

EFFECT OF ALOIN ON HEPATIC HISTOPATHOLOGY AND TNF- α EXPRESSION IN AN AGED MOUSE MODEL OF OBESITY AND TYPE 2 DIABETES MELLITUS

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Abstract

*Background: Obesity and type 2 diabetes mellitus are closely associated with chronic hyperglycaemia, insulin resistance, oxidative stress, and persistent inflammation, which may contribute to progressive hepatic injury. Aloin, an anthraquinone glycoside derived from Aloe species, has been reported to possess antioxidant and anti-inflammatory properties. Objective: This study aimed to evaluate the effect of aloin administered in combination with metformin on hepatic histopathology and tumour necrosis factor-alpha (TNF- α) expression in aged male mice with obesity and type 2 diabetes mellitus. Methods: This experimental study used a post-test-only control group design involving 32 male mice (*Mus musculus*) divided into two groups: a positive control group receiving metformin and a treatment group receiving metformin plus aloin. After 28 days of intervention, hepatic tissues were examined using haematoxylin-eosin staining and immunohistochemical assessment of TNF- α expression. Results: The treatment group showed relatively better-preserved hepatocyte morphology and more organised hepatic architecture than the positive control group, although several areas of cellular injury remained detectable. TNF- α immunoreactivity was less extensive and more scattered in the treatment group. The mean hepatic TNF- α value was lower in the treatment group than in the positive control group (81.244 ± 13.238 vs 99.352 ± 6.993). The difference between groups was statistically significant ($p = 0.011$). Conclusion: Aloin supplementation alongside metformin was associated with improved hepatic histological features and lower TNF- α expression in aged obese mice with type 2 diabetes mellitus. These findings support the potential role of aloin as an adjunctive agent in reducing diabetes-related hepatic inflammation and injury.*

Keywords: Aloin; Metformin; TNF- α ; Hepatic histopathology; Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin production or decreased sensitivity to insulin (Murtaningsih et al., 2021). Type 2 diabetes mellitus (T2DM) is the most common form of DM, characterized by insulin resistance and pancreatic β -cell dysfunction, leading to impaired carbohydrate, fat and protein metabolism (Nasution et al., 2018). Patients with T2DM often go undetected until serious complications occur, one of which is multi-organ damage, including the liver. Globally, the prevalence of DM continues to increase. The International Diabetes Federation (IDF) 2021 reported that 536 million people aged 20-79 years suffer from DM, with a global prevalence of 9.8%. It is estimated that this number will increase to 783 million by 2045 (International Diabetes Federation, 2021). Indonesia ranks 7th in the world with 10.7 million people with DM (6.2%), while in West Sumatra the prevalence reaches 1.6% (Permatasari et al., 2020). One of the main factors causing T2DM is obesity, which can increase insulin resistance and oxidative stress through excessive

production of Reactive Oxygen Species (ROS)(Sasmita et al.,2024). Excessive ROS stimulates the release of Tumour Necrosis Factor-alpha (TNF- α) and other proinflammatory cytokines, which play a role in the pathogenesis of insulin resistance and complications of T2DM(Yusuf,2022). TNF- α interferes with the insulin signalling pathway through serine phosphorylation at the insulin receptor, thereby inhibiting insulin action and exacerbating hyperglycemia. In addition, increased TNF- α and ROS in adipose tissue and hepatocytes also contribute to fatty liver (steatosis), inflammation (hepatitis), fibrosis, and cirrhosis(Gupta et al.,2019). Current T2DM treatment still relies on insulin and oral hypoglycemic drugs, which have limitations in the long term and have the potential to cause side effects. Therefore, alternative therapies that are safer and more effective are needed, one of which is the utilization of medicinal plants.

Aloe vera, especially the compound aloin, has been shown to have antioxidant and anti-inflammatory activities that can help protect the liver from oxidative stress due to obesity and T2DM(Dewi et al.,2020). Aloin works by suppressing TNF- α production, inhibiting NF- κ B activation, and increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH)(Bai J. et al.,2023).

