetative parts tested, observing the foam increase depending on the extract concentration (Table 2).

Table 1. Phytochemical profile

Class	Test	Leaf	Stalk	Branch
Alkaloids	Mayer	-	-	-
	Dragendorff	++	+	-
	Bertrand	-	+++	++
	Bouchadart	-	-	-
Phenolic Compounds	Ferric Chloride	+++	+++	+++
	Sodium hydroxide	+++	++	++
	Flavonoids			
	Ferric Chloride	+++	++	+++
	Sodium hydroxide	++	+++	+++
	Ferric Chloride	+++	++	+++
Flavonoids	Sodium hydroxide	++	+++	+++

(-) Negative, (+) Weak positive, (++) Moderate positive, (+++) Strong positive.

Table 2. Qualitative test of saponins.

H_2O	Extract	Leaves	Stalk	Branch
5	-	-	-	-
4	1	+	+	++
3	2	++	+	++
2	3	++	++	+++
1	4	+++	+++	+++
-	5	+++	+++	+++

(-) Negative, (+) Weak positive, (++) Moderate positive, (+++) Strong positive.

Biochemical results

Regarding body weight, there was no statistically significant difference between the groups analyzed (Figure 1A). The plant and plant extract did exert positive effects in the glucose levels. The animals fed a standard diet and treated with the plant and/or extract presented increased glucose levels (STD + PL / STD + HE) (STD + PL, 152.0 ± 4.88 *vs* STD + HE, 134.3 ± 6.69) (Figure 1B). However, in the HFD-fed mice, the animals that received only the plant (HFD + PL) had a decrease in glucose levels as compared to the extract (HFD + HE) (HFD + PL, 54.0 ± 3.0 *vs* HFD + HE, 113.3 ± 21.23).

The animals that received the extract (HE) and the plant (PL) had higher levels of albumin in the two diets (standard and HFD) (STD + VE, 1.56 ± 0.18 *vs* DST + LP, 3.12 ± 0.47 *vs* DST + HE, 3.17 ± 0.12). The increase in albumin was more significant in the animals that received the plant extract (Figure 1C).

Animals receiving a standard diet and the hydroethanolic extract presented high levels of plasma GPT as compared to the vehicle (STD + HE, 78.50 ± 5.69 *vs* STD + VE, 37.0 ± 2.97) (Figure 1D).

As for total cholesterol, the animals fed a standard diet that received the suspended plant and the extract displayed increased levels as compared to the vehicle (STD + PL, 123.7 ± 11.7 *vs* STD + VE, 41.0 ± 1.58) (STD + HE, 126.0 ± 5.50 *vs* STD + VE, 41.0 ± 1.58). The high-fat fed animals treated with the extract showed increased cholesterol levels (HFD + VE, 60.0 ± 5.03 *vs* HFD + HE, 102.3 ± 8.09) (HFD + PL, 55.5 ± 6.60 *vs* HFD + HE, 102.3 ± 8.09) (Figure 1E).

Regarding the HDL serum levels, the standard and high-fat diet fed mice treated with the *L. pacari* extract presented increased levels as compared to vehicle (STD + VE, 32.5 ± 4.26 *vs* STD + PL, 88.9 ± 7.59 *vs* STD + HE, 82.9 ± 5.61) (HFD + VE, 44.0 ± 5.27 *vs* HFD + PL, 38.3 ± 4.75 *vs* HFD + HE, 72.7 ± 5.77) (Figure 1F)

There were no significant differences between the levels of creatinine, GOT and triglycerides (Figure 1G - 1I).

Figure 1:

