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ARTIGO ORIGINAL

# *Efeito da infusão de Myrcia guianensis (Pedra-ume-caa) na glicemia e lipemia de ratos Wistar.*

*Effect of Myrcia guianensis (Pedra-ume-caa) infusion on Wistar rat glycemia and lipidemia.*

Palavras-chave: diabetes Mellitus, glicemia, metabolismo, *myrcia guianensis*, pedra-ume-caa, teste de tolerância à glicose.
Key words: diabetes Mellitus, blood glucose, glucose tolerance test, metabolism, *myrcia guianensis*, pedra-ume-caa.

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## *RESUMO*

*Sabe-se que a Myrcia guianensis possui propriedades antidiabéticas, porém, poucos estudos foram realizados descrevendo sua ação metabólica. Este estudo foi realizado com o intuito de se investigar os efeitos da infusão de folhas de Myrcia guianensis na glicemia e lipemia de ratos diabéticos induzidos por estreptozocina. O chá foi administrado aos animais por 15 dias e, periodicamente no 1º, 7º e 15º dia, os mesmos foram submetidos a uma sobrecarga de glicose (teste de tolerância à glicose) com acompanhamento dos parâmetros: peso, glicemia de jejum e lipídeos testes para análise histopatológica sem que qualquer alteração tenha sido encontrada. Em animais normais, um importante efeito hipoglicemiante foi induzido com a infusão, mesmo quando submetidos à sobrecarga de glicose. Uma queda mais sutil na glicemia foi observada durante todo o tratamento nos animais diabéticos submetidos à infusão, embora, não tenham sido observadas diferenças significativas. A principal alteração no metabolismo dos lipídeos ocorreu com os valores de triglicerídeos, os quais apresentaram uma diminuição nos níveis séricos nos animais normais e uma queda ainda maior nos diabéticos. Ao final do experimento, houve um decréscimo de ganho de peso nos animais diabéticos, sugerindo uma possível influência da infusão nos efeitos catabólicos desencadeados pelo diabetes mellitus. Os resultados apresentados confirmam a ação hipoglicemiante da infusão de Myrcia guianensis, revelaram sua ação hipotrigliceridemiante, a baixa toxicidade hepática e jejunal, sugerindo interessante potencial como ferramenta terapêutica alternativa para o tratamento do Diabetes mellitus.*

## *ABSTRACT*

*Myrcia guianensis is claimed to have anti-diabetic properties, however few studies aimed on describing its specific metabolic action. The present study was conducted to investigate the effect of Myrcia guianensis on the glycemia and lipidemia of streptozotocin-diabetic rats. The animals were treated with Myrcia guianensis leaf infusion for 15 days and, periodically, on the 1st, 7th, and 15th day, were exposed to a glucose overload (glucose tolerance test) and biochemical analysis as fasting glycemia and lipids (serum triglycerides and total cholesterol) were performed as well the weight measurement. Samples of liver and jejunum tissues were collected, at the end of*

*the infusion was observed even when submitted to glucose overload. A slight decrease in glycemia occurred throughout the entire treatment in the diabetic animals that were given the infusion, even though no significant differences were found. The main influence of the infusion on lipid metabolism concerned triglycerides values, triggering lower levels in normal animals and higher levels in diabetic animals. Later weight evaluation of the diabetic animals showed a lower increase in values, suggesting a possible influence of the infusion on catabolic effects triggered by diabetes. The results confirm the hypoglycemic Myrcea guianensis infusion action, reveling a hypotriglyceridemic action and a low hepatic and jejunum toxicity, suggesting to be an interesting alternative therapeutic tool in Diabetes mellitus treatment.*

## *INTRODUCTION*

*Diabetes Mellitus (DM) can be considered a heterogeneous syndrome characterized by an endocrine imbalance of the organism. Metabolic abnormalities trigger important consequences in carbohydrate, lipid and protein metabolism. Lipid abnormalities in diabetic patients are likely to play an important role in the development of atherogenesis[1-6]. Furthermore, considering both types (1 and 2), the most common cause of death in diabetic people is cardiovascular disease[5,7-10]. It has long been known that a reduction in hyperglycemia decreases the risk of micro vascular complications and is very likely to reduce the risk of macrovascular complications[5,11-13]. In this context, the study of the hypoglycemic effect of medicinal plants, as well as the investigation of their action on lipid metabolism, are relevant.*

