Intake of *Acrocomia aculeata* Reduces Body Weight Gain and Increases Glutathione Peroxidase Activity in the Kidneys of Wistar Rats

ABSTRACT

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| *Acrocomia aculeata* (Jacq.) Lodd. is a common palm tree in the Cerrado and Pantanal. The pulp of this fruit is rich in carbohydrates, lipids, proteins, and bioactive compounds such as xanthines, lutein, zeaxanthin, β-carotene, and tocopherol. The aim of this study was to evaluate (body and plasma) the murinometric and biochemical parameters and the enzymatic antioxidant system in the kidney of Wistar rats. The experimental protocol was approved by the ethics committee (nº 23108.013689/2019-58). Male Wistar rats (*Rattus norvegicus*), weighing approximately 200 g (8 weeks old), were fed *A. aculeata* pulp for 28 days. The animals were randomly divided into two groups: Control Group (received a vehicle dose) and 250AAc Group (received 250 mg/kg of *A. aculeata*). The animals had access to water and Labina® feed *ad libitum*. The 250 mg/kg AAc group had a reduction in body weight gain (28%) and lower water intake. In addition, they had an 18% reduction in urinary urea without changing the daily and total urinary flow. There was an increase in glutathione peroxidase activity in the kidneys of the 250 mg/kg AAc group. These data suggest that *Acrocomia aculeata* altered murinometric parameters related to body weight gain and water intake with a consequent reduction in urea levels possibly caused by the diuretic effect of the fruit. It improved oxidative parameters, evidenced by the increase in the glutathione peroxidase activity of antioxidant enzymes, improving oxidative stress. |

*Keywords: Acrocomia aculeata, kidney, rats, antioxidants system*

1. INTRODUCTION

The palm tree family (Arecaceae) comprises approximately 2,600 species (Lorenzi and Negrelle, 2006). *Acrocomia aculeata* (Jaqc.) Lodd. ex Mart. is a palm tree native to the Brazilian Cerrado and Pantanal regions, where it is particularly abundant in the state of Mato Grosso (MT). Its fruits, commonly known as bocaiuva or macaúba, are valued for their diverse applications, ranging from food uses to their emerging role as a raw material for biofuels (Hiane *et al.*, 2003; Duque *et al.*, 2025). Bocaiuva pulp is rich in carbohydrates, fiber, lipids, and proteins, and also contains a high concentration of bioactive compounds (Lorenzi and Negrelle, 2006; Lescano *et al*., 2015), particularly polyunsaturated fatty acids such as oleic acid (Hiane et al., 2006; Schex *et al*., 2018).

Studies indicate that *A. aculeata* pulp has a high carotenoid content, particularly β-carotene (dos Santos Correia *et al.*, 2022a) Moreover, its pulp flour is rich in phenolic compounds (Correia *et al.*, 2024a), making the fruit a natural source of antioxidants. These bioactive compounds exhibit antioxidant and anti-inflammatory properties, contributing to the prevention of chronic diseases (Correia *et al.*, 2024a). Antioxidants help neutralize reactive oxygen species (ROS), thereby reducing the risk of various pathologies.

Research on the oil extracted from the pulp of this fruit has demonstrated significant anti-inflammatory and diuretic potential (Lescano *et al.*, 2015; Jacobowski *et al.*, 2021). Microencapsulated oil has exhibited antiedematogenic and diuretic activities (Lescano *et al.*, 2015). Additionally, studies have highlighted the antioxidant properties of macaúba oils and their coproducts—such as pulp cake, leaves, and epicarp—through in vitro assays. The anti-inflammatory effects are closely associated with the phytochemicals present in pulp, potentially mediated by phenolic compounds (dos Santos Correia *et al.*, 2022a).

Previous studies conducted in our laboratory demonstrated that the mesocarp of *Acrocomia aculeata* from the state of Mato Grosso (MT) contains high levels of lipids, carbohydrates, phenolic compounds, and flavonoids (dos Santos Correia *et al.*, 2022a). These bioactive compounds, present in the fruit pulp, play a crucial role in its antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. The antioxidant properties of *A. aculeata* pulp (ACP) were confirmed in vivo through rodent studies (dos Santos Correia *et al.*, 2022a). After 28 days of pulp solution intake at doses of 250 and 500 mg/kg body weight, an improvement in antioxidant enzyme activity and a reduction in oxidative stress in the liver were observed. However, pulp consumption also led to changes in body composition, biochemical and metabolic parameters, oral glucose tolerance, and components of energy balance (dos Santos Correia *et al.*, 2022a).

