

Mechanism of Liver Damage Prevention and Interleukin-6 Decrease by Red Onion Extract: A Partial Least Squares Analysis in Diabetic Rats

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ABSTRACT

Worldwide, in 2025, it is estimated that 350 million people will suffer from diabetes mellitus. The presence of dysfunction and changes in hepatic cell mass indicates histological changes in the liver and triggers the IL-6 inflammatory process. The study aims to analyze the mechanism by which red onion extract prevents liver damage and reduces the level of IL-6. In this study, 24 white rats were divided into six groups. Five of the six groups were diagnosed as diabetic and given intraperitoneal alloxan injection. The study groups consisted of six groups: control group without diabetes (A); alloxan-induced group without therapy (B); alloxan-induced group treated with synthetic drug metformin dose of 45 mg/kg (C); alloxan-induced group treated with red onion extract dose 1 (D); alloxan-induced group treated with dose 2 (E); alloxan-induced group treated with dose 3 (F). The fixed hepatic organs were then examined using HE staining and IHC. Statistical analysis of the PLS test. The results showed the highest average necrosis in the group not given red onion extract, at 3,550. Giving red onion extract lowers the mean IL-6 by 2.550 compared to the group that did not receive it. The mechanism of providing red onion extract directly reduces necrosis, and the IL-6 t-value is greater than 1.96. This study concludes that administering red onion extract can prevent liver damage and reduce IL-6 levels. This study can inform the public that red onions may be developed as an alternative treatment for humans with DM.

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1. Introduction

The number of individuals with diabetes mellitus (DM) in Indonesia is steadily rising. Changes in people's lifestyles and diets contribute to the increase in this disease. The pancreas's ability to produce insulin or to desensitize insulin receptors decreases, causing DM. Metabolic disorders prevent glucose from being converted into glycogen and from entering cells, increasing blood glucose levels. Clinical manifestations include impaired lipid, carbohydrate, and protein metabolism, which then cause hyperglycemia, which will eventually lead to diabetes mellitus. Inflammation and activation of the innate immune system correlate with the pathogenesis of diabetes mellitus (Daryabor et al., 2020).

According to the 2018 Global Status Report on NCDs from the World Health Organization (WHO), non-communicable diseases (NCDs) account for 60% of deaths worldwide. Diabetes mellitus (DM) is the sixth leading cause of death. Approximately 1.3 million people die from diabetes, with 4% dying before the age of 70 (WHO, 2018). In 2021, 536.6 million people (10.5%) worldwide had DM, and this number is expected to increase to 783.2 million (12.2%) by 2045. Of all the countries in the world, Southeast Asia has the third-highest number of DM patients, with 90.2 million. Of the ten countries in Southeast Asia, Indonesia has the fifth highest number of DM patients, with 19.5 million people. DM is increasingly common in industrialized and developing countries. In Indonesia, 21.3 million people will have DM by 2030. According to Indonesia's 2018 Riskesdas findings, the prevalence of diabetes mellitus rose from 6.9% in 2015 to 8.5% in 2018. In Indonesia, the highest number of DM cases was reported in DKI Jakarta in 2018, with 40,210 cases (2.6%), East Java with 151,878 cases (2.0%), DI Yogyakarta with 14,602 cases (2.4%), and North Sulawesi with 9,542 cases (Shokeralla et al., 2025).

Oral antidiabetic drugs may be beneficial for patients who are allergic to insulin or who prefer not to use insulin injections in their diabetes management. However, their use must be understood to be fit for purpose and not cause hypoglycemia. Therefore, experts have developed a safe method for the traditional treatment of diabetes mellitus using herbal ingredients (Xiao & Luo, 2018). Red onion (*Allium ascalonicum* L) is one of the plants that humans often consume as an alternative. Red onions can be used as a medicinal aid to help lower sugar and cholesterol levels. Red onion can also be used to treat diabetes. Red onion bulbs, particularly those rich in quercetin, have been shown to exhibit hypoglycemic effects and may benefit individuals with diabetes mellitus (Shahrajabian et al., 2020). One-way oral hypoglycemic drugs work in people with diabetes is by inhibiting the enzyme that breaks down glucose in the small intestine, known as alpha-glucosidase. Blood glucose levels after a meal do not rise as quickly because alpha-glucosidase inhibitors prevent the small intestine from breaking down glucose. One of the most effective approaches to preventing the onset of diabetic complications has been demonstrated (Aoki et al., 2019). Indonesia has a variety of plants that can be used as medicines; therefore, research on these plants is necessary. This is because alpha-glucosidase inhibitors are found in some plant extracts. Onion bulb extract contains an alpha-glucosidase inhibitor found in naphthalene. Besides naphthalene, onion extracts also contain other compounds such as flavonoids, triterpenoids, and saponins, each of which has antioxidant and anti-inflammatory functions.