*Through the years, many drugs have been used in DM treatment. Insulin use began in 1921[14-16]; oral hypoglycemic agents have been part of the pharmaceutical therapeutic arsenal since the early 1940's[14].*

*However, the use of medicinal plants with hypoglycemic properties dates back much earlier than that. The use of plants in diabetes treatment dates from the Ebers papyrus (around 1550 BC). More than 400 species of vegetables have been described as anti-diabetic[17-19]. In Brazil, more than sixty vegetables have been used orally based on its hypoglycemic effects[19-24].*

*Since their effectiveness, minimal side effects and relatively low prices, the anti-diabetic plants may become an interesting alternative to classic anti-diabetic agents[25,26]. Therefore, studies are strongly necessary to investigate the use of phytotherapics with hypoglycemic effects, which also decrease the lipid alteration, associated with DM. Such studies may lead to the discovery of a drug that reduces these important causes of cardiovascular risk[14,27].*

*vegetable insulin, cambuí or cambuim, is a plant with hypotensive and anti-diarrheic effects, though mainly, an assured hypoglycemic effect.*

*Many studies have described the anti-diabetic effect of various species of Myrcia[18,28,31-33]. In 1929, Toledo described the hypoglycemic effect of Myrcia sphaerocarpa for the first time [(23)]. Grüne (1979)[31] suggested this effect was due to the inhibition of the intestinal absorption of glucose induced by the plant, according to animal-based research. Yoshikawa et al. (1998)[32] and Matsuda et al. (2002)[33] observed that Myrcia multiflora derivatives are potent aldose reductase and alpha-glucosidase inhibitors.*

*The present study suggests the evaluation of the Myrcia guianensis plant effects on fasting glycemia, post oral glucose overload glycemia and cholesterol and triglycerides blood levels in Wistar streptozotocin-diabetic rats. During a 15-day period, the animals were treated with an infusion of Myrcia guianensis leaves.*

## *MATERIALS AND METHODS*

*Materials*

*Myrcia guianensis dried leaves were obtained from the Amazon Forest and identified by morphological analysis.*

*Infusion preparation*

*4g of dried leaves of Myrcia guianensis were added to 300 ml of boiling water and infusion was allowed for 15 minutes, according to Bragança (1996)[14] and Swanston-Flatt et al. (1990)[34]. The animals from groups 2 and 4 received 0.42 mL infusion/100g of weight/day, corresponding to 0.0028g of the active principle of the plant[14]. Myrcia guianensis infusion was administered orally and always at the same time for 15 days. The control group (Group 1) and Group 3 received the same amount of distilled water at the same time for the same period. All animals received the infusion or water through a hand-made metal curve-feeding syringe. On the days of glucose overload administration (1st, 8th and 15th days) the time of administration was observed. The animals were given first the glucose solution; then the water or infusion and, finally, the timed blood samples were collected[35].*

*Animals*

*All experiments were performed on male Wistar rats aged 5 to 8 weeks (130g - 160g) (the according to experimental ethics procedures/ COBEA). The animals were acclimatized for 1-2 weeks before being used in the experiments, and given standard pelleted diet and ad libitum water They were randomly selected and kept in plastic cages (2 animals/cage) and maintained under a constant 12 hour light/12 hour dark cycle, at an environmental temperature of 21-25°C.*

*animals. The control group (Group 1) consisted of normal animals that received water throughout treatment period. Group 2 consisted of normal animals treated with Myrcia guianensis infusion. Groups 3 and 4 were formed by diabetic animals that were given water or the infusion, respectively.*

*Induction of experimental DM*

*Animals from Groups 3 and 4, fasting overnight, became diabetic after a single dose of streptozotocin (STZ) (60 mg $kg^{-1}$, i.p.). The STZ was freshly dissolved in citrate buffer (0.01M, pH 4.5) and kept at low temperature prior to use[36]. Diabetes was confirmed in the STZ-treated rats, by means of daily measurement of the fasting blood glucose concentration. Animals had free access to food and water after STZ administration.*

*GTT in normal and STZ-diabetic Wistar mice*

*Prior to the glucose tolerance test (GTT), the rats were overnight fasted. The oral glucose overload was performed with 50% glucose solution in a ratio of 3.0 $g.kg^{-137}$ and distilled water or Myrcia guianensis infusion was immediately administered. Blood samples were collected before the glucose load, at zero time, and at 30, 60 and 120 minutes later.*

