

The Effect of Soursop (*Annona muricata* L.) Leaf Extract on Hepatic Histopathology and Hepatocyte Index in *Rattus norvegicus* Rats with Diabetes Mellitus: An Experimental Study

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The Effect of Soursop (*Annona muricata* L.) Leaf Extract on Hepatic Histopathology and Hepatocyte Index in *Rattus norvegicus* Rats with Diabetes Mellitus: An Experimental Study

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ABSTRACT

Background: The oxidative stress caused by diabetes mellitus (DM) damages hepatocytes and reduces liver function. Flavonoids and acetogenins found in soursop (*Annona muricata* L.) leaves have hepatoprotective and antioxidant qualities. The purpose of this study was to assess how soursop leaf extract affected the hepatocyte index and hepatic histopathology in diabetic Wistar rats.

Methods: In a laboratory experiment, 16 male Wistar rats weighing 100–150 g and aged 2-3 months were given a high-fat diet and an injection of streptozotocin (27.5 mg/kg BW) to induce type 2 diabetes. The rats were placed in a randomized posttest-only control group. For 21 days, rats were split into two groups at random: a control group (n = 8) that received aquadest, and a treatment group (n = 8) that received soursop leaf extract at a dose of 100 mg/kg BW. Hematoxylin-eosin staining was used to analyze hepatic histopathology, and a quantitative evaluation of the hepatocyte index was conducted. The independent t-test and Mann Whitney U test were used to assess the data.

Results: There was a significant difference ($p=0.041$) in the hepatocyte index between the treatment and control groups. The treatment group had less hepatocyte deterioration than the control group, according to histopathological scoring ($p=0.037$).

Conclusion: Diabetic Wistar rats treated with soursop leaf extract showed better hepatocyte index and decreased hepatic degeneration, suggesting hepatoprotective potential.

Keywords: *Annona muricata*, soursop leaf extract, hepatocyte index, hepatic histopathology, diabetes mellitus

ABSTRAK

Latar belakang: Stres oksidatif akibat diabetes melitus (DM) merusak hepatosit dan menurunkan fungsi hati. Flavonoid dan asetonin yang terkandung dalam daun sirsak (*Annona muricata* L.) memiliki sifat hepatoprotektif dan antioksidan. Tujuan penelitian ini adalah untuk mengkaji pengaruh ekstrak daun sirsak terhadap indeks hepatosit dan histopatologi hati pada tikus Wistar diabetes.

Metode: Dalam sebuah percobaan laboratorium, 16 tikus Wistar jantan dengan berat 100–150 g dan berusia 2-3 bulan diberi diet tinggi lemak dan suntikan streptozotocin (27,5 mg/kg BB) untuk menginduksi diabetes tipe 2. Tikus-tikus tersebut ditempatkan dalam kelompok kontrol acak pasca-uji saja. Selama 21 hari, tikus dibagi menjadi dua kelompok secara acak: kelompok kontrol ($n = 8$) yang menerima akuades, dan kelompok perlakuan ($n = 8$) yang menerima ekstrak daun sirsak dengan dosis 100 mg/kg BB. Pewarnaan hematoksilin-eosin digunakan untuk menganalisis histopatologi hati, dan evaluasi kuantitatif indeks hepatosit dilakukan. Uji-t independen dan Uji Mann Whitney U digunakan untuk menilai data.

Hasil: Terdapat perbedaan indeks hepatosit yang signifikan ($p=0,041$) antara kelompok perlakuan dan kelompok kontrol. Kelompok perlakuan mengalami degenerasi hepatosit yang lebih sedikit dibandingkan kelompok kontrol, berdasarkan skor histopatologi ($p=0,037$).

Kesimpulan: Tikus Wistar penderita diabetes yang diobati dengan ekstrak daun sirsak menunjukkan indeks hepatosit yang lebih baik dan penurunan degenerasi hati, yang menunjukkan potensi hepatoprotektif.