According to research conducted by Randeria et al. (2019), inflammatory markers, such as cytokines, are potent indicators of the onset of diabetes mellitus in a group of mice. One of the major cytokines implicated in the development of type 2 diabetes is interleukin-6 (IL-6). In the majority of cells and tissues, the cytokine interleukin-6 is accountable for both immunologic and non-immunologic regulation (Uciechowski & Dempke, 2020). In the study, mice were exposed to IL-6 for two hours by Hu et al. (2018). The results showed that insulin uptake into the muscle was reduced. Previous research has primarily examined the effects of onions on the pancreas, insulin, and blood glucose levels. However, the hepatic-specific mechanisms are unknown. The liver is the first organ in the body after the gastrointestinal tract to be exposed to toxic substances, making it susceptible to damage. According to Kim et al. (2022), the liver clears harmful substances through its metabolic processes. The study aims to analyze the mechanism by which red onion extract prevents liver damage and reduces the level of IL-6. Considering the potential of red onion extract as an anti-inflammatory and antioxidant, it is necessary to investigate its effects on inflammation and necrosis by regulating IL-6 expression and hepatic histopathology. Therefore, it is crucial to examine the histopathological picture of the liver to determine the changes occurring in hepatic cells. The above information encourages research to determine mechanisms that prevent liver damage and reduce interleukin-6 using experimental animal models of diabetes mellitus, as well as red onion extract, to investigate necrosis and IL-6.

2. Materials and Methods

2.1 Materials

The materials used in this investigation consisted of pelleted feed for the experimental animals and distilled water, which was provided freely to the white rats. Alloxan, obtained from Aldrich (BCBN910V), was used to induce diabetes in white rats, and red onion extract and 0.5% CMC Na were used to treat the diabetes in these rats. In the immunohistochemical procedure, chemicals required for IL-6 expression included anti-IL-6 antibody (Ab 6672 IL-6), 10% PBS, 3% H₂O₂, labeled secondary antibody, streptavidin®, Diaminobenzidine (DAB), metallic red and green colors, blocking buffer ultra V (Sigma-Aldrich), ether, distilled water, and physiological sodium chloride.

The research tools used during the treatment included plastic animal cages, feeders, drinkers, sonde needles for rats, and Terumo syringe® syringes. The Easy Touch GCU glucometer and Easy Touch glucose test strips were used to measure blood glucose levels in white rats. Necroplasty used equipment such as surgical scissors, scalpels, tweezers, white rat trays, and small pots and lids for storing organs. A Glass objective, Olympus® CX-41 microscope glass cover, and Nikon Eclipse Ci microscope were also used.

2.2 Data Collection Procedures

This study is a basic experimental investigation that employed a randomised, separate pretest-posttest control group design in addition to a complete randomised design. This study was conducted at the Laboratory of the Faculty of Health, Universitas Nahdlatul Ulama Surabaya. It was ethically recognized by the Health Research Ethics Committee of Universitas Muhammadiyah Lamongan with Number 137/EC/KEPK-S2/04/2025. The study included 24 male white rats, with 4 in each group. In this study, there were six different groups: a non-diabetic control group; group B received no diabetes therapy; group C received synthesized metformin therapy; 200 mg of red onion extract was given to group D, while 400 mg of red onion extract was given to group E; and group F received red onion extract at a dose of 800 mg/kgBB. The variable in this study was red onion extract. White rats (*Rattus norvegicus*), the experimental animals in this study, were sacrificed through

inhalation anesthesia with ether. Afterwards, surgery was performed to retrieve the target organ, which was the liver. White rats from each group were treated for 18 days. Afterwards, their blood glucose was measured, and they were anaesthetized with ether. On the 19th day, they were sacrificed and dissected to remove the hepatic organs. After weighing and immersing the harvested heparin in a 10% concentration Buffer Neutral Formalin (BNF) fixation liquid in each labeled bottle, the heparin was inserted. The hepatic organs were then prepared.

