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Article

Antihyperglycemic Activity of Methanolic *Annona muricata* L. Leaf Extract Nanoemulsion in Alloxan Induced Mice

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Abstract. Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and oxidative stress-related organ damage, including liver injury. This study evaluated the antihyperglycemic activity and liver histopathological effects of a nanoemulsion containing methanolic extract of Annona muricata L. leaves in alloxan-induced rats. The nanoemulsion exhibited an average droplet size of 13.96 nm and a polydispersity index of 0.216. Thirty-five male rats were divided into seven groups: normal control, negative control, positive control (glibenclamide), nanoemulsion-treated groups (140 and 280 mg/kg body weight), and conventional extract-treated groups at the same dose. Blood glucose levels were measured on days 0, 3, and 17, while liver histopathology was evaluated using hematoxylin-eosin staining. Alloxan induction caused severe hyperglycemia and liver damage characterized by congestion, inflammatory cell infiltration, vacuolization, fatty degeneration, and necrosis. Both nanoemulsion and conventional extract treatments significantly reduced blood glucose levels compared with the negative control. Nanoemulsion at 280 mg/kg body weight produced the greatest antihyperglycemic effect, reducing blood glucose levels by 79.8%, whereas the conventional extract reduced them by 77.1% at the same dose. Histopathological findings also demonstrated improved liver structure and reduced tissue damage, particularly in nanoemulsion-treated animals. These results suggest enhanced biological performance and therapeutic potential.

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1. Introduction

Non-communicable diseases (NCDs) continue to be a significant global health issue, Diabetes Mellitus (DM) being one of the primary contributors to morbidity and mortality worldwide, including Indonesia [1]. Diabetes mellitus (DM) is a chronic metabolic disorder caused by deficiency in insulin secretion, decreased insulin sensitivity, or both, resulting in a persistent state of hyperglycemia. This condition not only affects glucose metabolism, but also contributes to damage to various target organs through the mechanisms of oxidative stress and chronic inflammation. Uncontrolled hyperglycemia triggers serious complications, such as microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (cardiovascular diseases), which are the leading cause of death in patients with diabetes [2-3]. Among the affected organs, the liver plays a central role in glucose regulation through gluconeogenesis and glycogenolysis; thus, secondary liver damage from hyperglycemia further exacerbates metabolic dysfunction. The multifactorial complexity of DM and its complications makes the development of more effective, safe, and accessible therapeutic strategies an ongoing clinical priority [4].

In recent decades, the use of medicinal plants as complementary therapy for diabetes mellitus has gained increasing attention. Natural products are widely studied due to their abundant availability and potential biological activities, although their effectiveness and safety still depend on the type of active compounds and the dosage used [5-6]. One plant that has been empirically used is Daun Sirsak (*A. muricata* L.), which are known to contain various secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and steroids. These compounds have been reported to have activities related to lowering blood glucose levels through various mechanisms, including increasing insulin sensitivity and modulating glucose metabolism [7-8]. The synergistic contribution of these phytochemicals supports the antihyperglycemic potential of A. muricata leaf extract as a whole-plant formulation.

However, the effectiveness of bioactive compounds from plants is often limited by their low water solubility, poor stability, and limited bioavailability after oral administration. These limitations can impact optimal therapeutic effects, necessitating formulation approaches that enhance the performance of these active compounds. One of the developing approaches is the nanoemulsion system, namely a dispersion system with droplet sizes in the nano range (20–200 nm) which is reported to increase the solubility and stability of several bioactive compounds and has the potential to improve absorption in biological systems [8-9]. Several studies have shown that nanoemulsion formulations can provide better biological responses than conventional extract forms, including in hyperglycemia models [10-11]. In addition to increasing bioavailability, the nanoemulsion system also has the potential to provide a more controlled release of active compounds and increase more even distribution to target tissues. This is relevant in DM therapy, considering that long-term treatment is often associated with limited drug absorption, fluctuations in glucose levels, and the risk of side effects such as hepatotoxicity and hypoglycemia due to the use of certain synthetic drugs [12-13].

A previously reported nanoemulsion formulation of methanolic A. muricata L. leaf extract (F1) exhibited favorable physicochemical characteristics, with a mean droplet size of 13.96 nm and a polydispersity index (PDI) of 0.216, indicating a homogeneous and stable colloidal system [14]. These parameters suggest that the formulation has a suitable size distribution to support enhanced bioavailability. However, direct experimental evidence regarding its antihyperglycemic efficacy and its effects on liver histopathological changes in a diabetes animal model remains limited and has not been systematically evaluated. Based on this, this study aims to evaluate the antihyperglycemic activity of a methanolic soursop leaf extract nanoemulsion and its association with changes in blood glucose

levels and liver histopathology in alloxan-induced mice. The results are expected to contribute to the development of a natural nanoemulsion-based delivery system as an alternative approach to diabetes therapy.

