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Manuscript originating from the course conclusion work entitled "Characterization of the biochemical effects of bacuri oil (*Platonia insignis*) in male mice", authored by Luana Oliveira Severo and supervised by Silvana Peterini Boeira, presented in August 2016 at the Universidade Federal do Pampa (Federal University of Pampa) – Campus Itaqui. Itaqui, RS, Brazil.

Biochemical effects of bacuri oil (Platonia insignis) in male mice

Efeitos bioquímicos do óleo de bacuri (Platonia insignis) em camundongos machos

Abstract

Introduction: The utilization of medicinal plants as a source of therapeutic products has been a part of human history. *Platonia insignis* is a fruit species native to the eastern Amazon, popularly known as bacuri. The oil extracted from bacuri seeds provides a chemical composition with a high content of active substances with healing, antioxidant, and anti-inflammatory effects. ***Objective***: This study aims to characterize the biochemical effects of bacuri oil as a possible preventive therapeutic alternative. ***Method***: 15 adult Swiss mice were used, divided into three groups. Bacuri oil at doses of 10 and 100 mg/kg and the vehicle (olive oil – 10 ml/kg) were administered orally for 30 consecutive days. After 24 hours of administration of the natural compound and vehicle, the animals were euthanized; blood collected by cardiac puncture and plasma used to perform biochemical analyses. The biochemical parameters were evaluated: glucose, total cholesterol, LDL (Low Density Lipoprotein) and HDL (High Density Lipoprotein) cholesterol, triglycerides, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma glutamyl transferase (GGT), urea , creatinine, and total proteins. ***Results***: Pretreatment with bacuri oil promoted beneficial effects on the biochemical parameters analyzed. *Conclusions*: In this way, the fruit can be used as a therapeutic alternative in the prevention of diseases.

Keywords: Fruit. Biochemistry. Health. Prevention

Resumo

Introdução: A utilização das plantas medicinais como fonte de pr odutos terapêuticos acompanha a história da humanidade. A *Platoniainsignis* é uma espécie frutífera nativa da Amazônia oriental, popularmente conhecida como bacuri. O óleo extraído das sementes do bacuri proporciona uma composição química com alto teor de substâncias ativas com efeitos cicatrizantes, antioxidantes e anti-inflamatórios. *Objetivo*: Este estudo visa caracterizar os efeitos bioquímicos do óleo de bacuri como possível alternativa terapêutica preventiva. ***Método***: Foram utilizados 15 camundongos Swiss adultos divididos em 3 grupos. O óleo de bacuri nas doses de 10 e 100 mg/Kg e o veículo (óleo de oliva – 10 ml/Kg) foram administrados via oral por 30 dias consecutivos. Após 24 horas da administração do composto natural e do veículo, procedeu-se a eutanásia dos animais; sangue coletado por punção cardíaca e o plasma utilizado para realizar as análises bioquímicas. Foram avaliados os parâmetros bioquímicos: glicose, colesterol total, colesterol LDL *(*LowDensityLipoprotein*)* e HDL *(*High Density Lipoprotein*)*, triglicerídeos, transaminase glutâmica oxalacética (TGO), transaminase glutâmica pirúvica (TGP), gama glutamil transferase (GGT), ureia, creatinina e proteínas totais. ***Resultados***: O pré-tratamento com o óleo de bacuri promoveu efeitos benéficos nos parâmetros bioquímicos analisados. ***Conclusões***: Desta forma, o fruto pode ser utilizado como uma alternativa terapêutica na prevenção de doenças.

Palavras-chave: Fruto. Bioquímica. Saúde. Prevenção.

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INTRODUCTION

Since ancient times, medicinal plants have been used as a source of therapeutic products. Brazil has a wide variety of tropical fruits, which arouse the interest of scientific communities in the study, conservation, and rational use of these resources. Foods of plant origin, especially fruits, play an role in human nutrition as they have nutritional and therapeutic effects. The Amazon region, especially, is rich in fruits with exotic flavors that demonstrate benefits to the organism.[1]

The fruit species *Platonia insignis* Mart. (Clusiaceae) is native to the Brazilian Amazon and is popularly known as bacuri. It is a fruit that is little known in the scientific community, but has been gaining prominence for providing anti-inflammatory, antioxidant and other activities, ranging from the use of edible pulp to by-products from the food industry, seeds and peels.[2,3]