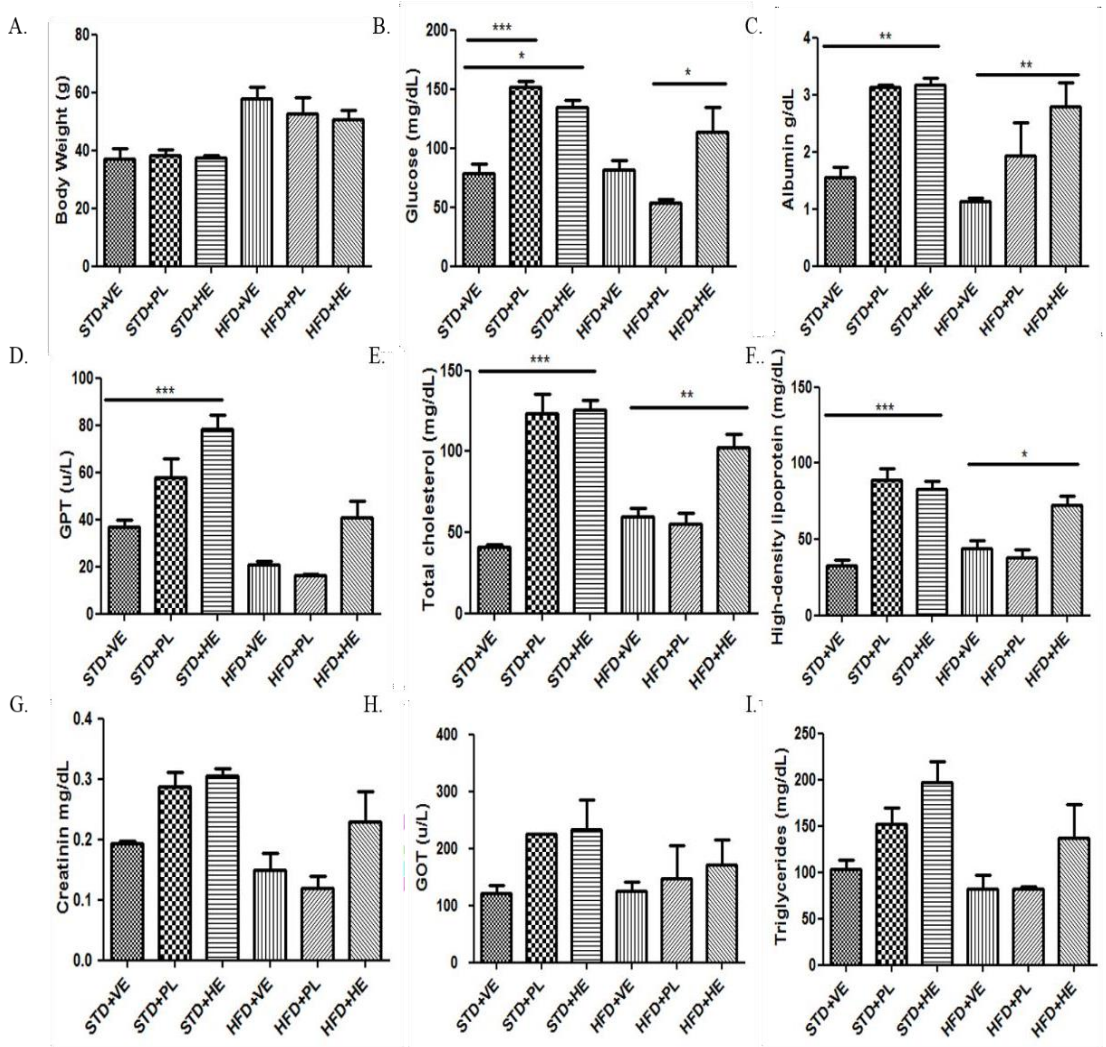

Figure 1. Body weight and biochemical profile A) Body weight (g); B) Fasting serum glucose (mg/dL); C) Albumin (g/dL); D) Glutamic pyruvic transaminase (GPT/u/L); E) Serum total cholesterol (mg/dL); F) Serum high-density lipoprotein (mg/dL); G)Creatinin (mg/dL); H) Glutamic-oxalacetic transaminase (GOT – u/L)); I) Serum triglycerides (mg/dL); * $p < 0.05$ versus indicated groups by the bars.High-fat diet and vehicle (HFD+VE); High-fat diet and hidroetanolic extract (HFD+HE); High-fat diet and plant leaves (HFD+PL); Standard dietand vehicle (STD+VE); Standard diet and hidroetanolic extract (STD+HE); Standard diet and plant leaves (STD+PL).* $p < 0.05$ versus indicated groups by the bars.**$p<0.01$ ***$p<0.001$.

Histology

From the hepatocytes analysis, it was possible to observe that the animals that received a high-fat diet and the plant (PL) had larger hepatocytes as compared to the others. No significant differences were observed among the standard-fed animals (Figure 2).