2. Research Method

2.1. Materials

In this study, the materials used were: Daun Sirsak (*Annona muricata L*), Methanol 96% (Merck), *Aquadest* (one lab), HCl 32% (merck), Mg powder (merck), H_2SO_4 2N, reagent Dragendroff, reagent Meyer and reagent Wagner, $FeCl_3$ 1%, CH_3COOH. The tools used are sample preparation: blenders, measuring flasks, measuring cups, rotary evaporators, dark glass bottles, filter paper, glass funnels, vials, analytical scales, spatulas, stirring rods, dropper pipettes, micropipettes, syringes 1 cc, oral feeding needles, Particle Size Analyzer (PSA) and light microscopes.

2.2. Preparation of Plant Extract and Nanoemulsion

Extract and nanoemulsion were obtained from previously optimized formulations and used without further modification [15].

2.3. Phytochemical Screening

Phytochemical screening was performed qualitatively to identify classes of secondary metabolites in the extract [15]. Flavonoid identification was performed using the Mg-HCl test, which is characterized by a color change from yellow to red. The alkaloids were tested using Dragendorff's, Mayer's, and Bouchardat's reagents, which yielded positive results through the formation of a white precipitate. Saponins were identified based on the formation of a stable foam after shaking and the addition of 2 N HCl. Tannins were tested using 1% $FeCl_3$, with a color change to green, blue, purple, or black serving as an indicator. Meanwhile, the identification of steroids and triterpenoids is performed using the Liebermann–Burchard reagent after extraction with n-hexane, characterized by a green color change for steroids and a red or purple color change for triterpenoids.

2.4. Antihyperglicemic Activity

This study used 35 male mice, which were divided into 7 groups, acclimated for 1 week in the laboratory, and fed a standard diet. The test animals were fasted for approximately 16 hours on day 0, after which their body weight was measured and their blood glucose levels were determined. Groups II–VII were administered an intraperitoneal injection of alloxan at a dose of 210 mg/Kg body weight. Subsequently, blood glucose levels were measured on day 3; animals with glucose levels ≥ 200 mg/dL were selected as test animals. The test animals were divided into several treatment groups, each consisting of five animals, and all treatments were administered orally.

Observations of test animals according to groups were carried out for 14 consecutive days. Blood glucose levels were measured on days 0, 3, and 17 after induction [16]. The percentage reduction in blood glukosa levels is calculated as follows:

$$\%Decreased = \frac{initial\ blood\ glucose\ level - final\ blood\ glucose\ level}{initial\ blood\ glucose\ level} \times 100\%$$

In addition, this research stage has also obtained approval from the Ethics Committee of the Faculty of Medicine and Health Sciences, University of Jambi (project number 1658/UN21.8/PT.01.04/2025).

Table 1. Treatment Group of Test Animals

Group	Treatment
Normal	not induced
K-	alloxan 210 mg/Kg body weight
K+	alloxan 210 mg/Kg body weight and Glibenclamide 5 mg/Kg body weight
N1	alloxan 210 mg/Kg body weight and nanoemulsion of extract sirsak leaf 140 mg/Kg body weight
N2	alloxan 210 mg/Kg body weight and nanoemulsion of extract sirsak leaf 280 mg/Kg body weight
E1	alloxan 210 mg/Kg body weight and extract of sirsak leaf 140 mg/Kg body weight
E2	alloxan 210 mg/Kg body weight and extract of sirsak leaf 280 mg/Kg body weight

Note: K- = Negative control, K+ = Positive Control, N1 = nanoemulsion dosage 140 mg/Kg body weight. N2 = nanoemulsion dosage 280 mg/Kg body weight, E1 = extract dosage 140 mg/Kg body weight, E2 = extract dosage 280 mg/Kg body weight.

2.5. Histopathology

Mice were euthanized through cervical dislocation after anesthesia with chloroform until they lost consciousness and showed no response to stimulation. A necropsy was then performed and the liver was carefully removed. The liver tissue was immediately placed in 10% Neutral Buffered Formalin (NBF) for 24 hours for fixation. After fixation, the tissue was cut to a thickness of approximately 0.2–0.4 cm and placed in a tissue cassette. The dehydration process was carried out in stages using 70%, 80%, 90%, 96%, and 100% ethanol, followed by clearing using xylene. The tissue was then infiltrated with paraffin and paraffin blocks were created through an embedding process. The paraffin blocks were cut using a microtome with a thickness of 4–7 μm, then the tissue slices were attached to a glass slide and stained with Hematoxylin-Eosin (H&E) for histopathological observation of the liver under a light microscope [17]. Then an examination was carried out using a microscope with 100X magnification and then continued with 400X magnification [18].

2.6. Data Analysis

Blood glucose measurement data were statistically analyzed using One-Way ANOVA (Analysis of Variance) to determine significant differences between treatment groups. If the ANOVA results showed a p-value <0.05, then a significant difference was considered, and post-hoc analysis was conducted using the Least Significant Difference (LSD) Test at a 5% significance level [19].