Its seed is not used in food but can be used in the production of soap or bacuri lard. Traditionally, the extraction of bacuri oil occurs with great difficulty, as the seeds are soaked in water for more than a year and then boiled, with the oil removed from the surface of the boiling water. However, with the advancement of technology and industrial improvements, this process can be optimized through techniques such as seed pressing, as well as cold extraction, from wild species from the Amazon Forest.[4] It is described that the oil has antioxidant, healing, and anti-inflammatory properties.[5]

Studies show that bacuri oil has been used in Brazilian folk medicine for therapeutic purposes, such as for the treatment of eczema, herpes, and diarrhea. About its antioxidant capacity, bacuri promotes the detoxification of free radicals and modulates the antioxidant enzymes, thus presenting a potent protective action against the emergence and development of degenerative processes associated with various diseases.[6-10] This activity is related to its chemical constituents with antioxidant properties such as vitamin C (ascorbic acid) and vitamin E (tocopherols), flavonoids, anthocyanins, and polyphenols.[11]

Other benefits of consuming Bacuri are related to several factors, especially the fatty acid content. In this sense, phytochemical studies related to fruit peels indicate the presence of several compounds such as palmitic, oleic, linoleic, stearic, caprylic and myristic acids, alcohols, hydrocarbons, and esters.[12]

Given the above, the objective of the present study was to evaluate the beneficial effects of bacuri oil (*Platonia insignis*) through serum biochemical analyzes in male mice. It was considered that it could be used as a therapeutic alternative, playing a role as a functional food in human health.

METHOD

The experiment was conducted at the Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas (Laboratory of Pharmacological and Toxicological Evaluations applied to bioactive molecules)- (LAFTAMBIO Pampa) at the Universidade Federal do Pampa (UNIPAMPA) – Campus Itaqui, in the period January/February 2016. The experiments were conducted in accordance with the principles and procedures described by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee for the use of animals at UNIPAMPA (protocol number 013/2016).

For the experiment, 15 male Swiss mice were used, three months old and weighing 25-35 grams. The animals were obtained from the Central Animal Facility of the Federal University of Santa Maria (UFSM). The animals were housed in polypropylene boxes under controlled light conditions (12-hour light/dark cycle), controlled temperature (22 ± 2 °C), with water and food *ad libitum* (fed with standard commercial food).

The mice were randomly divided into three groups (n=5), comprising the following treatments: bacuri oil at doses of 10 and 100 mg/kg and the vehicle (olive oil – 10 ml/kg). Borges brand extra virgin olive oil was used in the experiment,while bacuri oil was supplied by Amazon Oil Indústria e Comércio Ltda. (Ananindeua, PA, Brazil), which followed the methodology of pressing the seeds of wild species from the Amazon Forest.

Bacuri oil at different doses and the vehicle were administered orally, via gavage, for 30 consecutive days. After 24 hours of administration of the natural compound or olive oil, all animals received a dose of pentobarbital (180 mg/kg, intraperitoneal), and blood was collected by cardiac puncture and transferred to tubes containing heparin (anticoagulant). After centrifuging the samples, the plasma was used to perform biochemical analyses.

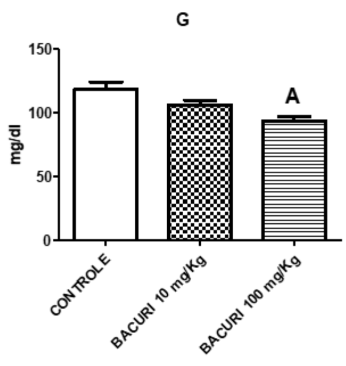
The following biochemical parameters were evaluated: glucose, total cholesterol, LDL cholesterol (Low Density Lipoprotein), HDL cholesterol (High Density Lipoprotein), triglycerides, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma glutamyl transferase (GGT) , urea, creatinine, and total proteins. All analyzes followed the methodology described for each Labtest Diagnóstica brand kit.

To statistics the GraphPad Prism 5 program was used. One-way analysis of variance (ANOVA) was performed, followed by the Newman-Keuls test. The significance level considered was $p<0.05$

RESULTS

Based on the results obtained, it is possible to understand the beneficial effects of the pretreatment with the bacuri oil in male Swiss mice. Among the biochemical variables analyzed, a statistically significant reduction in glucose levels was observedin animals treated with a dose of 100 mg/kg of Bacuri oilcompared to the control group (Figure 1).