METHODS

This study was conducted within the scope of internal medicine and pharmacology, focusing on the microscopic effect of aloin on hepatic function in male mice (*Mus musculus*) modelling the elderly with obesity and diabetes mellitus type 2. The research employed a laboratory experimental approach using a Post-Test Only Control Group Design. The sample size was calculated using the Federer formula, resulting in 16 mice per group, with a total of 48 mice divided into three groups: the negative control group (no treatment), the positive control group (received metformin), and the treatment group (received a combination of metformin and aloin extract). Mice were fed a high-fat diet until they reached a body weight of 30–40 grams and had random blood glucose levels ≥ 176 mg/dL. Blood glucose measurements were performed on days 0, 14, and 28. To increase blood glucose levels, intraperitoneal streptozotocin (STZ) induction was administered 3 days before the pre-test, with an STZ dose of 2 mg per mouse. The negative control group only performed elderly modeling. The positive control group received metformin at a dose of 2.6 mg per mouse, a daily dose of 2 mL metformin, because it is given to mice at $2 \text{ mL} \times = 0.2 \text{ mL}$, while the treatment group received a combination of metformin and aloin extract (30 μg / kg BB). Aloin and metformin were given for 28 days, in the morning, along with feeding. Aloin was administered 60 minutes after metformin administration. After 28 days, the male mice were euthanized using the cervical dislocation method, and the liver was taken for histopathological analysis. The process of tissue slide preparation consists of three stages: paraffin block preparation, sectioning, and staining. The final step was staining and mounting the samples for microscopic examination. Hematoxylin-Eosin (HE) staining was used, followed by Immunohistochemical (IHC) staining using N-Histophin DABV2 reagent. Histopathologic examination of kidney slides was performed using a microscope at 100x and 400x magnification. Analysis included observing the presence of TNF- α expression and assessing histologic damage. Assessment was done for each group by comparing the treatment group

with the control group.

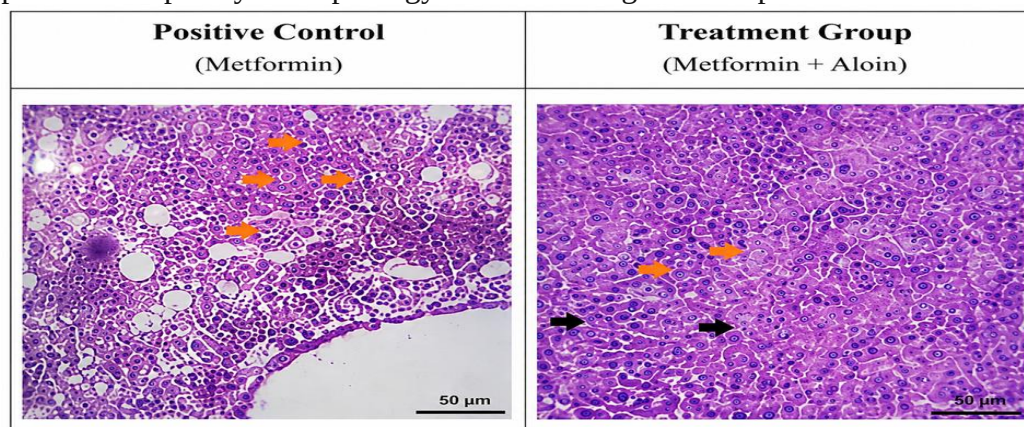
RESULTS AND DISCUSSION

Histopathological examination of the hepar sections revealed distinct morphological differences between the positive control group receiving metformin and the treatment group receiving a combination of metformin and aloin. The positive control group exhibited more pronounced hepatocellular alterations, including disruption of the normal hepatic architecture, cellular degeneration, karyolysis, and inflammatory cell infiltration. Several hepatocytes showed irregular cellular organisation, while the presence of clear spaces within the tissue indicated more extensive structural alterations. These findings suggest that hepatic injury remained evident in mice receiving metformin alone. Chronic hyperglycaemia, obesity, and insulin resistance may contribute to hepatic injury by increasing oxidative stress, disrupting lipid metabolism, and sustaining inflammatory responses. Persistent metabolic disturbances may progressively alter hepatocyte structure and promote the development of metabolic hepar injury in type 2 diabetes mellitus (Dharmalingam, 2018). Similar histopathological changes have been reported in experimental models of diabetes, in which prolonged hyperglycaemia was associated with increased oxidative stress, inflammatory activity, hepatocellular degeneration, and disruption of normal hepatic architecture. Studies involving streptozotocin-induced diabetic animals have also demonstrated inflammatory cell infiltration, apoptotic cellular changes, increased hepatic TNF- α expression, and elevated markers of hepar injury, supporting the relationship between chronic metabolic disturbances and progressive hepatic damage (Mahfoz, 2022).