On the other hand, animals treated with a 500 mg/kg dose of *A. aculeata* pulp (ACP) consumed more food and energy, which led to increased energy gain and lipid accumulation, while carcass protein content decreased. These animals also exhibited elevated total hepatic lipid levels and reduced PPARα expression, suggesting that impaired fatty acid utilization in the liver may contribute to hepatic lipid deposition (Correia *et al.*, 2024a).

Moreover, supplementation with 500 mg/kg ACP altered carbohydrate metabolism. From day 15 onwards, blood glucose concentrations during the absorptive phase were significantly elevated and remained high through day 28. In the oral glucose tolerance test, the 500 mg/kg ACP group displayed a more pronounced glycemic peak at 30 minutes and a greater area under the curve (AUC), indicating diminished glucose tolerance in these animals (Correia *et al.*, 2024a).

Conversely, animals receiving 250 mg/kg ACP showed improvements in the glutathione antioxidant system, with no significant changes in metabolic parameters such as glucose tolerance or energy balance (Correia *et al.*, 2024a). Given these findings and the reported diuretic potential of oil extracted from *A. aculeata* pulp, it is therefore pertinent to investigate renal metabolism in ACP‐treated animals.

Acting as both an antiedematogenic and diuretic agent, *A. aculeata* pulp may have significant therapeutic potential in the treatment of kidney disorders. The kidney is a vital organ with essential metabolic and endocrinological functions, including blood filtration and homeostasis maintenance through the removal of toxins and other substances via urine (Irazabal and Torres, 2020). Moreover, it is highly susceptible to oxidative damage. Acute renal failure occurs when there is a temporary, partial, or total loss of kidney function, whereas chronic renal failure refers to irreversible and permanent insufficiency. Kidney dysfunction alters specific plasma biomarkers, most notably urea and creatinine (Zsom *et al.*, 2022). Reduced kidney function is characterized by a glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m² or the presence of kidney damage markers, such as albuminuria (albumin-to-creatinine ratio ≥ 30 mg/g) (Zhang and Parikh, 2019).

Oxidative stress plays a key role in the progression of various kidney diseases(Ratliff *et al.*, 2016a; Irazabal and Torres, 2020). The excessive production of reactive species is the primary driver of oxidative stress, resulting in an imbalance between antioxidants and pro-oxidants. The kidneys are particularly susceptible to redox imbalances and oxidative stress, as reactive oxygen species (ROS) significantly influence the physiological regulation of renal function (Dennis and Witting, 2017; Irazabal and Torres, 2020). Consequently, studies investigating antioxidant agents present promising therapeutic strategies for the treatment and prevention of kidney diseases. This research aims to assess renal function parameters in rats following the ingestion of *A. aculeata* pulp. Our hypothesis is that the high concentration of total phenolic compounds in the fruit pulp (dos Santos Correia *et al.*, 2022a) may enhance the redox system and improve renal function markers. To test this hypothesis, we evaluated the effects of *A. aculeata* pulp consumption on i) murinometric, ii) biochemical, and iii) oxidative parameters.

2. methodology

**2.1 Collection and preparation of the fruit**

The exsiccata of botanical material from *A. aculeata* is registered in the herbarium of the Federal University of Mato Grosso under number 44.463. The fruit collections took place in the state of Mato Grosso, in the municipality of Poconé (16° 16’21.6” S 56° 37’34.5” W, Poconé-MT). After collection, they were sanitized and pulped. The pulp was frozen at a temperature of -20°C and subsequently freeze-dried until a constant weight. Then, the pulp was crushed until completely ground.