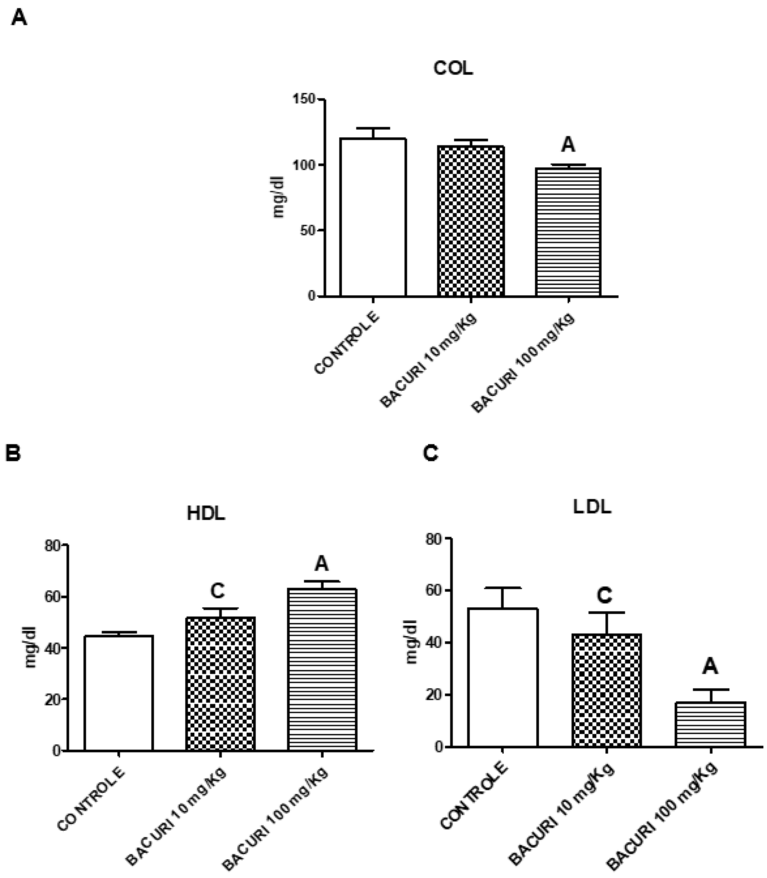
Figure 1. Effect of pretreatment with bacuri oil (10 and 100 mg/kg) on serum glucose (G) levels in mice. Data are expressed as mean ± standard error for a number = 5 animals per group. A: indicates a significant difference ($p < 0.05$) compared to the control group.



Regarding the analyzed lipid profile, a significant decrease in total cholesterol was observed in 100 mg/kg of Bacuri oil (Figure 2A). In relation to LDL cholesterol, a decrease was obtained in the two groups pretreated with bacuri oil, and at a dose of 100 mg/kg the reduction was higher, as shown in Figure 2B.As for HDL cholesterol, there was a significant increase in the two doses of bacuri oil used compared to the control group, and at a dose of 100 mg/kg, the increase was more expressive, as shown in Figure 2C.

Statistical analysis of serum triglyceride levels revealed no differences between the groups treated with bacuri oil and in relation to the control group. Therefore, these data were not demonstrated in the article

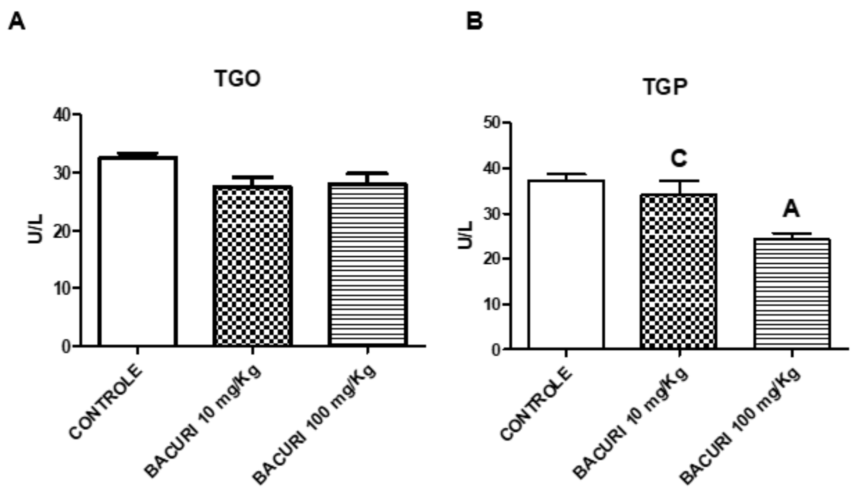
Figure 2. Effect of pre-treatment with bacuri oil (10 and 100 mg/Kg) on serum levels of total cholesterol (COL) (A), HDL cholesterol (B) and LDL cholesterol (C) in mice. Data are expressed as mean ± standard error for a number = 4 animals per group. A: indicates a significant difference ($p < 0.05$) compared to the control group. C: Indicates a significant difference ($p < 0.05$) compared to the bacuri 100 mg/Kg group