Figure 2:

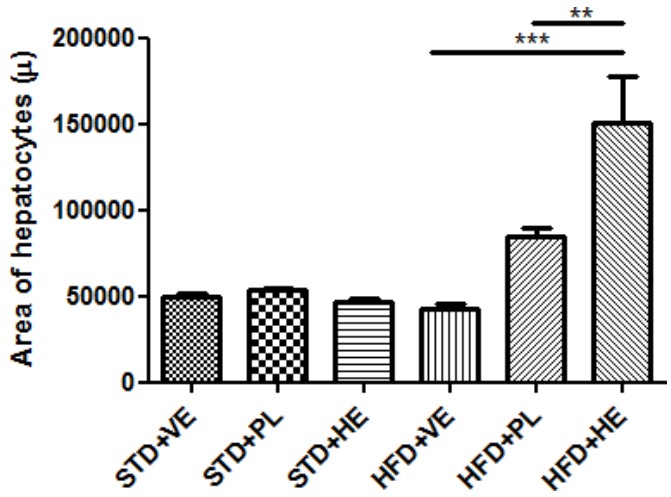

Figure 2: Area of hepatocytes. High-fat diet and vehicle (HFD+VE); High-fat diet and hidroetanolic extract (HFD+HE); High-fat diet and plant leafs (HFD+PL); Standard dietand vehicle (STD+VE); Standard diet and hidroetanolic extract (STD+HE); Standard diet and plant leafs (STD+PL).* $p < 0.05$ versus indicated groups by the bars.**$p<0.01$ ***$p<0.001$.

Interestingly, the histological analysis displayed that the animals treated with the extract or the plant presented a greater amount of tissue necrosis (Figure 3).

Figure 3:

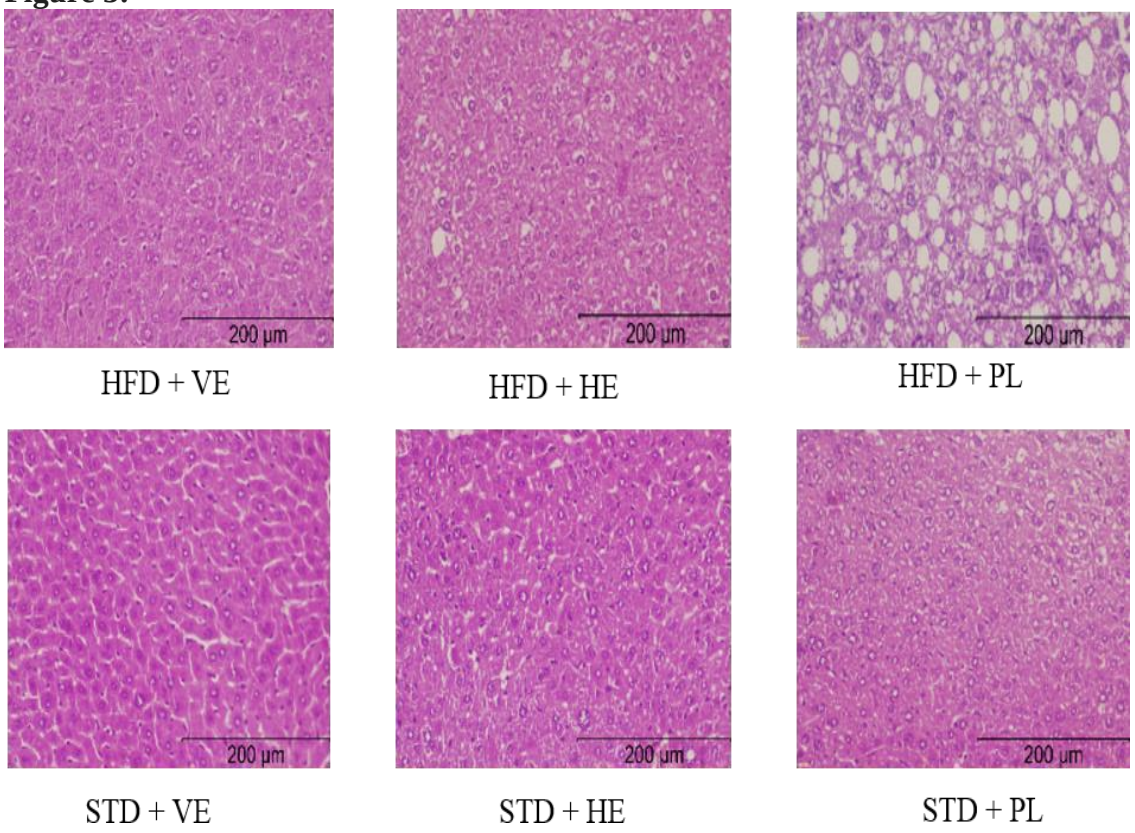

Figure 4: Histological analysis. High-fat diet and vehicle (HFD+VE); High-fat diet and hidroetanolic extract (HFD+HE); High-fat diet and plant leafs (HFD+PL); Standard dietand vehicle (STD+VE); Standard diet and hidroetanolic extract (STD+HE); Standard diet and plant leafs (STD+PL).

Discussion

There is an increase in the use of medicinal plants and herbs due to their low cost, high availability and minimal risk of side effects compared to allopathic medicines. In this context, the therapeutic activities of the plants of the Cerrado stand out for the high biodiversity, possessing an extensive range of raw materials and bioactive molecules to be explored.

The alternative of the use of popular plants as therapeutic resources has encouraged several researchers to follow a line of study based on popular culture, being the Cerrado region an option for new discoveries, with this, we chose a plant of popular culture and that has few metabolism studies.

Phytochemical analyzes of *L. pacari* showed metabolites such as saponins, flavonoids and phenolic compounds. Studies by Galdino (12) showed that, in addition to these, this plant species presents the presence of tannins, triterpenes, flavonoids, steroids and

alkaloids. In addition, phytochemical differences between the bark, leaves and branches were not found.

In the present study, an increase in the serum glucose levels of the animals that received the high fat diet and the plant was observed, there was an increase also in the animals that received the standard diet and only the plant, this suggests that the plant should not be used in treatment for diabetes until further studies are performed. The same was observed for albumin levels, which were increased in HE-treated mice. Albumin is a protein produced by the liver and has the function of transporting molecules, such as fatty acids, also represents most of the protein's plasma content and is responsible for blood osmorality. There were also changes in the serum levels of GPT that were increased in the animals that received the treatment with HE. Such increase may have been caused by the existence of ethanol in the extract composition, further experiments are required to evaluate the influence of the extract on GPT.

In total cholesterol, there was a significant increase in the animals that made use of the plant, both as an extract and in the form of decoction. Such increase may be related to the presence of phytosterols, necessitating a more specific study for the steroid quantification that the plant possesses. Differences were also observed in serum HDL levels in the extracts treated mice. These differences may be related to phenolic compounds that are abundant in the plant. HDL acts in the reverse transport of cholesterol and has antioxidant, anti-inflammatory, fibrinolytic and endothelial protection functions (13).

In the analyzes of body weight and creatinine there were no significant statistical differences, creatinine is a marker of renal irregularities, when very high may indicate renal failure. Statistically significant results were not found in the triglycerides and GOT analysis. Triglycerides is a fatty acid that acts as a reserve of energy in the body, when not used, it can be stored as fat form in adipose tissue. High serum triglyceride levels may indicate possible development of coronary heart disease. GOT is an enzyme that markers for liver and muscle damage, as there was no alteration of the GOT but there was GPT, more studies are necessary to explain the alteration of one data and not change of another.

Macroscopic analysis of the liver indicated alterations in tissue morphology of the animals that received the high fat diet and were treated with the extract, fraction and animals treated with the decoction. It is emphasized that one animal of the HFD + HE group presented polyps, and one of the HFD + PL animals presented a tumor in the hepatic region. Histological analysis was also observed. These changes were histologically verified, in which

focal necrosis (characterized by fields with absence of nucleus) and hydropic degeneration were observed, characterizing toxicity. In the study of Da Cruz (14), using higher doses of *L. pacari*, and the same dosage (10mg / kg) and animal model for the extract, no toxic effects were observed in the 28 day period in macroscopic, biochemical and hematological. The differences for these results can be attributed to the amount of animals used in the study (3 animals per dose to be tested), the toxicity analysis method (OECD vs. planting time) and the use of dry extract (lyophilized).

Based on these findings, it is important to continue the studies on the toxicity of the plant in the metabolism *in vivo*, because in some communities oral intake of this species is performed. As there were divergent results regarding biochemical parameters, further studies are required for the safe and effective use of *L. pacari*.