Kata kunci: *Annona muricata*, ekstrak daun sirsak, indeks hepatosit, histopatologi hepar, diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is a long-term metabolic disease marked by persistently high blood sugar levels brought on by deficiencies in either the action or secretion of insulin, or both. Increased production of reactive oxygen species (ROS) over time due to high blood glucose can

cause oxidative stress, which harms several organs. Because it is essential to the metabolism of fats and carbohydrates, the liver is particularly vulnerable in diabetes mellitus (DM); oxidative stress in DM is linked to hepatocyte degeneration, inflammatory infiltration, and liver function impairment. Research shows that diabetic rats given streptozotocin (STZ) exhibit significant hepatic histological alterations, including cytoplasmic vacuolization and disorganized hepatocyte organization.^{1,2}

The hepatocyte index, also known as hepatocyte integrity, is a crucial indicator of the health of liver tissue since it shows the number and state of functional hepatocytes. Hyperglycemia, weakened antioxidant defenses, and elevated lipid peroxidation are all correlated with decreased hepatocyte index and histological deterioration in diabetes animals.^{3,4} Antioxidant treatments have been investigated to lessen the liver damage brought on by diabetes mellitus. According to review studies, oxidative stress and inflammation play a major role in the processes that cause hepatic lesions in people with diabetes, and substances that can alter redox balance have the potential to preserve the structure and function of the liver.^{5,6}

Traditional medicine recognizes soursop (*Annona muricata* L.) for its hepatoprotective and antidiabetic properties. Bioactive substances found in its leaves, seeds, and fruits, including as flavonoids, acetogenins, and phenolic compounds, have been shown in a variety of animal models to have anti-inflammatory, antioxidant, and glucose-lowering properties. For instance, "Ameliorative Effect of *Annona muricata* Extract" reduced liver damage in diabetic mice by having protective histological effects.^{7,8} According to a different study, *A. muricata* seed methanol extract dramatically decreased hyperglycemia and shown hepatoprotective effects in Wistar rats with diabetes caused by alloxan.⁹ Fewer research, however, provide thorough evaluations of both histology and the quantitative hepatocyte index using standardized experimental design; most investigations concentrate on biochemical markers.

Thus, the purpose of this study is to examine how soursop leaf extract affects hepatic histopathology and hepatocyte index in a diabetic *Rattus norvegicus* model. This study aims to provide more solid data on whether *A. muricata* can lessen the hepatic alterations brought on by diabetes mellitus by employing a randomized post-test only control group design.

METHOD

Study Design

This study used a randomized posttest-only control group design and was conducted entirely in a laboratory. The investigation was carried out at Udayana University's Faculty of Medicine's Integrated Biomedical Laboratory.

Experimental Animals

In all, sixteen healthy male Wistar rats (*Rattus norvegicus*) weighing between 100 and 150 g and two to three months of age were used in this investigation. Healthy appearance, age between two and three months, and body weight within the specified range were requirements for admission. Rats that fell ill or passed away during the trial were not allowed to participate (dropout criteria). The animals were kept in wire-topped plastic cages of 30 by 40 by 40 cm, with a 12-hour light/dark cycle and a temperature of 30 ± 1 °C. They have unlimited access to food and drink. Prior to the trial, all animals were given a week to acclimate.

Induction of Diabetes Mellitus

Rats were given a high-fat diet and then given a single intraperitoneal injection of streptozotocin (STZ) at a dose of 27.5 mg/kg body weight, dissolved in 0.1 M citrate buffer (pH 4.5), to develop type 2 diabetes mellitus. A glucometer was used to measure fasting blood glucose levels in order to verify the induction of diabetes.

Grouping and Treatment

Rats were divided into two groups of eight at random:

- Control group (K): received aquadest orally (placebo) for 21 days.
- Treatment group (P): received soursop (*Annona muricata* L.) leaf extract orally at a dose of 100 mg/kg body weight for 21 days.

Histopathology and Hepatocyte Index Examination

Animals were put to sleep with ketamine (60 mg/kg BW, intramuscular) on day 59, and they were then beheaded as sacrifices. After being removed, the livers were embedded in paraffin, fixed in 10% buffered formalin, sectioned at a thickness of 4-5 µm, and stained with hematoxylin-eosin. Hepatocyte index: quantitatively determined at various fields of view using light microscopy. Hepatocyte degeneration was scored histopathologically as follows: 4 (very severe, >75%), 2 (moderate, 26–50%), 3 (severe, 51–75%), and 0 (none).

Statistical Analysis

The Shapiro-Wilk test was used to check the data for normality. An independent t-test was used to compare the control and treatment groups based on normally distributed data (hepatocyte index). Descriptive presentation was used for non-normally distributed data (histopathological scores). P-values less than 0.05 were regarded as statistically significant.

RESULT

Hepatocyte Index

In comparison to the control group, the treatment group's mean hepatocyte index was greater. An independent t-test statistical analysis revealed a significant difference between the groups ($p = 0.041$), suggesting that giving diabetic rats leaf extract from *Annona muricata* enhanced the integrity of their livers.

Histopathological Findings

Histological analysis showed that the control group had significant degenerative abnormalities, including disordered hepatic cords, nuclear modifications, and cytoplasmic vacuolization (Figure 1A). The therapy group, on the other hand, showed less hepatocyte degradation, more normal hepatocyte shape, and comparatively intact hepatic architecture (Figure 1B).