*Biochemical analysis of blood*

*Peripheral blood samples from the tail vein at intervals of 7 days, so that glycemia was measured at the 4 times described above and serum triglycerides and cholesterol levels were measured only during fasting.*

*All biochemical parameters were enzymatically determined by Accutrend GTC, Roche Diagnostics GmbH, d-68298 Mannheim-Germany, with the range of glucose values equal to 20 and 600mg/dL (1.1-33.3 mmol/L), triglycerides equal to 70 and 600mg/dL (0.8-6.86 mmol/L) and total cholesterol ranging from 150 to 300 mg/dL (3.88 a 7.76 mmol/L).*

*Histopathological analysis*

*At the end of the treatment period ($15^{th}$ day), pieces of liver and jejunum were extracted from randomly chosen animals, from each of the 4 groups, and kept in buffered formol 10% solution and performed for histological analysis.*

*Statistical analysis*

*The results are shown as means SEM with n = 6. Changes in the body weight, blood glucose, total cholesterol and triglycerides were compared by parametric ANOVA test followed by Tukey test. α-values of 0.05, $p<0.05$ (*); $p<0.01$ (**) and $p<0.001$ (***) were considered significant.*

## *RESULTS AND DISCUSSION*

*Glycemic Evaluation*

*and fourth day after STZ administration, and was confirmed by daily fast glycemic monitoring. After induction, the animals showed classical symptoms of type 1 DM, such as, hyperphagia, polydipsia and loss of body weight. Control diabetic animals showed an average glycemia of 363 27 mg/dL, while non-diabetic animals, which were given water, showed an average 152 8 mg/dL, at the beginning of the treatment, on the fourth day after induction.*

*Glycemia was verified on the $1^{st}$, $8^{th}$ and $15^{th}$ days and followed a consistent pattern all over the experiment. Analysis of the GTT of normal animals, on the last day of treatment (Figure 1), showed that Group 2 animals always showed lower glycemia levels than those of Group 1. The referred analysis also indicated that this reduction was significant ($p < 0.001$ for the times $T_{15}$ and $T_{30}$ and $p < 0.01$ for $T_{60}$), and that, both animal groups, had their glycemic peaks at $T_{15.}$ In normal animals, we observed an important hypoglycemic effect induced by the infusion, even when submitted to glucose overload ($T_{15}$ in Figure 1).*

*Glycemia occurrence in the diabetic animals subjected to glucose overload on the $1^{st}$ (A), $8^{th}$ (B) and $15^{th}$ (C) days of treatment is shown in figure 2. On the first day, a significant difference of 0.05 (*) between the glycemic curve of animals that were given water (Group 3) or the infusion (Group 4), at 15, 60 and 120 min, could be observed. Even though there is no significant statistical difference between glycemia levels for groups 3 and 4, at any other given time, a faint decrease in the glycemia of Group 4 could be noted. The levels of glycemia in the diabetic animals (Groups 3 and 4) were significantly different from the ones found in normal animals (Groups 1 and 2), and their peak occurred at $T_{30}$ in the diabetic glycemic curves, i.e., a later peak, characteristic of DM, which reflects a lack of insulin.*

*The present results are in agreement with Yoshikawa et al. (1998)[32] work that showed the methanolic extract and ethyl acetate-soluble portions of the leaves of Myrcia multiflora DC plant also inhibited the increase of serum glucose level in sucrose-loaded rats and in alloxan-induced diabetic mice. Also Pepato et al. (1993)[28], found a positive effect of the diabetic state of streptozotocin-diabetic rats treated with aqueous leaf extracts of Myrcia uniflora which presented reduced hyperglycaemia, polyphagia, polydipsia, urine volume and the urinary excretion of glucose and urea. In contrast, Russo et al. (1990)[38], found that Myrcia uniflora infusion given to a group of healthy subjects and a group of type II diabetic patients showed no acute or chronic effects on plasma glucose levels or glycated haemoglobin in either group. The differences in results found in those works, could be explained by the amount of extract used, by the extraction of infusion preparation, the species studied or other protocol influences.*

*As observed in here, the data suggest that the*

*Myrcia multiflora DC[32,33] and Myrcia uniflora[28]. It can be explained due to the action of flavanone glucosides (myrciacitrins I and II) and acetophenone glucosides (myrciaphenones A and B) that show an inhibitory activity on aldose reductase and alpha-glucosidase enzymes, as were identified in Myrcia multiflora DC extracts[33]. Aldose reductase inhibition is a quite interesting pharmacological target of action since it has been identified as the first enzyme involved in the polyol pathway of glucose metabolism which converts glucose into sorbitol, linked to tissue-based pathologies associated with diabetes complications[39]. On the other hand, alpha-glicosidase inhibition directly decreases glucose intestinal absorption, justifying the results presented in Figures 1 and 2.*