In contrast, the treatment group receiving metformin in combination with aloin demonstrated relatively better preservation of hepatocyte morphology and a more organised hepatic architecture. Most hepatocytes retained more distinct cellular boundaries and visible nuclei, with a relatively uniform arrangement throughout the observed tissue. Although several areas of hepatocellular degeneration, karyolysis, and nuclear alteration remained detectable, these changes appeared less extensive than those observed in the positive control group. In the representative photomicrographs, the black arrows indicate relatively preserved hepatocytes, whereas the orange arrows indicate areas of hepatocellular degeneration, karyolysis, or inflammatory cell infiltration. These observations are consistent with experimental evidence indicating that improvements in oxidative balance and inflammatory regulation may be accompanied by reduced hepatocellular injury and better preservation of hepatic tissue architecture. Previous studies involving antioxidant and anti-inflammatory bioactive compounds in diabetic animal models have reported improvements in hepar histology alongside reductions in lipid peroxidation, inflammatory cell infiltration, and TNF- α expression (Alrefaei, 2025). Therefore, the relatively preserved hepatic morphology observed in the combination group suggests that aloin may provide additional hepatocellular protection when administered alongside metformin. Nevertheless, the persistence of cellular alterations indicates that hepatic injury was not completely resolved during the intervention period.

Representative haematoxylin–eosin-stained hepar sections are shown in Figure 1. The positive control group showed more extensive disruption of hepatic

architecture, hepatocellular degeneration, karyolysis, and inflammatory cell infiltration. In contrast, the treatment group demonstrated relatively better-preserved hepatocyte morphology and a more organised hepatic architecture.



Notes: Black arrows indicate normal hepatocyte architecture or preserved cells.
Orange arrows indicate areas of hepatocellular degeneration, karyolysis, or inflammatory cell infiltration.

Figure 1. Representative Histopathological Appearance Of Hepatic Tissue In The Positive Control And Treatment Groups

The positive control group received metformin, whereas the treatment group received metformin plus aloin. Black arrows indicate relatively preserved hepatocytes, while orange arrows indicate areas of hepatocellular degeneration, karyolysis, or inflammatory cell infiltration. Haematoxylin–eosin staining; scale bar = 50 μm

Immuohistochemical examination revealed different patterns of hepatic tumour necrosis factor-alpha (TNF- α) immunoreactivity between the two groups. The positive control group exhibited more extensive brown immunostaining, with numerous visually identifiable TNF- α -positive cells distributed throughout the hepatic tissue. In contrast, the treatment group showed less extensive and more scattered brown immunostaining, accompanied by relatively more uniform hepatic architecture. The arrowheads in the representative immunochemical photomicrographs indicate cells with positive TNF- α immunoreactivity. Increased TNF- α activity is closely associated with hepatic inflammation and may aggravate insulin resistance by interfering with insulin-signalling pathways, thereby contributing to persistent hepatocellular injury (Vachliotis, 2023). Experimental studies have also demonstrated that diabetes-associated hepatic injury is accompanied by increased TNF- α expression and the activation of other inflammatory mediators. Conversely, improvements in endogenous antioxidant defence may reduce inflammatory activity and support the preservation of hepatic architecture (Bashir et al., 2020). Thus, the less extensive TNF- α immunostaining observed in the treatment group may indicate a lower hepatic inflammatory response following the addition of aloin to metformin.

Representative immunochemical findings of hepatic TNF- α expression are presented in Figure 2. The positive control group exhibited more extensive brown immunostaining and a greater number of visually identifiable TNF- α -positive cells, whereas the treatment group showed less extensive and more scattered brown immunostaining.