Table 1 summarizes the distribution of hepatocyte degeneration scores. While the treatment group showed a shift toward milder degeneration (scores 1–3), the majority of rats in the control group displayed very severe degeneration (score 4). A significant difference between the groups was confirmed by the Mann-Whitney U test ($p = 0.037$).

To better visualize the differences between groups, Figure 2 presents the comparison of hepatocyte degeneration scores as a bar chart. In line with the preventive effect of sour sop leaf extract, the treatment group displayed a greater distribution throughout mild to severe categories, whereas the control group primarily displayed extremely severe deterioration.

Table 1. Hepatocyte degeneration score distribution between treatment (P) and control (K) groups

Degeneration score	Control (K), n (%)	Treatment (P), n (%)
0 (jaringan normal)	0 (0%)	0 (0%)
1 (mild, ≤25%)	0 (0%)	1 (12,5%)
2 (moderate, 26–50%)	0 (0%)	1 (12,5%)
3 (severe, 51–75%)	2 (25%)	4 (50%)
4 (very severe, >75%)	6 (75%)	2 (25%)
Total	8 (100%)	8 (100%)

Mann–Whitney U test showed a significant difference in hepatocyte degeneration scores between groups ($p = 0.037$). K = control; P = treatment.

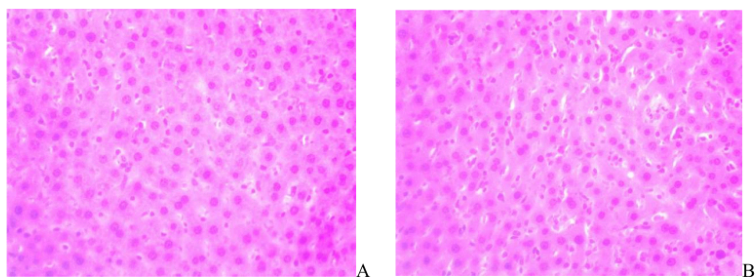


Figure 1. Histopathological features of the liver (H&E staining, 40×).

(A) Control group: This histological appearance is consistent with moderate hepatocyte degeneration in the diabetic condition without therapeutic protection. The observed changes, including cloudy swelling, cytoplasmic vacuolization, and lobular disorganization, indicate the presence of significant oxidative stress.

(B) Treatment group: This histological appearance shows hepatocytes in a relatively better condition, with only mild degeneration (minimal cloudy swelling). This finding can be interpreted as a more evident protective effect of the extract therapy in this group.

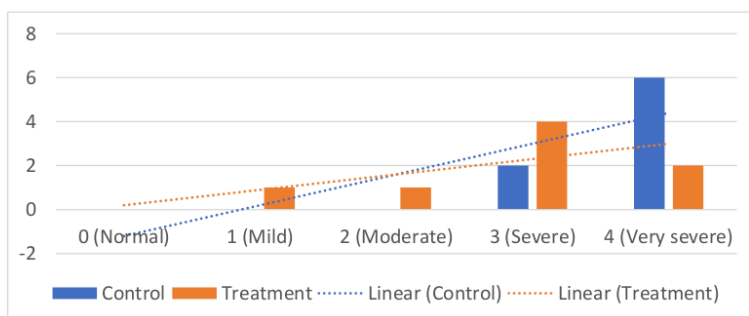


Figure 2. Comparison of hepatocyte degeneration scores between groups.

The bar chart illustrates that most control rats exhibited very severe degeneration (score 4), while the treatment group displayed a shift toward milder scores (1–3).

DISCUSSION

An improvement in the hepatocyte index and a decrease in the hepatocyte degeneration scores show that the leaf extract of *Annona muricata* considerably reduced the hepatic damage in diabetic rats. These results support and add to earlier studies on *A. muricata*'s hepatoprotective properties in both diabetic and non-diabetic mice.

In 2021, a study titled "Ameliorative Effect of *Annona muricata* Extract on Hyperglycemia Induced Hepatic Damage in Type 2 Diabetic Mice" examined the effects of 50 or 100 mg/kg BW of *A. muricata* extract over a nine-week period in diabetic mice that were fed a high-fat diet and STZ. According to the study, the extract improved insulin signaling (IRS-1, GLUT2), decreased oxidative stress markers (4-HNE, protein carbonyls), and improved hepatic morphology, including decreased lipid droplets and improved lipophagy (p-AMPK, LC3 conversion), in addition to lowering fasting blood glucose and HbA1c.⁸ This lends credence to the notion that *A. muricata* protects hepatocytes by regulating their metabolism and maintaining their structural integrity.