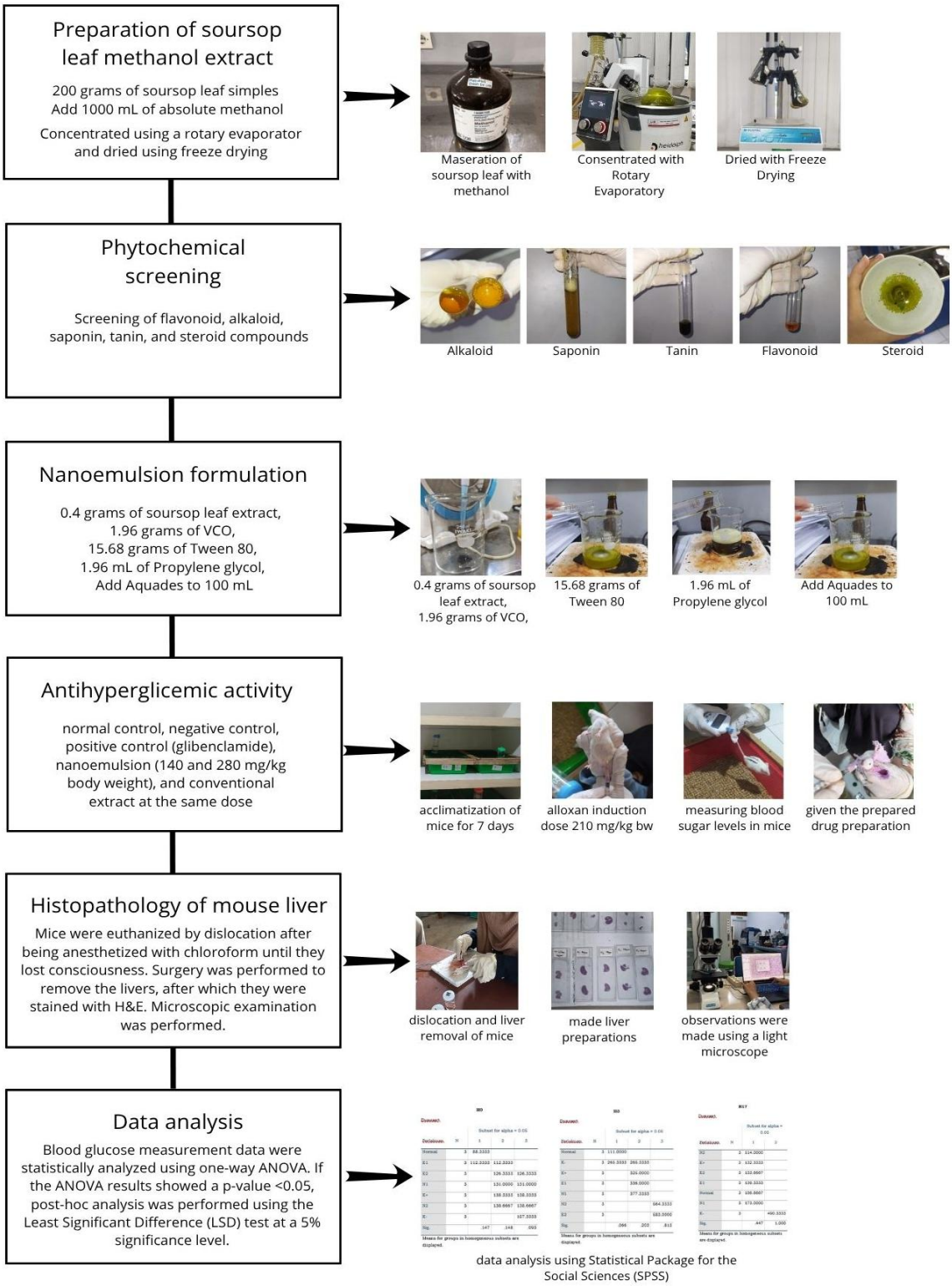


Figure 1. Schematic/Flowchart of Research

3. Results and Discussion

3.1 Phytochemical Screening and Nanoemulsion

Phytochemical screening of the methanol extract of A. muricata L. leaves was carried out qualitatively to identify the presence of major secondary metabolites. The results of the analysis showed the presence of flavonoids, alkaloids, saponins, tannins, and steroids (Figure 1). Identification of these compounds is based on characteristic color changes or the formation of precipitates upon the addition of specific reagents according to standard methods. However, the biological effectiveness of these compounds is often limited by their low water solubility and limited oral bioavailability. Therefore, the extract was formulated into a nanoemulsion system as an approach to improve its physicochemical characteristics and support its biological performance.

(a) (b) (c) (d) (e)

Figure 2. Phytochemical Screening Result of *A.muricata* L. Methanol Extract; (a) Flavonoids, (b) Alkaloids with Dragendroff Reagent, (c) Saponins, (d) Tannins, (e) Streoids.

In nanoemulsion formulations, VCO is used as the oil phase due to its good solubility with various bioactive compounds. Its hydrophobic nature allows the formation of nanoemulsion droplet cores, which can protect the active compounds from degradation due to environmental factors such as light, temperature, and digestive enzymes [20]. Tween 80 was chosen as the surfactant due to its excellent emulsifying capacity and its suitability for oil-in-water nanoemulsion systems. As a nonionic surfactant with a high hydrophilic-lipophilic balance (HLB ≈ 15), Tween 80 effectively reduces the interfacial tension between the oil and water phases, thus facilitating the formation of nano-sized droplets with better physical stability. The steric barrier formed by Tween 80 around the oil droplets can prevent coalescence and phase separation, thus maintaining the integrity of the nanoemulsion during storage and gastrointestinal transit [21].