**2.2 Animals and treatment**

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The experimental protocol was approved by the Ethics Committee of the Federal University of Mato Grosso (UFMT) (nº 23108.013689/2019-58). Male Wistar rats *(Rattus norvegicus)* (6-10 animals/group), weighing approximately 200 g at 8 weeks age, from the Central Animal House of UFMT, were randomly divided in two groups: Control Group (C) and 250 mg/kg *A. aculeata* pulp group (250Aac Group). Control group received the vehicle (distilled water), while group 250Aac received the aqueous solution of the *A. aculeata* pulp by orogastric gavage. The chosen dose was based on previous studies with fruit pulp developed by Correia et al. (2024) (Correia *et al.*, 2024a)The animals were fed with commercial Labina® food and water *ad libitum* and kept in individual cages, in a 12-hour light-dark cycle and at a temperature of 22 ± 2°C. Water intake, food consumption, urinary volume and body weight were monitored daily for 28 days. At the end of the treatment, the animals were euthanized, and the kidneys, blood and urine samples were collected for oxidative and enzymatic analyses. To calculate the total intake of phenolic compounds ingeriada a partir da polpa da *A. aculeata*, a concentration of 50 mg of the gallic acid equivalent by 100g of *A. aculeata* pulp was considered(dos Santos Correia *et al.*, 2022b) and estimated from the dose (250 mg of pulp) and total body weight in kilograms.

**2.3 Total lipid, cholesterol and triglyceride contents in the kidney**

"The total lipid content was extracted from the kidney using the Folch method (Folch, Lees and Sloane Stanley, 1957). Cholesterol and triglyceride contents were determined by a spectrophotometric method using commercial kits (Quibasa, Belo Horizonte, Minas Gerais, Brazil)."

**2.4 Biochemical parameters in urine, serum and plasma**

"Serum and urinary concentrations of urea and creatinine were determined by an enzymatic-colorimetric method using specific commercial kits. (Labtests®, Gold Analisa® and BioTécnica® Kits).

**2.5 Calculation of daily creatinine clearance**

Daily creatinine clearance (DCD) was performed using the formula: DCD= [U/P x V/min], where, U: urinary creatinine concentration (mg/dL), P: plasma creatinine concentration (mg/dL), V: urine volume (mL) and min: urine collection time. Data were expressed in mL.min-1 (Kanaan, S. *et* *al*. 2014).

$$Creatinine clearance =\frac{Urinary cretinine \left(mg.ml^{-1}\right)}{Serum creatinine \left(mg.ml^{-1}\right) } × \frac{Urinary volume}{1440} $$

**2.6 Kidney antioxidant activity**

**2.6.1 Determination of oxidative damage markers**

The measurement of the thiobarbituric acid reactive species contents (TBARS) were carried out using the colorimetric method by Percário & Vital (PERCARIO, VITAL and JABLONKA, 1994). Its content of malondialdehide (MDA) was determin in 535 nm and express in µmol MDA g-1 of tissue. Carbonyl proteins concentration was determin according to Odetti(Odetti, 1996). Content of Carbonil was determined in 370nm and express in nmol protein carbonyl. mg-1 of protein. The proteins were measured according to the method of Bradford (Bradford, 1976).

**2.6.2 Enzymes antioxidants activity**

Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) were determined in 250mg of the kidney homogeneizated in sodium phosphate buffer (0.1 mol. L-1, pH 7.0, with EDTA 1 mmol. L-1, DTT 3 mmol.L-1 and PMSF 1 mmol.L-1), after centrifugation at 1000 g for 10 minutes at 4°C. The activity of the enzymes SOD, GPx and GR were determined using commercial Randox spectrophotometric kits (Randox Laboratories Ltd., Antrim, UK). The results were expressed in U.mg-1 of proteins. To determine catalase activity, a homogenate was prepared using 20 mg of kidney in Tris-HCl buffer (50 mmol. L-1, pH 7.4) after centrifugation according to method described by AEBI, H. (Aebi, 1984) using hydrogen peroxide (0.3M). The results were expressed as U.mg of proteins. The proteins in were measured according to the method of Bradford (Bradford, 1976).

**2.7 Statistical analysis**

Results were presented as mean and mean standard error. Statistical analyzes were performed after data normality using Kolmogorov-Smirnov test. Comparisons were made using Student-t test, with a significance level of p<0.05.

3. results and discussion

This study aimed to evaluate the effects of ingesting 250 mg/kg of *A. aculeata* pulp on renal function and redox parameters in the kidneys. *A. aculeata* is a palm species widely distributed in the Central-West region of Brazil, and its fruit is notable for its high content of starch, fiber, lipids, and phenolic compounds, as well os its strong antioxidant potential, as demonstrated in both in vitro (dos Santos Correia *et al.*, 2022a) and in vivo (Correia *et al.*, 2024b) studies. Previous research by our group observed effects on hepatic antioxidant enzymes in animals administered 250 and 500 mg/kg of *A. aculeata* pulp. However, in the group receiving 500 mg/kg, animals exhibited increased energy intake and body fat accumulation, along with reduced hepatic PPARα expression, which may contribute to impaired lipid metabolism in the liver (Correia *et al.*, 2024b).