The analysis of specific liver enzymes that rule out the possibility of liver toxicity from bacuri oil also makes up the body of results of this work. In this sense, a significant decrease in GPT enzymatic levels was observed in the group treated with 100 mg/kg of bacuri oil in relation to the control. In contrast, the group treated with 10 mg/kg of bacuri oil showed increase in the GPT enzyme compared to the group treated with 100 mg/kg (Figure 3B). Regarding the GOT enzyme, no significant results were observed (Figure 3A).

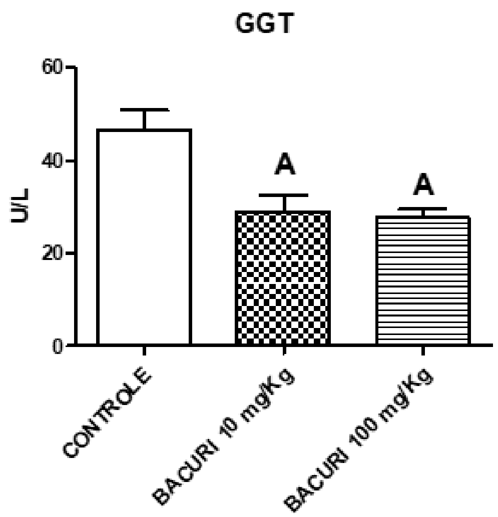
Figure 3. Effect of pre-treatment with bacuri oil (10 and 100 mg/kg) on the levels of the liver damage enzymes GOT and GPT in mice. Data are expressed as mean ± standard error for a number = 5 animals per group. A: indicates a

significant difference ($p < 0.05$) compared to the control group. C: Indicates a significant difference ($p < 0.05$) compared to the bacuri 100 mg/Kg group.



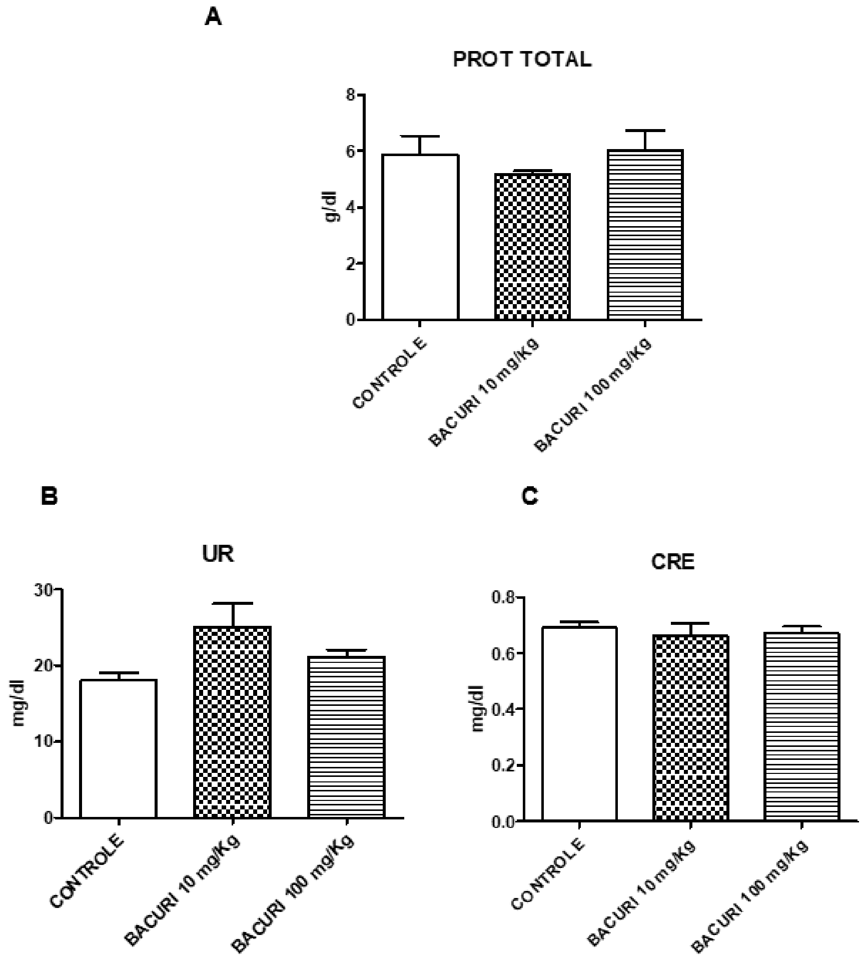
Regarding GGT (Figure 4), there was a significant reduction of this enzyme in the groups pretreated with bacuri oil (10 and 100 mg/kg) compared to the control group, demonstrating no liver damage.