The nanoemulsion used in this study (F1) is a formulation that has been optimized in previous research [15]. The formulation had an average droplet size of 13.96 nm with a polydispersity index (PDI) of 0.216, indicating a relatively homogeneous and stable particle size distribution. This system is an oil-in-water (O/W) type and did not exhibit phase separation during storage, indicating good physical stability.

The small droplet size and low PDI values allow for increased surface area and interaction with biological membranes. This is thought to contribute to the differences in antihyperglycemic responses observed between the nanoemulsion and conventional extract groups in this study, although the underlying mechanisms require further study.

3.2 Antihyperglicemic Activity

Measurement of blood glucose levels (Table 2; Figure 2) showed significant differences between groups on days 0, 3, and 17 ($p<0.05$). In the normal group, glucose levels gradually increased throughout the observation period, but remained within physiological limits (<200 mg/dL). This reflects that glucose homeostasis is maintained well without alloxan induction through normal metabolic regulation.

In contrast, the negative control group (K−) showed a very high increase in glucose levels reaching 490.33±189.9 mg/dL on day 17. This condition clearly indicates the occurrence of severe hyperglycemia due to alloxan induction. This is in accordance with the mechanism of alloxan which

can damage pancreatic β cells through the formation of free radicals, thereby significantly reducing insulin secretion [20].

Table 2. *One-Way ANOVA Post Hoc Duncan* Test of Decreased Blood Glucose in Mice

Treatment Group	Blood Glucosa Levels in Mice		
	H0	H3	H17
Normal	88,33±20,4[a]	111,00±11,5[a]	156,66±17,9[a]
K+	138,33±18,7[bc]	325,00±120,5[b]	132,33±29,3[a]
K-	157,33±34,4[c]	265,33±75,7[ab]	490,33±189,9[b]
N1	131,00±1,0[bc]	377,33±186,7[b]	173,00±80,5[a]
N2	138,66±5,0[bc]	564,33±50,1[c]	114,00±21,6[a]
E1	112,33±10,4[ab]	336,00±67,5[b]	139,33±47,7[a]
E2	126,33±21,7[bc]	583,00±27,7[c]	133,66±45,5[a]
Sig.	0,016	0,000	0,001

In the positive control group (K+), blood glucose levels increased on day 3, then decreased on day 17 to approach the normal range. This pattern indicates an improvement response after the initial hyperglycemic phase, reflecting the effectiveness of the therapeutic agent in suppressing the increase in glucose levels.

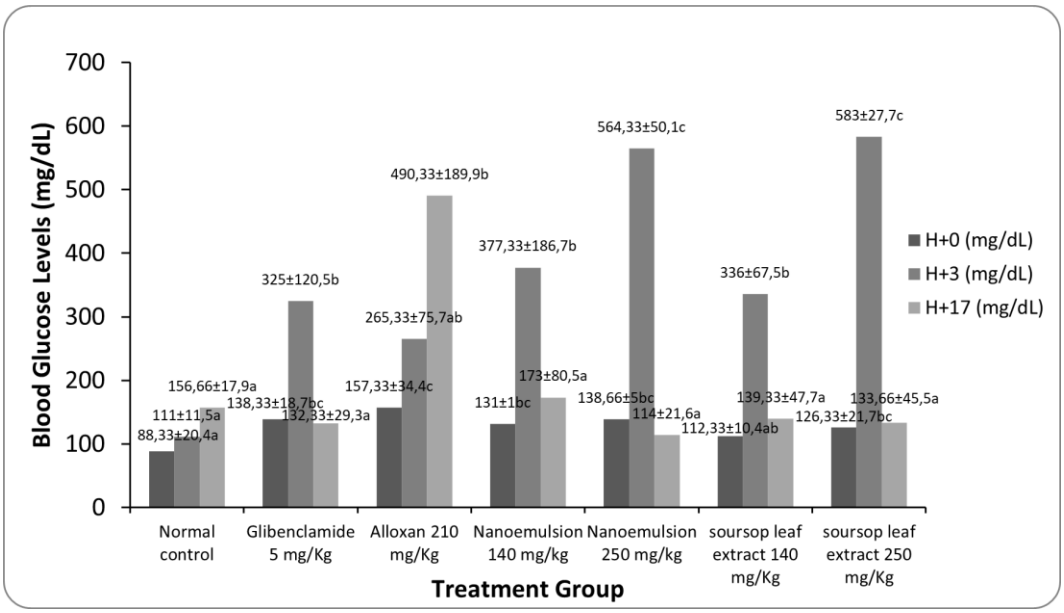


Figure 3. Diagram of Activity Methanol Extract on Blood Glucose Levels of Alloxan-Induced Mice. Different Letter Notations Indicate Differences Between Treatments Based on Duncan's Extended Test.