*Lipid Evaluation*

*The most common profile of lipid alteration in humans and rats with a diabetic condition is hypertriglyceridemia, low HDL-cholesterol and increased LDL-cholesterol levels[3-5,40].*

*In these research, the levels of total cholesterol, for all samples, in any given treatment group, were lower than the detection limit used (150mg/dL). This result is acceptable since previous studies have showed that total cholesterol values are not affected by DM[41,42]. Furthermore, colesterol values lower than 150 mg/dL are expected in normal and diabetic Wistar rats as described in other researches[43-45]. The most probable cholesterol alterations would be a decrease in HDL-cholesterol fractions in addition to an increase in triglycerides levels[5,40,46].*

*Nevertheless triglycerides values suffered significant alteration. Figure 3 describes the triglyceridemia of normal and diabetic animals that received water and infusion during the 15-day experiment. As expected, a significant increase in triglyceridemia was found in diabetic animals (Groups 3 and 4). Comparison of TG values from diabetic and normal animals show that TG levels were higher in diabetic animals than in normal animals, at all times, with values significantly higher ($p< 0.001$) for Group 4. Hypertriglyceridemia is one of the most common abnormalities found in DM[3-6, 26, 40, 47], because of the higher VLDL liver production and the decrease in its clearance[40,47]. When considering the normal animals (Groups 1 and 2), a significant increase ($p<0.001$) in TG levels from the 1st to the 15th day can be seen. We also noted that TG values were always lower for the animals of Group 2 than those of Group 1, with a significant difference only on the 15th day ($p<0.001$). This fact suggests that the infusion may disturb normal lipid metabolism, retreating serum TG, which may be related to hypoglicemic effect of Myrcia guianensis, that could decreases the substrate proffer for liver TG synthesis.*

*Unlike the normal animals, Group 3 animals showed TG values significantly lower than the animals treatment. Such situation was observed in the whole period of the treatment, with no variance of statistical significance. The different profile of TG in diabetic and normal animals treated with infusion suggests that hypertriglyceridemic effect of DM is more efficient than the action of the infusion.*

*It can be conceivable that the presence of flavonoids in Mycia guianensis infusion can contribute to TG levels decrease. Several researchers have demonstrated that flavonoids act as reducers of lipid activities[40,44,48-50]. It has been reported that Myrcia multiflora has flavonoids (myrciacitrins, quercitrin, desmanthin-1 and guaiajaverin) besides flavanone glucosides (myrciacitrins I and II) and acetophenones glucosides (myrciaphenones A and B)[32]. Studies with flavonoids are underway to further elucidate their mechanism of action.*

*Weight Evaluation*

*Weight increment during treatment is demonstrated in percentages in Figure 4. Normal animals increased significantly their weights from the 1st to the 15th day of treatment ($p<0.001$). However, no statistical difference was found in the comparison with normal animals given the infusion or water. In contrast, diabetic animals had no significant weight gain during the 15 days of the treatment, in accordance with the catabolic state prevailing in DM. The only significant difference ($p<0.05$) observed in this parameter occurred on the 15th day, during which the weight of animals given the infusion was higher than that of the animals given water, suggesting a possible protection from the catabolic effects triggered by the infusion.*

*Histopathological analysis*

*Histopathological analysis did not show any alteration in either of the two types of tissue specimens analyzed, liver or jejunum, suggesting that the Myrcia guianensis infusion, at least, considering the conditions performed in these experiment, has low toxicity.*