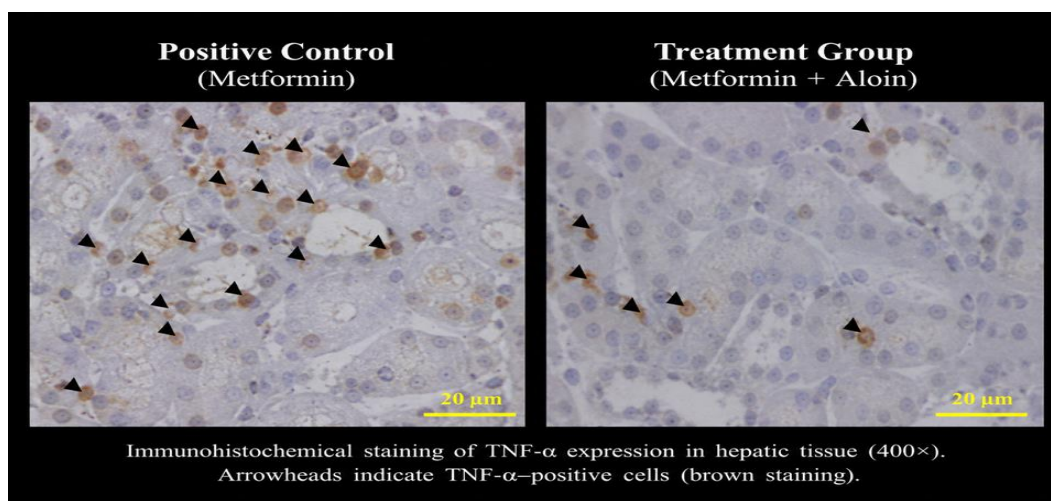


Figure 2. Representative Immunohistochemical Expression of TNF- α In Hepatic Tissue From The Positive Control And Treatment Groups

The positive control group received metformin, whereas the treatment group received metformin plus aloin. Black arrowheads indicate TNF- α -positive cells identified by brown immunostaining. Original magnification: 400 $\times$; scale bar = 20 μ m.

The observed histopathological and immunohistochemical findings may be associated with the antioxidant and anti-inflammatory properties of aloin. Aloin is an anthraquinone glycoside derived from Aloe species and has been reported to possess antioxidant, anti-inflammatory, antimicrobial, and other pharmacological activities(Xiao J. et al.,2022). Under chronic hyperglycaemic conditions, excessive production of reactive oxygen species may induce lipid peroxidation and damage cellular membranes, proteins, and DNA. Oxidative stress may also activate inflammatory signalling pathways and increase the production of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, thereby contributing to progressive hepatocellular injury. Experimental evidence has demonstrated that aloin may reduce hepatic malondialdehyde levels, increase superoxide dismutase activity, decrease the expression of TNF- α , IL-1 β , and IL-6, and increase the expression of the anti-inflammatory cytokine IL-10. Aloin has also been reported to attenuate hepatic inflammation and fibrotic changes through modulation of the TGF- β /Smad signalling pathway(Bai J.et al.,2023). Although the experimental model used by Bai et al. differed from the obesity- and diabetes-associated model employed in the present study, these findings support the potential role of aloin in limiting oxidative and inflammation-related hepatic injury.

The potential benefit observed in the treatment group may also reflect complementary effects between metformin and aloin. Metformin primarily improves glucose metabolism, reduces hepatic glucose production, and enhances insulin sensitivity, whereas aloin may provide additional antioxidant and anti-inflammatory activity. Metformin has also been reported to reduce oxidative stress, inflammatory cytokine production, and hepatocellular injury in experimental models. In a murine model of hepatotoxicity, metformin reduced hepatic TNF- α and IL-6 levels, improved endogenous antioxidant activity, and decreased hepatocyte necrosis(Zhang Y.et al.,2021). Furthermore, combination therapies involving metformin and other bioactive compounds have been associated with greater improvements in oxidative stress, inflammatory activity, lipid metabolism,

and hepatic histopathology than metformin monotherapy in experimental settings (Jamal et al., 2026). Therefore, the relatively preserved hepatocyte morphology and less extensive TNF- α immunoreactivity observed in the treatment group may reflect the combined metabolic effects of metformin and the antioxidant and anti-inflammatory properties of aloin.