Treatment groups N1, N2, E1, and E2 showed a similar trend, namely a spike in glucose levels on the 3rd day which was then followed by a decrease on the 17th day. Even though there was a fairly high increase, the value at the end of the observation was not significantly different from the normal group, but was significantly different compared to K-. Treatment group N2 and E2 showed a more consistent decrease in glucose levels on day 17, indicating a trend towards a better response at higher doses. However, because this study did not use advanced pharmacodynamic analysis, the dose-response relationship can only be concluded as a trend. The antihyperglycemic effect observed in the

treatment group is thought to be related to the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins. Flavonoids have antioxidant and anti-inflammatory activities that play a role in reducing oxidative stress and modulating insulin signaling pathways such as PI3K/Akt and AMPK [21-22]. In addition, flavonoids can also inhibit the enzymes α-amylase and α-glucosidase, thereby reducing glucose absorption in the intestine [23].

Alkaloids are reported to have antihyperglycemic activity through inhibition of carbohydrate digestive enzymes and increased regulation of glucose metabolism [24]. Meanwhile, tannins can reduce glucose absorption through inhibition of digestive enzymes, and saponins play a role in stimulating insulin secretion and regulating lipid and glucose metabolism [24-25].

The superior effectiveness of nanoemulsions compared to conventional extracts is thought to be related to their physicochemical characteristics, particularly their extremely small droplet size (13.96 nm) and relatively homogeneous particle size distribution (PDI 0.216). The nanometer-scale droplet size results in a significantly larger specific surface area compared to conventional extracts. This increased surface area allows for more intensive contact between bioactive compounds and gastrointestinal fluids and epithelial membranes, thereby accelerating the intestinal dissolution process and increasing the amount of active compounds available for absorption.

At the molecular level, this increased bioavailability can increase the concentration of active compounds reaching the systemic circulation. By increasing the absorption of active compounds through the nanoemulsion interaction system, the bioactive compounds are thought to be more optimally linked to their target molecules compared to conventional extracts.

Furthermore, oil-in-water nanoemulsion systems can help maintain the stability of bioactive compounds during digestion and protect them from degradation due to pH changes and enzymatic activity in the digestive tract. Nanodroplets also have the potential to increase intestinal membrane permeability through better interaction with the mucosal layer, thereby increasing the fraction of compounds that can pass through the intestinal epithelium. This mechanism can explain why the nanoemulsion group, especially the 280 mg/Kg body weight dose (N2), showed a greater reduction in blood glucose levels compared to the conventional extract at the same dose.

These mechanisms collectively explain the trend of decreased blood glucose levels observed in the treatment group. Furthermore, these improvements in biochemical parameters were accompanied by qualitative improvements in liver histopathology, indicating reduced necrosis and inflammation compared to the negative control group. Overall, the reduction in blood glucose levels in the treatment group was the result of the synergistic contribution of these various bioactive compounds. However, because no direct molecular mechanisms were tested, this explanation is interpretive based on the supporting literature.

3.3 Histopathology

In this study, histopathological observations used hematoxylin-eosin (HE) staining (Table 3). Using hematoxylin-eosin (HE) staining, it is possible to clearly identify changes in tissue morphology, where the cell nucleus is stained blue and the cytoplasm is red to orange [26]. In the normal group, liver tissue exhibited intact lobular architecture with a central vein located in the center of the lobule. Hepatocytes appeared radially arranged, forming cords or plates of cells that led from the central vein to the portal area. Hepatocytes had round, distinct nuclei, and sinusoids were seen arranged regularly between the cords. This histological arrangement indicated that the liver structure and function were in normal physiological condition without any signs of tissue damage [27].

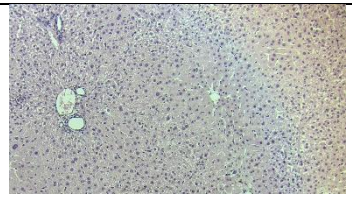
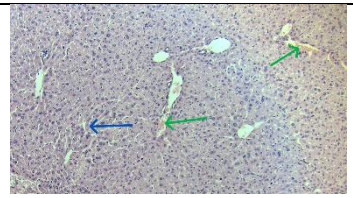
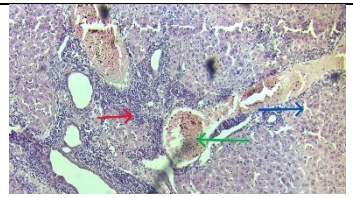
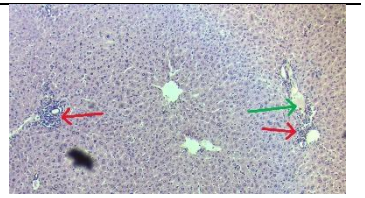
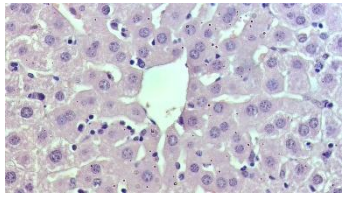
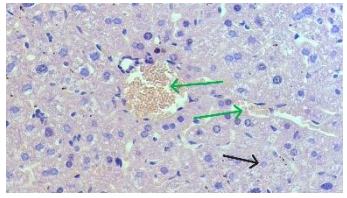
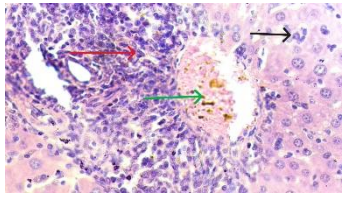
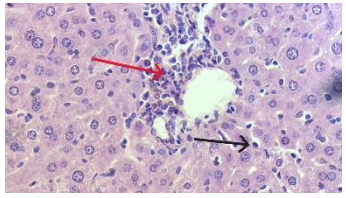
Alloxan induction triggered significant changes in both biochemical and histological parameters. The high blood glucose levels in the negative control group (K-) up to day 17 correlated with liver structural damage characterized by congestion, inflammatory cell infiltration, vacuolation, and necrosis. This condition indicates that hyperglycemia plays a role in increasing oxidative stress that causes hepatocyte cell damage. This mechanism is related to alloxan's ability to produce free radicals that damage cell membranes and organelles, thus disrupting cellular function [21].