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4. CONCLUSÕES

Avaliamos no presente estudo os efeitos da *Lafoensia pacari* no metabolismo de camundongos com obesidade induzida por dieta. Os principais resultados demonstraram que o extrato desta espécie parece ser tóxico para os animais, alertando então para precaução do consumo oral da mesma. Mais estudos devem ser realizados afim de se comparar os efeitos das diferentes partes da planta, e estabelecer os órgãos mais ricos em compostos terapêuticos que culminam em menor prejuízo para a planta quando extraídos. Atualmente, existem poucos estudos que comprovem a segurança da *L. pacari*no metabolismo ou na utilização *in vivo*, portanto estudos adicionais são encorajados.

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ANEXO A – AUTORIZAÇÃO DO INSTITUTO ESTADUAL DE FLORESTAS – IEF

GOVERNO DO ESTADO DE MINAS GERAIS
SECRETARIA DE ESTADO DE MEIO AMBIENTE E DESENVOLVIMENTO SUSTENTÁVEL
INSTITUTO ESTADUAL DE FLORESTAS
DIRETORIA DE PROTEÇÃO À FAUNA
GERÊNCIA DE PROJETOS E PESQUISAS

AUTORIZAÇÃO PARA PESQUISA CIENTÍFICA NO ESTADO DE MINAS GERAIS

Número da Autorização	Data da Emissão	Prazo de Validade
107/2017	29/12/2017	29/12/2018

INFORMAÇÕES DO RESPONSÁVEL E DO PROJETO

Título do Projeto	"Potencial terapêutico e farmacológico de espécies vegetais nativas da Bacia do Rio pandeiros no tratamento de doenças metabólicas: incentivo à preservação da flora".							
Instituição	Universidade Federal de Minas Gerais							
Responsável	Sergio Henrique Sousa Santos						**CPF**	055.482.156-71
Logradouro	Rua Cosme e Damião							
Nº/KM	260	**Complemento**			**Bairro/Localidade**	Santo Expedito		
Município	Montes Claros			**UF**	MG	**CEP**	39401-502	**Cx. Postal**
Telefone	(38) 2101-7710		**Celular**	(38) 99912-1055				
E-mail	sergiosousas@hotmail.com							

INTEGRANTES DA EQUIPE

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Júnio Cota Silva	UFMG	067.256.616-88	Pesquisador Colaborador
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Janaina Ribeiro Oliveira	UNIMONTES	068.789.696-70	Colaboradora
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Daniel Silva Moraes	UNIMONTES	098.038.796-50	Colabordor
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Fábio Ribeiro dos Santos	UFMG	108.799.876-00	Colaborador

INFORMAÇÕES DAS ATIVIDADES

Tipo de Atividade:	( ) Captura	(X) Coleta	(X) Transporte	( ) Sem Coleta/Captura	
	( )Abiótica	( ) Microrganismos	( ) Fungo	(X) Botânica	( ) Invertebrados
	( )Anfíbios	( ) Répteis	( ) Aves	( ) Mamíferos	( ) Ictiofauna

OBSERVAÇÕES	Esta autorização permite coleta/transporte botânico de sementes, folhas e ramos das espécies citadas na tabela de estimativa de coleta. Esta autorização não permite coleta de espécies ameaçadas. Esta autorização permite até cinco (05) integrantes da equipe a cada campanha.

LOCAL DA ATIVIDADE – EM UNIDADE DE CONSERVAÇÃO ESTADUAL

Unidade de Conservação	Responsável pela UC	Contato (Telefone e e-mail)	Endereço da UC	Assinatura do Responsável pela UC

Assinatura do responsável pela Autorização	Número do Processo SIGED/SIPRO – IEF/DFAU/GPROP
Janaina A. B. Aguiar Gerente de Projetos e Pesquisas do IEF Masp: 1131566-0	SIGED 00000241 2101 2018

Cidade Administrativa Tancredo Neves, Edifício Minas - Gerência de Projetos e Pesquisas - 1° and
Rodovia João Paulo II, 4143, Bairro Serra Verde - CEP 31630-900
Telefones: (31) 3915-1326 E-mail: pesquisa.gprop@meioambiente.mg.go

Refúgio de Vida Silvestre Estadual do Rio Pandeiros	Neilton Viana Neves	(38) 3621-0100 neilton.viana@meioambiente.mg.gov.br	BR 479, Vila da CEMIG, S/nº Distrito de Pandeiros. CEP: 39.490-000	
APA Estadual do Rio Pandeiros	Altenfelder Martins da Fonseca	(38) 3625-6222 (38) 3625-6205 altenfelder.fonseca@meioambiente.mg.gov.br	Rua Joaquim Borges Monteiro nº 180 – Bonito de Minas CEP: 39.490-000	

Esta autorização será válida apenas com a autenticação do responsável pela(s) Unidade(s) de Conservação.