An additional pertinent study is *Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation* (2018), which demonstrates how long-term hyperglycemia causes lipid peroxidation (MDA, TBARS), elevated ROS, and activation of inflammatory cytokines (TNF- α , IL-6, IL-1 β), all of which contribute to hepatocyte apoptosis, degeneration, and eventually liver dysfunction.¹⁰

In addition, the 2015 study "Protective effects of *A. muricata* Linn. leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of STZ-treated diabetic rats" discovered that *A. muricata* extracts enhanced antioxidant enzyme activities (SOD, CAT, GSH), reduced ROS, TBARS, and improved lipid profiles (lower TG, LDL), and raised serum insulin in STZ-induced diabetic subjects.¹¹ The histological improvements seen in this study, where decreased hepatocyte degeneration and improved hepatocyte index suggest structural preservation as downstream effects of reduced oxidative damage and improved metabolic management, are probably connected to this biochemical protection.

Mechanistically, the effects seen in this study could be explained by multiple pathways:

- Oxidative stress reduction: Through glucose auto-oxidation, mitochondrial failure, NADPH oxidase activation, advanced glycation end products (AGEs) binding to RAGE, and other mechanisms, hyperglycemia increases the production of free radicals. The imbalance is exacerbated by decreased antioxidant defenses (such as GSH, SOD, and CAT). *A. muricata* seems to upregulate these antioxidant systems, reducing lipid peroxidation (TBARS, MDA) and ROS, thereby preventing damage to cellular membranes and organelles in hepatocytes.^{12,13}
- Control of inflammation: Increased ROS frequently sets off inflammatory reactions with cytokines (TNF- α , IL-6, and IL-1 β), which either directly or indirectly cause hepatocyte damage by attracting immune infiltrates. Certain research (such as Effect of Graviola and Ginger extract) demonstrate that *A. muricata* alters the production of apoptotic proteins (Bax, Bcl-2, and p53) and inflammatory markers in the liver.¹⁴
- Better insulin signaling (IRS-1, GLUT2), decreased insulin resistance, decreased HbA1c, and enhanced lipid metabolism (less steatosis/lipid droplets) all contribute to better

metabolic regulation, which lessens lipotoxic stress in hepatocytes. Similar to the 2021 study, *A. muricata* also improved energy balance through AMPK, which is known to shield hepatic cells, and the expression of autophagy markers (lipophagy).^{8,15}

- Potential function of cell survival/death pathways although apoptosis and pyroptosis markers were not measured in this study, other recent research suggests that hyperglycemia can cause ischemia-reperfusion injury, hepatocyte pyroptosis (through the NLRP3 inflammasome), and inhibition of protective pathways such as Nrf2 and mitophagy in diabetic livers.^{16,17} These could be pertinent to additional research on *A. muricata*.

Limitations and Suggestions

It is important to acknowledge the limitations of this study. First, any dose-response effects were not investigated because just one dosage of *A. muricata* leaf extract (100 mg/kg BW) was administered. Second, hematoxylin–eosin staining was used for histological examination. This method is sufficient for identifying hepatocyte degeneration, but it does not offer molecular-level information.

Future studies could further clarify the mechanisms behind *A. muricata*'s hepatoprotective benefits by evaluating various dosages and utilizing supplementary methods like immunohistochemistry (e.g., caspase-3, TGF- β). Longer observation times might also yield more details about the extract's capacity to stave against chronic liver damage.

Implications

These findings suggest that leaf extract from *A. muricata* may be used as a hepatoprotective adjuvant in diabetic patients. Its antioxidant, anti-inflammatory, and metabolic modulatory properties may help stop or slow the course of diabetes-induced liver damage. Though further research is required in the areas of safety, pharmacokinetics, human trials, and extract standardization (active components), these findings suggest potential therapeutic advancement in herbal medicine.

CONCLUSION

By significantly improving the hepatocyte index and reducing histological degeneration in comparison to controls, this study shows that *Annona muricata* leaf extract has hepatoprotective benefits in streptozotocin-induced diabetic rats. These results imply that *A. muricata*'s anti-inflammatory and antioxidant qualities may protect hepatocyte integrity in the face of oxidative stress and hyperglycemia. The therapeutic potential of *A. muricata* as a supplemental method in protecting the liver against diabetes-induced harm requires more research with longer treatment durations, dose-response evaluations, and molecular analysis.

CONFLICT OF INTERESTS

The authors affirm no conflict of interest in this study.

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