2.2.1 Method for Counting the Number of Necrotizing Cells

Hematoxylin-eosin (HE) staining was used to prepare histopathological hepatic tissue. The number of necrotic cells was counted microscopically using a quantitative method, in which all necrotic cells in a single preparation were observed at 400× magnification.

2.2.2 The Process of Observing IL-6 Expression

IL-6 expression was demonstrated by immunohistochemical (IHC) staining, as evidenced by a positive response to the anti-IL-6 rat monoclonal antibody. The positive cell value for each field of view is the average of the number of positive cells. Counts per five high-power fields (HPF) were observed with a light microscope at 400 times magnification, and the number of IL-6-positive cells was counted when they produced a brownish color in the cytoplasm.

2.3 Data Analysis

The first step will be to examine normalcy and homogeneity using the Levene and Kolmogorov-Smirnov tests. Once the data appear normal and homogeneous, the one-way ANOVA will be used to compare groups. The Tukey HSD test is used to determine the significance of differences between treatment groups. Using Partial Least Squares (PLS), red onion extract was administered to each group of variables (i.e., IL-6 and necrosis), comparing the treatment and control groups to determine the causal relationship and describe the mechanism by which red onion extract lowers Interleukin-6 and prevents liver damage.

3. Results

3.1 Blood Glucose Reduction Brought on by Red Onion Extract Administration

Blood glucose levels (GDP Post) after red onion extract treatment (measured on the 19th day after administration of red onion extract) were measured from tail blood using a glucometer. The distribution of the mean and standard deviation of the blood glucose level (GDP Post) is shown here.

Table 1: Average Blood Glucose Level After Consumption of Red Onion Extract

Group	Mean $\pm$ SD	Maximum	Minimum
A	102.250 $\pm$ 11.087 ^a	112	88
B	257.000 $\pm$ 10.456 ^d	267	247
C	111.750 $\pm$ 3.304 ^{ab}	115	108
D	122.000 $\pm$ 5.292 ^b	129	117
E	116.500 $\pm$ 12.234 ^{bc}	128	100
F	108.750 $\pm$ 6.850 ^{ac}	115	99

The means for each group are presented in Table 1. Group A (102.250 $\pm$ 11.087), Group B (257.000 $\pm$ 10.456), Group C (111.750 $\pm$ 3.304), Group D (122.000 $\pm$ 5.292), Group E (116.500 $\pm$ 12.234), and Group F (108.750 $\pm$ 6.850). All groups of experimental animals given onion extract in different doses after treatment experienced a decrease in fasting blood sugar levels (GDP Post), which is below the standard limit (<126 mg/dL) which does not result in hyperglycemia, namely D (122.000 mg/dL), E (116.500 mg/dL), and F (108.750 mg/dL). The highest blood glucose level was in group B, which was only intervened with alloxan, at 257.000 mg/dL. Group A, which received no intervention and served as the negative control group, had the lowest fasting blood sugar level. Blood glucose levels were 108.75 mg/dL in Group F, which received 800 mg/kg BW of red onion extract daily.

A data normality test using the Shapiro-Wilk test yielded a p-value greater than 0.05, indicating that blood glucose levels in all groups have a normal distribution ($p > 0.05$). Group A ($p=0.485$), group B ($p=0.145$), group C ($p=0.513$), group D ($p=0.689$), group E ($p=0.671$), and group F ($p=0.362$). An ANOVA test was conducted on the groups because the blood glucose levels showed a normal and homogeneous distribution, with a p-value of 0.094. The calculation results showed significant differences in all groups (ANOVA, $p = 0.000$); therefore, the analysis was carried out using the Least Significant Differences (LSD) test to determine the differences between groups. In group A, blood glucose levels compared with each

treatment group mostly showed a difference ($p < 0.05$), namely in group B (LSD, $p = 0.000$), group D (LSD, $p = 0.005$), and group E (LSD, $p = 0.035$), while in group C (LSD, $p = 0.145$) and group F (LSD, $p = 0.312$) showed no difference. In group B, blood glucose levels showed a significant difference compared with those of each group ($p < 0.05$) in groups A, C, D, E, and F (LSD, $p = 0.000$). Likewise, with group C, blood glucose levels compared with each group showed a significant difference ($p < 0.05$) in group B (LSD, $p = 0.000$), while group A (LSD, $p = 0.145$), group D (LSD, $p = 0.118$), group E (LSD, $p = 0.457$), and group F (LSD, $p = 0.0637$) showed no difference.