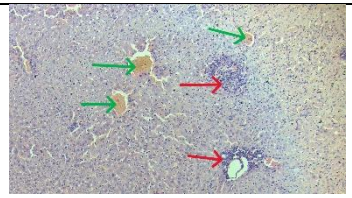
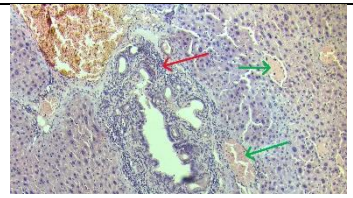
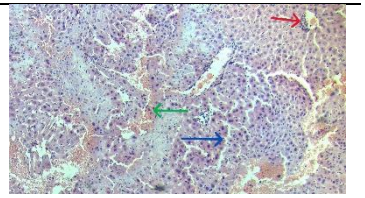
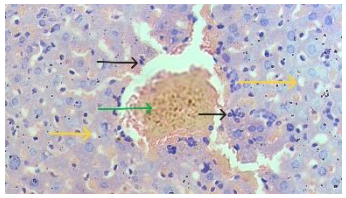
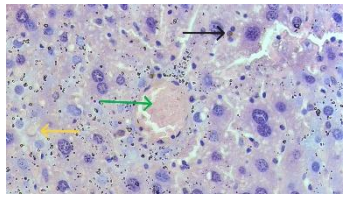
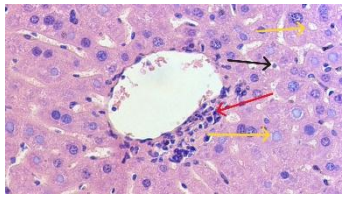
The positive control group (K+) showed a decrease in blood glucose levels on day 17, followed by improvement in histopathology, although mild damage was still found. This indicates that glucose control directly contributes to the reduction in liver tissue damage. Treatment groups N1, N2, E1, and E2 showed a pattern of increased blood glucose levels in the initial phase followed by a decrease at the end of the observation. These changes occurred in conjunction with improvements in liver histology, characterized by reduced necrosis, inflammation, and congestion compared to the K- group. Fatty degeneration and vacuolation were still found in some groups, indicating that the tissue recovery process was not yet fully optimal.

A better response was seen in the N2 and E2 groups, which showed a more significant decrease in blood glucose levels on day 17. This condition is consistent with the relatively better histopathological picture compared to the other treatment groups, thus indicating a stronger protective effect. This can be attributed to the characteristics of the preparation used, where the optimized nanoemulsion formulation allows for better dispersion of the active compounds and enhances interaction with biological systems. The association between improved blood glucose levels and tissue structure suggests that modulation of glucose metabolism plays a crucial role in maintaining hepatocyte integrity. This histopathological improvement is likely related to reduced oxidative stress due to the activity of bioactive compounds, particularly flavonoids and polyphenols, which have been reported to have protective effects on tissue through antioxidant and anti-inflammatory mechanisms [28-29]. However, because the histopathological analysis in this study is qualitative, the conclusions that can be drawn are still limited to observations of tissue morphology. In line with these findings, there was a consistent relationship between improvements in blood glucose levels and improvements in liver histological structure in the treatment group, indicating that glycemic control contributes to the restoration of liver tissue integrity.

The improvement in liver histopathology observed in the nanoemulsion treatment group, particularly reduced necrosis, inflammatory cell infiltration, and congestion, is thought to be mediated by the antioxidant and anti-inflammatory activity of bioactive compounds in A. muricata extract. This is supported by Zeweil et al [30] who demonstrated that A. muricata extract was able to suppress TNF-α and IL-1β mRNA expression and improve the immunohistochemical structure of damaged tissue. This mechanism involves the role of flavonoids and acetogenins in inhibiting inflammatory pathways, increasing endogenous antioxidant capacity, and maintaining cell membrane integrity from damage due to oxidative stress.

Table 3. Representative Histopathological Images of Liver Tissue in Experimental Groups (H&E Staining; 100× and 400× Magnification)

Groups	Normal	K+	K-	N1
100× magnification				
400× magnification				

Groups	N2	E1	E2
100× magnification			
400× magnification			

Notes: Normal group shows preserved hepatic architecture. Negative control (K−) exhibits severe cellular damage, including necrosis and inflammatory infiltration. Positive control (K+) and treatment groups (N1, N2, E1, E2) demonstrate varying degrees of cellular improvement, indicated by reduced necrosis and better structural organization. Black arrow (→) = Necrosis, Green arrow (→) = Congestion, Red arrow (→) = Imflammatory cell infiltration, Blue arrow (→) = Vacuolization, yellow arrow (→) = fatty degeneration or abnormal accumulation of fat.