Figure 4. Effect of pretreatment with bacuri oil (10 and 100 mg/kg) on plasma levels of the GGT enzyme in mice. Data are expressed as mean ± standard error for a number = 5 animals per group. A: indicates a significant difference ($p < 0.05$) compared to the control group.



In addition to evaluating the possibility of liver toxicity from bacuri oil, some markers of kidney function were also measured.Serum parameters total proteins (Figure 5A), urea (Figure 5B), and creatinine (Figure 5C) did not change during the 30 consecutive days of treatment with different doses of bacuri oil.

Figure 5. Effect of pretreatment with bacuri oil (10 and 100 mg/Kg) on kidney damage parameters total proteins (PROT TOTAL) (A), urea (UR) (B), and creatinine (CRE) (C) in mice. Data are expressed as mean ± standard error for a number = 5 animals per group.



DISCUSSION

There is an increase in the number of studies involving the chemical, biological, and pharmacological properties of bacuri. The studies focus on the fat extracted from the seeds, which in popular medicine is used to treat skin problems, diarrhea, earaches, spider and snake bites, rheumatism, and arthritis, and as a healing agent.[13,14]

Furthermore, it is necessary that national scientific communities value the use of native products and serve as a more viable therapeutic alternative in terms of minimizing drug and food interactions, and disease prevention. However, the mechanisms and processes that elucidate such effects are not fully understood in the literature, highlighting the importance of developing work with native fruits.

Scientific literature has demonstrated different biological activities for bacuri. The potential as a cardioprotective,[4,14] vasorelaxant,[15] leishmanicidal,[8] immunomodulatory[16] and for glycemic control,[10] among others, has reported in several studies. The present work seeks to corroborate other studies on the beneficial potential of bacuri oil and provide data that prove that, despite differences in doses, treatment times and experimental animals used, this compound can be considered a bioactive ally in disease prevention.

Furthermore, this study compared the results of the serum biochemical parameters with other studies to establish a safe range of administration, and positive effects and possible toxicity levels.

Based on our results, it is possible to understand the beneficial effects of pretreatment with bacuri oil. A decrease in glucose levels and lipid parameters such as total cholesterol, LDL, and an increase in HDL were observed. Regarding liver parameters, a decrease in GPT was observed at a dose of 100 mg/kg and, on the other hand, an increase in the enzyme at a dose of 10 mg/kg. Renal function markers were also evaluated, and total proteins, urea and creatinine did not change during pretreatment.

Diabetes mellitus is a chronic disease characterized by high glycemic levels, due to disturbances in the metabolism of lipids, carbohydrates and proteins caused by the absence or deficiency in insulin secretion.[17] Therefore, it is necessary to evaluate the effects of natural alternatives to balance or reduce hyperglycemia levels. In our work, pretreatment with bacuri oil promoted a reduction in blood glucose levels. This result is relevant, given the incidence of diabetes in the population and the associated comorbidities. Furthermore, it is necessary to carry out more detailed studies to measure the hormone insulin and verify glucose levels in longer treatments with bacuri oil.