3.2 Prevention of Liver Damage by Administration of Red Onion Extract

The distribution and standard deviation of the mean reduction in necrosis indicate that red onion extract was administered to prevent liver damage.

Table 2: Reduced Necrosis Rate After Consumption of Red Onion Extract

Group	Mean $\pm$ SD	Maximum	Minimum
A	2.100 $\pm$ 0.258 ^a	2.4	1.8
B	3.550 $\pm$ 0.341 ^b	4.0	3.2
C	2.750 $\pm$ 0.191 ^c	3.0	2.6
D	2.700 $\pm$ 0.258 ^d	3.0	2.4
E	1.650 $\pm$ 0.443 ^d	2.2	1.2
F	1.300 $\pm$ 0.258 ^c	1.6	1.0

The mean values for each group are presented in Table 2. Group A had a value of 2.100 ± 0.258 , group B had a value of 3.550 ± 0.341 , group C had a value of 2.750 ± 0.191 , group D had a value of 2.700 ± 0.258 , group E had a value of 1.650 ± 0.443 , and group F had a value of 1.300 ± 0.258 . Group B, which was treated only with alloxan, showed the most necrosis. Group F, which received red onion extract at 800 mg/kg BW daily, exhibited the lowest necrosis, with an average necrosis-positive cell count of 1,300. Group F also experienced a lower number of necrosis-positive cells. The size of necrosis was measured through histopathology.

The Shapiro-Wilk data normality test yielded a p-value greater than 0.05, indicating that the prevention of liver damage through decreased necrosis is usually normally distributed, with the mean necrosis across all groups also normally distributed ($p > 0.05$). Group A ($p = 0.972$), group B ($p = 0.850$), group C ($p = 0.272$), group D ($p = 0.972$), group E ($p = 0.798$), and group F ($p = 0.972$). An ANOVA test was performed on the groups because the mean necrosis results exhibited a regular and homogeneous distribution, with a p-value of 0.443 ($p > 0.05$). The calculation results showed significant differences in all groups (ANOVA, $p = 0.000$). The analysis was then conducted using the Least Significant Difference (LSD) test to determine differences between groups. In group A, the mean necrosis compared with each treatment group showed differences in most groups ($p < 0.05$), namely in group B (LSD, $p = 0.000$), group C (LSD, $p = 0.007$), group D (LSD, $p = 0.012$), group E (LSD, $p = 0.049$), and group F (LSD, $p = 0.002$). In group B, the mean necrosis compared with each group showed a significant difference ($p < 0.05$) in all groups, namely group A (LSD, $p = 0.000$), group C (LSD, $p = 0.002$), group D (LSD, $p = 0.001$), group E (LSD, $p = 0.000$), and group F (LSD, $p = 0.000$).

3.3 Reduction of Interleukin-6 by Administration of Red Onion Extract

The distribution of the mean and standard deviation of the number of IL-6-positive cells shows the effect of red onion extract on this number.

Table 3: Average Number of IL-6 Positive Cells After Consumption of Red Onion Extract

Group	Mean $\pm$ SD	Maximum	Minimum
A	1.750 $\pm$ 0.341 ^a	2.2	1.4
B	8.450 $\pm$ 0.191 ^d	8.6	8.2
C	2.750 $\pm$ 0.341 ^c	3.2	2.4
D	4.400 $\pm$ 0.864 ^d	5.6	3.6
E	4.150 $\pm$ 0.550 ^d	4.8	3.6
F	2.550 $\pm$ 0.341 ^c	3.0	2.2

Table 3 shows the average of each group. Group A (1.750 ± 0.341), group B (8.450 ± 0.191), group C (2.750 ± 0.341), group D (4.400 ± 0.864), group E (4.150 ± 0.550), and group F (2.550 ± 0.341). The mean number of IL-6-positive cells was highest in group B, the only group that received alloxan intervention. At the same time, the lowest mean of IL-6-positive cells was in group A, which did not receive any intervention and served as the negative control group. The treatment group that approached the negative control group was group F, which received a dose of 800 mg/kg BW/day of shallot extract, with a mean positive cell IL-6 of 2.550. In the group given shallot extract, the number of IL-6-positive cells tends to decrease. IL-6 expression was measured immunohistochemically using anti-IL-6 antibodies. IL-6 expression was observed in five fields of view using a microscope at 400x magnification.