4. Conclusion

Methanol extract of soursop leaves (Annona muricata L.) contains various bioactive compounds that potentially contribute to antihyperglycemic activity. Alloxan induction in mice causes an increase in blood glucose levels accompanied by liver histopathological changes in the form of congestion, inflammatory cell infiltration, vacuolation, and necrosis. Administration of soursop leaf extract nanoemulsion at doses of 140 and 280 mg/Kg body weight showed a significant decrease in blood glucose levels compared to the negative control, and was followed by qualitative improvement in liver histology. Compared with conventional extracts, the nanoemulsion formulation showed a tendency to provide a more consistent response to reduce blood glucose levels. These findings indicate that soursop leaf extract nanoemulsion has potential as a candidate for a natural-based antihyperglycemic agent with protective effects on liver tissue. However, further research with a quantitative approach and additional biochemical parameters is needed to confirm its mechanism and effectiveness more comprehensively.

5. Acknowledgement

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References

[1] Pratiwi, J. A. D., Huang, C. T., Juber, N. F., & Liu, J. J. (2024). Associations between diabetes mellitus and subsequent non-communicable diseases in Indonesia. *Discover Social Science and Health*, *4*(1), 30.

[2] Padhi, S., Nayak, A. K., & Behera, A. (2020). Type II diabetes mellitus: a review on recent drug based therapeutics. *Biomedicine & Pharmacotherapy*, *131*, 110708.

[3] Lestari, L., & Zulkarnain, Z. (2021). Diabetes Melitus: Review etiologi, patofisiologi, gejala, penyebab, cara pemeriksaan, cara pengobatan dan cara pencegahan. In *Prosiding Seminar Nasional Biologi* (Vol. 7, No. 1, pp. 237-241).

[4] Nogueira-Ferreira, R., Oliveira, P. F., & Ferreira, R. (2024). Liver metabolism: The pathways underlying glucose utilization and production. In *Glycolysis* (pp. 141-156). Academic Press.

[5] Abdullah, D., Oktriana, A., & Nova, R. (2025). Obat Tradisional Untuk Penyakit Kronik Diabetes Melitus: Literature Review. *Journal of Public Health Science*, *2*(1), 27-33.

[6] Widiastuti, T. C., Khuluq, H., Handayani, E. W., Wulandari, A. W. S., Hemas, E., Kurniawan, I., & Yuliana, J. (2022). Pemanfaatan tanaman obat untuk mengatasi penyakit diabetes melitus di Kota Kebumen. *Jurnal Farmasi Klinik dan Sains*, *2*(1), 87-96.

[7] Sugiharto, M. I., Bintari, Y. R., & Damayanti, D. S. (2021). Mekanisme senyawa aktif daun sirsak (Annona muricata Linn.) sebagai anti diabetes: studi in silico. *Jurnal Kedokteran Komunitas (Journal of Community Medicine)*, *9*(2).

[8] Zubaidi, S. N., Mohd Nani, H., Ahmad Kamal, M. S., Abdul Qayyum, T., Maarof, S., Afzan, A., ... & Mediani, A. (2023). Annona muricata: Comprehensive review on the ethnomedicinal, phytochemistry, and pharmacological aspects focusing on antidiabetic properties. *Life*, *13*(2), 353.

[9] Dista, R., Larasati, C., Ayuningsih, S., Anggraeni, N., & Batubara, I. (2022). Formulation and Characterization of Sungkai Leaf Extract Nanoemulsion (Peronema canscens Jack). *Jurnal UIN Alauddin*, *10*(2), 192-200.

[10] Indratmoko, S. (2020). Optimasi Formula Self Nano Emulsifying Drug Delivery System (SNEDDS) Ekstrak Etanol Daun Sirsak (Annona muricata) sebagai Antibakteri (Stapylococcus aureus) dengan Metode Simplex Lattice Design. *Pharmaqueous: Jurnal Ilmiah Kefarmasian*, *2*(2), 125-134.

[11] Rahmayanti, T., Fawwaz, M., Febriyanti, A., & Iswanto, A. H. (2023). Evaluasi Penerapan Lean Six Sigma Terhadap Ruang Operasi Di Rumah Sakit: Literature Review. *An-Najat*, *1*(2),