Table 1. Descriptive Statistics, Normality Test, and Comparison of Hepatic TNF- α Expression Between Groups

Group	N	Mean $\pm$ SD	Minimum	Maximum	Shapiro- Wilk p- value	Independent t-test p- value
Positive Control (Metformin)	16	99.352 $\pm$ 6.993	87.297	111.766	0.974	0.011
Treatment (Metformin + Aloin)	16	81.244 $\pm$ 13.238	54.431	99.665	0.911	—

Statistical analysis demonstrated a significant difference in hepatic TNF- α measurements between the positive control group receiving metformin and the treatment group receiving metformin plus aloin ($p = 0.011$). The treatment group showed a lower mean hepatic TNF- α value than the positive control group (81.244 ± 13.238 vs 99.352 ± 6.993). This quantitative finding is consistent with the descriptive immunohistochemical observations, in which the treatment group exhibited less extensive and more scattered TNF- α -positive staining. Taken together, these findings suggest that the addition of aloin to metformin may be associated with reduced hepatic TNF- α expression and lower inflammatory activity. However, the biological interpretation remains dependent on the definition and direction of the immunohistochemical measurement scale. Therefore, the measurement unit, scoring criteria, number of microscopic fields, regions of interest, and image-analysis procedure should be clearly described to ensure the reproducibility and validity of the findings. Standardised assessment is important because inflammatory changes are more appropriately evaluated by integrating histopathological findings with quantitative immunohistochemical, biochemical, and molecular measurements rather than relying solely on representative tissue images (Pham et al., 2007) (Meyerholz, 2018).

Overall, the combination of metformin and aloin was associated with relatively improved hepatocyte morphology, better preservation of hepatic architecture, less extensive TNF- α immunoreactivity, and significantly lower hepatic TNF- α measurements compared with metformin alone. These findings may reflect complementary metabolic, antioxidant, and anti-inflammatory mechanisms. However, the remaining areas of hepatocellular degeneration and nuclear alteration indicate that hepatic injury was not completely resolved during the intervention period. The persistence of these alterations may be related to the severity of the metabolic disturbance, the duration of treatment, or incomplete recovery from chronic oxidative and inflammatory injury. In addition, the absence of an aloin-only group limits the ability to determine whether the observed effects resulted from the independent activity of aloin or its interaction with metformin. Future studies should include metformin-only, aloin-only, and combination-treatment groups;

employ standardised quantitative histopathological and immunohistochemical methods; and evaluate additional markers, including alanine aminotransferase, aspartate aminotransferase, malondialdehyde, superoxide dismutase, glutathione, IL-6, IL-10, and NF- κ B. Further mechanistic and quantitative investigations are required to confirm the potential hepatoprotective and anti-inflammatory effects of aloin in diabetes-associated hepatic injury.

CONCLUSION

The combination of metformin and aloin was associated with better preservation of hepatic morphology and lower hepatic inflammatory activity than metformin monotherapy in the experimental model. Histopathological examination demonstrated relatively more organised hepatic architecture and less extensive hepatocellular degeneration, karyolysis, and inflammatory cell infiltration in the treatment group. These morphological findings were supported by less extensive TNF- α immunoreactivity and a significantly lower mean hepatic TNF- α value in the metformin-plus-aloin group than in the metformin-only group (81.244 ± 13.238 vs 99.352 ± 6.993 ; $p = 0.011$). Collectively, these findings suggest that aloin may provide additional hepatoprotective and anti-inflammatory effects when administered alongside metformin, potentially through complementary antioxidant, anti-inflammatory, and metabolic mechanisms. However, residual hepatocellular alterations indicate that hepatic injury was not completely resolved during the intervention period. Furthermore, the absence of an aloin-only group limits the ability to distinguish the independent effects of aloin from its potential interaction with metformin. Therefore, further studies incorporating an aloin-only group, standardised quantitative histopathological and immunohistochemical assessments, and additional markers of hepatic function, oxidative stress, and inflammation are required to confirm the mechanisms and therapeutic potential of this combination.

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