LOCAL DA ATIVIDADE – FORA DE UNIDADE DE CONSERVAÇÃO ESTADUAL (apenas para material botânico)	
Município(s)	Não se aplica

TRANSPORTE – DESTINO DO MATERIAL COLETADO	
Instituição(ões)	ICA - UFMG
Endereço(s)	Montes Claros - MG

Outras Observações e Ressalvas:

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2. O pesquisador titular deverá contatar a administração dessa unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade de conservação, quando for o caso;
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4. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
5. O titular da autorização e os membros de sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos e empregar esforços de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condições *in situ*, quando for o caso;
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   - de espécies ameaçadas de extinção em lista oficial federal, salvo quando constante de projeto específico autorizado pelo SISBIO;
   - de espécies ameaçadas de extinção em lista oficial estadual, salvo quando constante de projeto específico autorizado pelo IEF;
   - de fauna e flora em áreas de domínio privado, sem o consentimento expresso ou tácito do proprietário nos termos do Código Civil;
7. Esta autorização não permite transporte interestadual e internacional de material biológico;
8. Esta autorização não dispensa o cumprimento da legislação que dispõe sobre o acesso ao patrimônio genético, sobre a proteção e o acesso ao conhecimento tradicional associado e sobre a repartição de benefícios para conservação e uso sustentável da biodiversidade. Veja maiores informações em www.mma.gov.br;
9. O titular desta autorização, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização suspensa ou revogada pelo IEF e o material biológico coletado apreendido nos termos da legislação em vigor;
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12. O pesquisador deverá estar sempre acompanhado desta autorização para apresentá-la às autoridades, quando solicitado.

Assinatura do responsável pela Autorização	**Número do Processo SIGED/SIPRO – IEF/DFAU/GPROP**
Janaina A. B. Aguiar Gerente de Projetos e Pesquisas do IEF Masp: 1131566-0	

ANEXO B - SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO – SISGEN

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A6B40FC

A atividade de acesso ao Patrimônio Genético/CTA, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A6B40FC**
Usuário:	**Universidade Federal de Minas Gerais**
CPF/CNPJ:	**17.217.985/0001-04**
Objeto do Acesso:	**Patrimônio Genético/CTA**
Finalidade do Acesso:	**Pesquisa**

Espécie

Davilla elliptica

Acosmium dasycarpum

Lafoensia pacari

Davilla elliptica, Lafoensia pacari, Acosmium dasycarpum.

Fonte do CTA

CTA de origem não identificável

Título da Atividade:	**Potencial Terapêutico e Farmacológico de Espécies Vegetais Nativas da Bacia do Rio Pandeiros no Tratamento de Doenças Metabólicas: Incentivo à Preservação da Flora.**

Equipe

Bruna Mara Aparecida de Carvalho	**UFMG**
Diego Vicente da Costa	**UFMG**

Junio Cota Silva	UFMG
Igor Viana Brandi	UFMG
João Marcus Oliveira Andrade	Unimontes
Janaina Ribeiro Oliveira	Unimontes
Amanda Souto Machado	Unimontes
Deborah de Farias Lelis	Unimontes
Daniela Fernanda de Freitas	Unimontes
Daniel Silva Moraes	Unimontes
Luis Paulo Oliveira	Unimontes
Natália Gonçalves Ribeiro	Unimontes
Jaciara Neves Sousa	Unimontes
Victor Hugo Dantas Guimarães	Unimontes
Fábio Ribeiro do Santos	UFMG
Alfredo Maurício Batista de Paula	Unimontes
André Luiz Sena Guimarães	Unimontes
Daniele Cristina Moreira	Unimontes

Parceiras Nacionais

22.675.359/0001-00 / Universidade Estadual de Montes Claros

Data do Cadastro: **06/11/2018 16:17:33**

Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético

Situação cadastral conforme consulta ao SisGen em **15:35** de **28/12/2018**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