39-47.
[12] Dewi, M. K., Chaerunisaa, A. Y., Muhaimin, M., & Joni, I. M. (2022). Improved activity of herbal medicines through nanotechnology. *Nanomaterials*, *12*(22), 4073.
[13] Dewi, N. L. K. A. A., Prameswari, P. N. D., Cahyaningsih, E., Megawati, F., Agustini, N. P. D., & Juliadi, D. (2022). Pemanfaatan tanaman sebagai fitoterapi pada diabetes mellitus. *Usadha*, *1*(3), 31-42.
[14] Puspitasari, R. D., Sarani, D., Erliana, D., & Ekasari, M. (2025). Formulation and Characterization of Soursop Leaf extract (Annona muricata L.) Nanoemulsion Using VCO and Tween 80. *KOVALEN: Jurnal Riset Kimia*, *11*(2), 54-62.
[15] Reubun, Y. T. A., Kumala, S., Setyahadi, S., & Simanjuntak, P. (2020). Pengeringan beku ekstrak herba pegagan (Centella asiatica). *Sainstech farma*, *13*(2), 113-117.
[16] Pangesti, C., Nopiyanti, V., & Widyasti, J. H. (2021). Uji aktivitas antihiperglikemia ekstrak etanol daun delima putih (Punica granatum L.) pada mencit jantan (Mus musculus L.) yang diinduksi aloksan. *Jurnal Farmasi (Journal of Pharmacy)*, *10*(2), 1-7.
[17] Mairinoja, L., Heikelä, H., Blom, S., Kumar, D., Knuuttila, A., Boyd, S., ... & Poutanen, M. (2023). Deep learning–based image analysis of liver steatosis in mouse models. *The American Journal of Pathology*, *193*(8), 1072-1080.
[18] Sijid, S. A., Muthiadin, C., Zulkarnain, Z., & Hidayat, A. S. (2020). Pengaruh pemberian tuak terhadap gambaran histopatologi hati mencit (Mus musculus) ICR jantan. *Jurnal Pendidikan Matematika dan IPA*, *11*(2), 193.
[19] Kusuma, G. P. O. R. (2021). Uji Antihiperglikemia Ekstrak Etanol Daun Sirsak (Annona muricata L.) Terhadap Kadar Gula Darah Mencit Jantan (Mus musculus) yang Diinduksi Glukosa. *Jurnal Natur Indonesia*, *19*(1), 1-5.
[20] Mah, S. H., Sundrasegaran, S., & Lau, H. L. N. (2024). Topical nanoemulsion hydrogel formulated from supercritical fluid extracted palm-pressed fiber oil and virgin coconut oil with antioxidant and antibacterial effects. *Journal of Oleo Science*, *73*(4), 489-502.
[21] Santamaría, E., Maestro, A., Vilchez, S., & González, C. (2023). Study of nanoemulsions using carvacrol/MCT-(Oleic acid-potassium oleate)/Tween 80®-water system by low energy method. *Heliyon*, *9*(6).
[22] Chen, J., Li, R., Knapp, S., Zhu, G., Whitener, R. L., Leiter, E. H., & Mathews, C. E. (2022). Intergenomic and epistatic interactions control free radical mediated pancreatic β-cell damage. *Frontiers in Genetics*, *13*, 994501.
[23] Sok Yen, F., Shu Qin, C., Tan Shi Xuan, S., Jia Ying, P., Yi Le, H., Darmarajan, T., ... & Salvamani, S. (2021). Hypoglycemic effects of plant flavonoids: a review. *Evidence-Based Complementary and Alternative Medicine*, *2021*(1), 2057333.
[24] Bouyahya, A., Balahbib, A., Khalid, A., Makeen, H. A., Alhazmi, H. A., Albratty, M., ... & El Omari, N. (2024). Clinical applications and mechanism insights of natural flavonoids against type 2 diabetes mellitus. *Heliyon*, *10*(9).
[25] Lam, T. P., Tran, N. V. N., Pham, L. H. D., Lai, N. V. T., Dang, B. T. N., Truong, N. L. N., ... & Tran, T. D. (2024). Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: a systematic review of in vitro studies. *Natural Products and Bioprospecting*, *14*(1), 4.
[26] Anshika, Pandey, R. K., Singh, L., Kumar, S., Singh, P., Pathak, M., & Jain, S. (2022). Plant bioactive compounds and their mechanistic approaches in the treatment of diabetes: a review. *Future Journal of Pharmaceutical Sciences*, *8*(1), 52.
[27] Yuan, Y., Shou, K. Y., Wu, S. Q., Zhao, J. Y., Liu, Y., Wang, Y. J., ... & Dai, W. T. (2026). Multifaceted therapeutic potential of saponins in type 2 diabetes mellitus: mechanisms targeting insulin resistance and diabetic complications. *Frontiers in Pharmacology*, *17*, 1793697.

[28] Prusty, M. R., Dinesh, R., Sheth, H. S. K., Viswanath, A. L., & Satapathy, S. K. (2023). Nuclei segmentation in histopathology images using Structure-Preserving color normalization based ensemble deep learning frameworks. *Computers, Materials and Continua*, *77*(3), 3077-3094.

[29] Acharya, P., Chouhan, K., Weiskirchen, S., & Weiskirchen, R. (2021). Cellular Mechanisms of Liver Fibrosis: Pragyan Acharya, Komal Chouhan, Sabine Weiskirchen, Ralf Weiskirchen:[Ressource électronique]. *Frontiers in Pharmacology*, *12*(671640).

[30] Zeweil, M. M., Khafaga, A. F., Mahmoud, S. F., Wasef, L., Saleh, H., Elrehim, A. M. A., ... & Ghoneim, H. A. (2024). Annona Muricata L. extract restores renal function, oxidative stress, immunohistochemical structure, and gene expression of TNF-α, IL-β1, and CYP2E1 in the kidney of DMBA-intoxicated rats. *Frontiers in pharmacology*, *15*, 1348145.