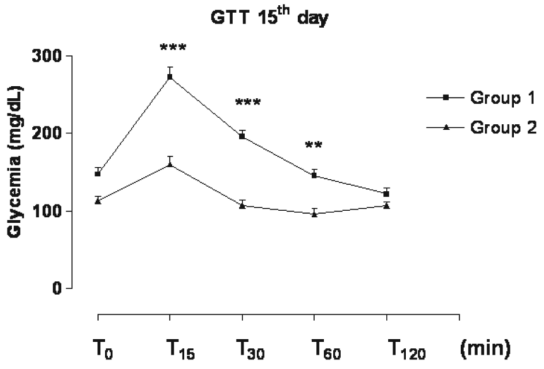
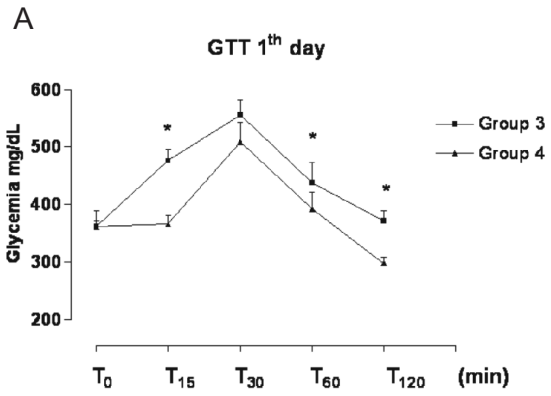
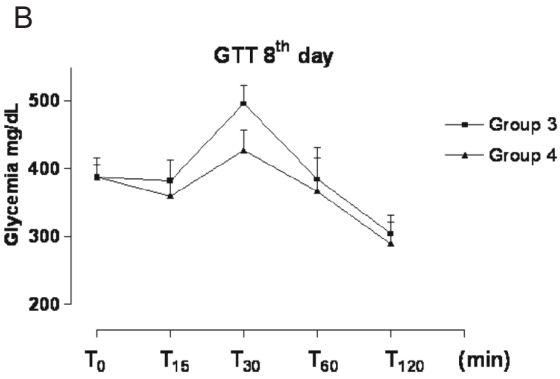



***Figure 1:*** *Glycemic Tolerance Test (GTT) in normal animals on the 15th day of treatment. Glycemic values of rats that were given water (Group 1) or Myrcia guianensis infusion (Group*



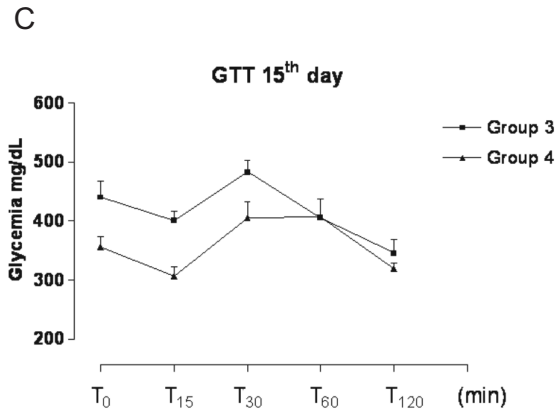




***Figure 2:*** *Glycemic Tolerance Test (GTT) in diabetic animals on the 1st (A), 8th (B) and 15th (C) day of treatment. Glycemic values of diabetic rats that were given water (Group 3) or Myrcia guianensis infusion (Group 4) at zero time and 15, 30, 60 and 120 minutes after glucose overload.*

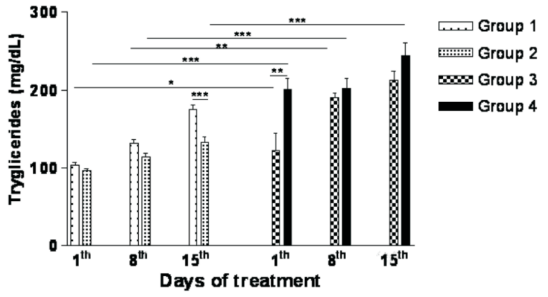



***Figure 3:*** *Triglyceridemia of normal and diabetic animals on the 1st, 8th and 15th day of treatment. Triglycerides values for rats given water or Myrcia guianensis infusion during fasting (T0).*

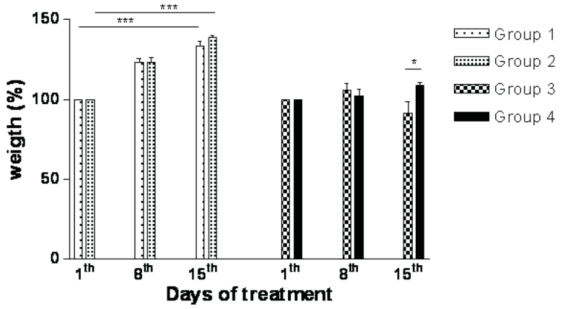



***Figure 4:*** *Weight evolution during treatment. Weight of normal and diabetic animals on the 1st, 8th e 15th days of treatment.*

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