In the presente study, treated group ingest 1.02 ± 0.04 mg galic acid equivalent related to 250mg/kg of *A aculeata*. The ingestion of *A. aculeata* at a dose of 250 mg/kg for 28 days reduced weight gain and water intake in rats (Fig. 1). A 12% reduction in the area under the curve for weight gain was observed, along with a 28% decrease in total weight gain in the 250AAc group. Food intake remained unchanged.



**Figure 1 - Effect of 250 mg/kg *A. aculeata* supplementation on body weight curve (A, B and C), water intake (D and E) and food intake (F).**  **Data are presented as mean ± SEM, n = 6–8 rat/group. \*P<0.05 versus control**.

Male and female rats (Wistar) received by gavage 2000 mg/kg of oil extracted from the pulp of *A. aculeata* (OPAC) for the acute toxicity test and 125, 250, 500 or 1000 mg/kg of OPAC for subacute toxicity tests demonstrate the absence of acute and subacute toxicity after oral exposure to *A. aculeata* oil in rats for 28 days (Traesel *et al.*, 2014), as well absence of toxicity in terms of cytotoxicity, genotoxicity, and mutagenicity (Traese *et al.*, 2015).

Plasma creatinine and urea concentrations showed no statistically significant differences in the 250AAc group compared to the control. Similarly, daily creatinine clearance and urinary creatinine levels remained unchanged (Fig. 2). However, animals receiving 250 mg/kg of *A. aculeata* pulp for 28 days exhibited an 18% reduction in urinary urea compared to the control.



**Figure 2 - Effect of 250 mg/kg *A. aculeata* supplementation on plasma urea (A) and creatinine (B), urinary urea (C) and creatinine (D) and Creatinine Clearence (E).**  **Data are presented as mean ± SEM, n = 6–8 rat/group. \*P<0.05 versus control**.

Urea is closely associated with liver and kidney functions. Its synthesis depends on factors such as dietary protein intake, gastrointestinal bleeding, and proteolysis (Bankir *et al.*, 1996). Alongside urea, creatinine is widely used to assess renal function. It can be measured in plasma and urine, and serves as a marker of glomerular filtration rate (GFR) through creatinine clearance. This is because creatinine—a catabolic product of creatine—is completely excreted by the kidneys, is not reabsorbed, and undergoes minimal secretion. As a result, serum creatinine concentration is inversely proportional to GFR (Bankir *et al.*, 1996; Zsom *et al.*, 2022)

As urea is also a marker of liver function, the reduction in urinary concentration may be related to a reduction in its synthesis in the liver. However, this is a hypothesis that needs further investigation.

Supplementation with 250 mg/kg of *A. aculeata* for 28 days did not affect total or daily urinary flow, nor did it alter total or relative kidney weight compared to the control group. Additionally, no statistically significant differences were observed in the plasma or urinary urea/creatinine ratio (Table 1).

**Table 1. Urinary flow, kidney weight and urea/creatinine ratio of rats treated with 250 mg/kg *A. aculeata* for 28 days.**

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| **Parameters** | **Groups** |
| **Control** | **250AAc** |
| **Total Urinary Flow (mL (100 g)-1)** | 87.0 ± 3.0 | 95.8 ± 2.0 |
| **Daily Urinary Flow (mL (100 g)-1)** | 3.1 ± 0.2 | 3.3 ± 0.2 |
| **Kidney (g)**  | 2.84 ± 0.14 | 2.80 ± 0.09 |
| **Kidney (g (100 g)-1)** | 0.79 ± 0.01 | 0.88 ± 0.03 |
| **Plasmatic urea/creatinine ratio** | 136.9±22.3 | 117.3±39.9 |
| **Urinary urea/creatinine ratio**  | 57.8±4.5 | 53.0±4.3 |

*Values are expressed as means ± SEM (n=8); Student’s T test.*

Administration of 250 mg/kg of *A. aculeata* pulp for 28 days did not alter total lipid content, triglycerides, or cholesterol levels in the kidneys compared to the control group (Table 2). However, total protein content in the kidneys was statistically reduced by 19% in rats treated with *A. aculeata* pulp at this dosage.