ANEXO C - SISTEMA DE AUTORIZAÇÃO E INFORMAÇÃO EM BIODIVERSIDADE – SISBIO

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Comprovante de registro para coleta de material botânico, fúngico e microbiológico

Número: 66693-1	Data da Emissão: 06/11/2018 16:54:26

Dados do titular

Nome: Sérgio Henrique Sousa Santos	CPF: 055.482.156-71

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Táxons autorizados

#	Nível taxonômico	Táxon(s)
1	Espécie	Plantae > Angiospermae > Dicotyledoneae > Lythraceae > Lafoensia > Pacari
2	Espécie	Plantae > Magnoliophyta > Magnoliopsida > Dilleniales > Dilleniaceae > Davilla > Elliptica
3	Espécie	Plantae > Magnoliophyta > Magnoliopsida > Lamiales > Boraginaceae > Cordia > Verbenacea
4	Espécie	Plantae > Magnoliophyta > Magnoliopsida > Asterales > Asteraceae > Lychnophora > Ericoides

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Sistema de Autorização e Informação em Biodiversidade - SISBIO

Comprovante de registro para coleta de material botânico, fúngico e microbiológico

Número: 66693-1	Data da Emissão: 06/11/2018 16:54:26

Dados do titular

Nome: Sérgio Henrique Sousa Santos	CPF: 055.482.156-71

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

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ANEXO D – Normas para a publicação no periódico *Life Sciences*

LIFE SCIENCES

DESCRIPTION

Life Sciences is an international journal publishing articles that emphasize the **molecular**, **cellular**, and **functiona*l*** **basis of therapy**. The journal emphasizes the understanding of mechanism that is relevant to all aspects of human disease and translation to patients. All articles are rigorously reviewed.

The Journal favors publication of full-length papers where modern scientific technologies are used to explain **molecular**, **cellular** and **physiological mechanisms**. Articles that merely report observations are rarely accepted. Recommendations from the Declaration of Helsinki or NIH guidelines for care and use of laboratory animals must be adhered to. Articles should be written at a level accessible to readers who are non-specialists in the topic of the article themselves, but who are interested in the research. The Journal welcomes reviews on topics of wide interest to investigators in the **life sciences**. We particularly encourage submission of brief, focused reviews containing high- quality artwork and require the use of mechanistic summary diagrams.

Manuscripts should present novel preclinical findings addressing questions of **biological significance** to **human disease**. Studies that fail to do so may be rejected without review. Quantitative conclusions must be based on truly quantitative methods. *Life Sciences* does not publish work on the actions of biological extracts of unknown chemical composition. Compounds studied must be of known chemical structure and concentration. The study must be reproducible; materials used must be available to other researchers so they can repeat the experiment. Clinical studies may be considered if they expand understanding of mechanism, but the journal does not encourage clinical trial reports.

Four common reasons for rejection include: out of scope (the manuscript does not conform to the goal of identification of mechanisms related to therapy for human disease); too preliminary (manuscript is based on a limited amount of experimental data diminishing significance); lack of novelty (manuscript is well done but does not address a significant question); unidentified structure (actions of biological extracts of unknown chemical composition).

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INTRODUCTION

Life Sciences is an international journal publishing articles that emphasize the molecular, cellular, and functional basis of therapy. All articles are rigorously reviewed. The Journal favors publication of full-length papers where modern scientific technologies are used to explain molecular, cellular and physiological mechanisms. Articles that merely report observations are rarely accepted. Articles should be written at a level accessible to readers who are non-specialists in the topic of the article themselves, but who are interested in the research.
The Journal welcomes reviews on topics of wide interest to investigators in the life sciences. We particularly encourage submission of focused reviews containing high-quality artwork and mechanistic diagrams.

IMPORTANT INFORMATION

• Submission of a paper will be held to imply that the manuscript contains original unpublished work and is not being submitted for publication elsewhere.
• Manuscripts should present novel findings addressing significant biological questions. Studies that fail to do so may be rejected without review.
• Quantitative conclusions must be based on truly quantitative methods.
• Life Sciences does not publish work on the actions of biological extracts of unknown chemical composition. Compounds studied must be of known chemical structure and concentration.
• The study must be reproducible; materials used must be available to other researchers so they can repeat the experiment.

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