In a study by Luz et al.,[17] pre-treatment with omega-3 helped to improve the secretion of insulin, hormone responsible for maintaining blood glucose homeostasis. This effect would be related to the antioxidant characteristic of essential fatty acids, which would act to reduce inflammation in liver tissue, leading to an improvement in the insulin signaling pathway in the liver of mice.In this sense, bacuri oil is considered an important antioxidant, as it is composed of essential fatty acids.[18] It has an average of 64% saturated fatty acids, 34% monounsaturated and 2% polyunsaturated, the main ones being representatives oleic, linoleic, stearic, and palmitic acids.[19]

Dyslipidemias are considered one of the main determining factors for the development of cardiovascular diseases. High concentrations of triglycerides, cholesterol and a decrease in HDL tend to increase the risk of developing diseases, such as atherosclerosis. Therefore, an adequate alimentation, rich in fruits, vegetables and vegetables, contributes to improving serum lipid profile levels.[20,21]

In our study, pretreatment with bacuri oil at doses of 10 and 100 mg/kg promoted beneficial changes, demonstrated by the increase in HDL and the decrease in total and LDL cholesterol. These data do not corroborate with Lutosa et al.,[16] who, in a 14-day treatment with hexane extract from *Platonia insignis* seeds, at a dosage of 2.0 g/kg in Wistar rats did not observe significant changes in the same parameters analyzed. It is worth noting that differences in experimental protocol (species, sex, dose, route of administration, treatment time) contribute to explaining these incompatibilities in results.

Toxicity studies are important to rule out any possibility of harmful effects of natural compounds, since it is in the liver metabolization of these products that active metabolites harmful to the organism may be produced. There are few studies that evaluate the hepatotoxicity of the bacuri oil in experimental protocols. Thus, GOT and GPT enzymes can be excellent biomarkers of liver damage, and when altered, they would indicate hepatotoxicity.[22]

During pretreatment for 30 consecutive days at doses of 10 and 100 mg/kg of bacuri oil, no increases in the analyzed liver enzymes were observed. Also, it can be inferred that the dose of 100 mg/kg is more effective, as it promoted a more significant decrease in the GPT enzyme. These results corroborate data from other studies that used bacuri oil and grape seed oil, which have constituent elements similar to bacuri.[16,23] In these studies, no statistically significant differences were found in the serum concentrations of enzymes related to liver damage, showing that the administration of the bacuri oil did not affect liver metabolism during pretreatment.

The GGT enzyme originates from the hepatobiliary system and is widely used to diagnose liver diseases. Its elevation is associated with chronic stimulation of the hepatocyte microsomal system and presence of cholestasis.[24] In our experimental protocol, a significant reduction in this enzyme was found at doses of 10 and 100 mg/kg, demonstrating no liver damage.Thus, the set of data related shows that the use of bacuri oil does not bring any harm to the liver.

Epidemiological studies have demonstrated considerable growth in cases of chronic kidney disease as a public health problem, as part of the population has some degree of loss of kidney function. The chronic inflammatory state usually found in the kidneys is identified as an independent factor for malnutrition and changes that lead to increased muscle protein catabolism.[25,26]

Our study allowed to evaluate some markers of kidney damage, such as total proteins, urea, and creatinine. Total protein measurement is used to check nutritional status and protein loss; Urea and creatinine are compounds excreted by the kidneys and are considered the most sensitive markers for evaluating the functional capacity of the kidneys.[26]

In our study, there was no significant change in these markers, demonstrating that the bacuri oil does not cause harmful effects on the renal system at the administered doses. In a similar study, garcinielliptone isolated from the hexane extract of *Platonia insignis* seeds (5.000 mg/kg) was used for 30 days, and no changes were observed in the same parameters analyzed.[5]

It is a point to consider that bacuri oil has beneficial effects were demonstrated in this work, through the analysis of several biochemical parameters. Over the years, several studies have elucidated the mechanisms involved in which the oil from this fruit acts, causing such effects.[4] In this sense, one of the elements that may be related to this beneficial capacity is the fact that bacuri is a potent antioxidant and act by increasing total antioxidant capacity and reducing oxidative stress. Such activity is related to the presence of compounds derived from terpenes, xanthones and phenolics, in addition to saturated and unsaturated fatty acids.[4]

CONCLUSION

The results demonstrate that the pretreatment with bacuri oil at doses of 10 mg/Kg and 100 mg/Kg for 30 consecutive days in male Swiss mice promoted beneficial effects on the levels of glucose, total cholesterol, HDL, LDL, liver and kidney markers. In this way, this study contributed to further elucidation of the beneficial effects of this oil and the possibility of use as a therapeutic alternative to disease prevention.

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Contributors

Boeira SP, creator of the project; Gomes MGG, acted as executor of the experiment and analysis and interpretation of data; Severo LO, executor of the experiment, analysis, and interpretation of data, and writing of the article; Backes L, worked on organizing references; Madalosso, LM helped review the text; Balok FRM carried out statistics and final review of the text.

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