**Table 2. Kidney lipids and protein of rats treated with 250 mg/kg A. aculeata for 28 days.**

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| --- | --- |
| **Parameters** | **Groups** |
| **Control** | **250AAc** |
| **Total lipids (mg g-1 of tissue)** | 32.16 ± 1.81 | 31.31 ± 4.12 |
| **Triglycerides (mg g-1 of tissue)**  | 4.49 ± 0.29 | 4.26 ± 0.48 |
| **Total cholesterol (mg g-1 of tissue)** | 3.01 ± 0.12 | 3.02 ± 0.17 |
| **Total Protein (mg g-1 of tissue)** | 3.94 ± 0.21 | 3.20 ± 0.08**\*** |

*Values are expressed as means ± SEM (n=8).\* P<0.05 versus control Student’s T test.*

Water intake may be influenced by thirst-regulatory mechanisms or osmotic balance, resulting in increased water retention (Franci, 1994). Although urinary flow remained unchanged, both water intake and urinary urea excretion were reduced. We believe that since urinary urea is the final product of protein metabolism, differences in protein handling could explain the lower urinary urea levels. Notably, despite equivalent protein ingestion between groups, both urinary urea excretion and total kidney protein content were decreased.

Conversely, the reduced water intake—coupled with unchanged urinary output—in the treated group may reflect the diuretic properties of *A. aculeata* pulp, as reported by other investigators working with this species (Lescano *et al.*, 2015; Correia *et al.*, 2024a). Furthermore, the decreased urinary urea concentration, without any alteration in plasma urea levels, corroborates this interpretation. In Figure 3, no statistically significant differences were observed in the levels of carbonylated proteins (3A) or TBARS (3B). Regarding antioxidant status, the enzymatic activity of catalase (3C), superoxide dismutase (3D), and glutathione reductase (3F) remained unchanged in the 250AAc group compared to the control. However, the activity of glutathione peroxidase increased by 52% in the kidneys of rats receiving 250 mg/kg of *A. aculeata* pulp for 28 days.

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**Figure 3 - Effect of 250 mg/kg *A. aculeata* supplementation on kidney malondialdehyde (A), Carbonylated proteins (B), Catalase activity (C), Superoxide Dismutase activity (D), Glutathione peroxidase activity (E) and Glutathione Reductase activity (F).**  **Data are presented as mean ± SEM, n = 5-6 rat/group. \*P<0.05 versus control**.

Oxidative stress arises from an imbalance between the production of reactive species and antioxidant defenses (Sies, 2015). Reactive oxygen species (ROS) are generated as byproducts of biochemical reactions taking place in the mitochondria, plasma membranes, peroxisomes, and the endoplasmic reticulum membrane. To protect cell membranes from ROS-induced damage, cells employ a defense system composed of both enzymatic and non-enzymatic antioxidants. In the renal tubules, ROS formation can cause local damage and contribute to pathologies such as glomerulosclerosis and tubulointerstitial fibrosis (Ratliff *et al.*, 2016b).

Animals supplemented with 250 mg/kg of *A. aculeata* exhibited increases only in hepatic GPx and GR activities, while the activities of the other enzymes remained unchanged (Correia *et al*., 2024a). In our study, we observed an increase in GPx activity in the kidneys of animals that received 250 mg/kg. Glutathione peroxidase (GPx) plays a critical role in the antioxidant defense by catalyzing the reduction of hydrogen peroxide to water, thereby neutralizing its cytotoxic effects and preventing oxidative damage. Conversely, GPx inactivation or diminished activity can disrupt redox homeostasis and impair cellular functions (Miyamoto *et al.*, 2003). In that way, the enhancement of the renal enzymatic antioxidant system suggests an improvement in the REDOX balance, which may be attributed to the regular, moderate intake of dietary phenolic compounds. In fact, the ingestion of phenolic compounds has been shown to stimulate the cellular defense system by increasing the synthesis of endogenous antioxidant enzymes (Forester and Lambert, 2011; D’Arcy, 2020), as also observed in patients with kidney disorders (Dennis and Witting, 2017).

4. Conclusion

In summary, regular ingestion of *A. aculeata* pulp resulted in reduced weight gain and water intake, which in turn led to lower urinary urea excretion, while urinary volume and creatinine clearance remained unchanged, indicating preserved renal function. Moreover, the increased glutathione peroxidase activity observed in the 250 mg/kg ACP group demonstrated enhanced enzymatic antioxidant capacity in the kidneys, thereby preventing the onset of oxidative stress.

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