Using the Shapiro-Wilk data normality test, the number of IL-6-positive cells in each group exhibited a normal distribution ($p > 0.05$), indicating that IL-6 expression is typically distributed in a usual manner. Group A ($p=0.850$), group B ($p=0.272$), group C ($p=0.850$), group D ($p=0.577$), group E ($p=0.650$), and group F ($p=0.850$). An ANOVA test was conducted on the groups because the results showed that the number of IL-6-positive cells was normally distributed, with a p-value of 0.201 ($p > 0.05$). The calculation results showed a significant difference across all groups (ANOVA, $p=0.000$), so the analysis was carried out using the Least Significant Differences (LSD) test to determine differences between groups. In group A, the number of IL-6 positive cells compared with each treatment group showed a difference in all groups ($p < 0.05$), namely in group B (LSD, $p=0.000$), group C (LSD, $p=0.010$), group D (LSD, $p=0.000$), group E (LSD, $p=0.000$), and group F (LSD, $p=0.033$). In group B, the number of IL-6-positive cells compared with each of the other groups showed a significant difference ($p < 0.05$) in all groups, namely groups A, C, D, E, and F (LSD, $p = 0.000$).

3.4 Mechanisms of Liver Damage Prevention and Interleukin-6 Decrease

The objectives of this study, namely the mechanisms that prevent liver damage and decrease Interleukin-6 levels caused by the use of shallot extract, were addressed through path analysis of the relationships among variables. The conceptual framework of this study served as the basis for the path analysis. The Smart Partial Least Squares (SmartPLS) version 3.3.2 program application was used to conduct the path analysis of the relationship. The t-statistic at the 5% significance level is used to determine the significance of the path analysis. If the t-statistic value is greater than 1.96, then the path analysis is considered significant. In this study, each variable has only one construct and one indicator, so the Standardised Loading Factor (SLF), Composite Reliability (CR), and Average Variance Extracted (AVE) values are not required for the analysis. In the inner model, path coefficients and significance values (t-statistic) are considered. Figure 1 shows the structural model, and Figure 2 shows the results of the path analysis of the relationship between variables in this study:

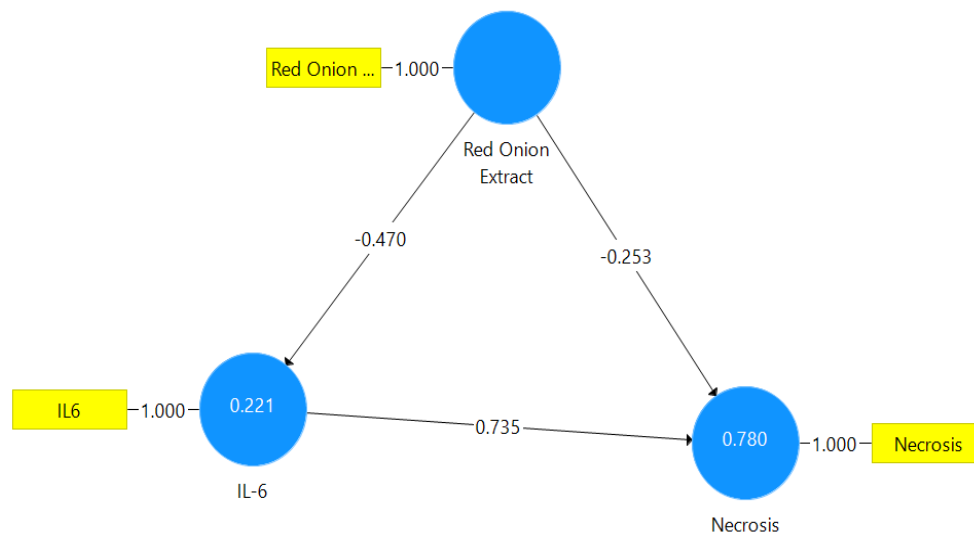


Figure 1: Inner Model with Path Coefficient Value

The results of pathway analysis of the mechanism of preventing liver damage and reducing interleukin-6 by administering shallot extract are shown in Figure 1:

1. The test value p-value = 0.000 ($p\text{-value} < 0.05$), indicating that red onion extract has a significant effect on IL-6, with a path coefficient $\beta = -0.470$ (the greater the coefficient, the closer the relationship). Administering red onion extract can reduce IL-6 expression. The negative sign of the standardized coefficient value indicates that as the red onion extract variable increases, the IL-6 expression variable decreases, and vice versa. If the red onion extracts variable decreases, the IL-6 expression variable increases.
2. The test value of p-value = 0.000 ($p\text{-value} < 0.05$), indicating that the effect of red onion extract on necrosis is significant, with a path coefficient $\beta = -0.253$ (the greater the coefficient, the closer the relationship). Administering red onion extract can reduce IL-6 expression. The negative sign of the standardized coefficient value indicates that as the red onion extract variable increases, the IL-6 expression variable decreases, and vice versa. If the red onion extracts variable decreases, the IL-6 expression variable increases.
3. Test value p-value = 0.000 ($p\text{-value} < 0.05$). IL-6 expression has a significant effect on necrosis, with a path

coefficient $\beta = 0.735$ (the greater the value, the closer the relationship). The positive sign of the standardized coefficient value indicates that if the IL-6 expression variable increases, the necrosis variable increases, and vice versa. If the IL-6 expression variable decreases, the necrosis variable decreases.

In Figure 1, this model analysis examines the structure of each research variable based on the path coefficient values shown. Meanwhile, the R-squared (R2) value shown in Figure 1 is the value of the great diversity of the dependent variable. R2 IL-6 = 0.221; R2 necrosis = 0.780. The magnitude of the effect of giving shallot extract on preventing liver damage and reducing interleukin-6 is necrosis (78%) and IL-6 (22.1%).

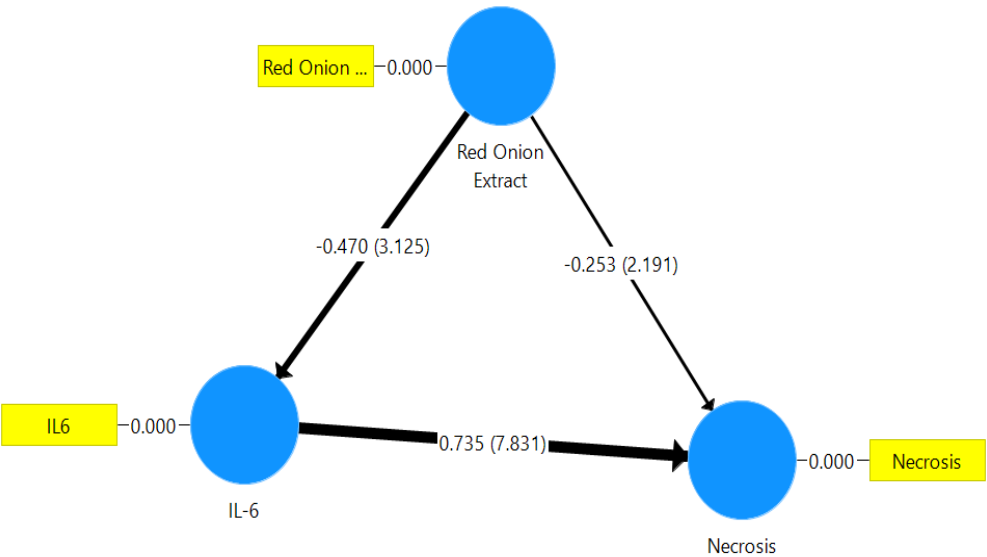


Figure 2: Mechanisms of Prevention of Liver Damage and Reduction of Interleukin-6 by Administration of Onion Extracts

In Figure 2, the inner model analysis is conducted to examine the influence among variables according to the research conceptual framework and the strength of that influence, thereby answering how administering shallot extract prevents liver damage and reduces Interleukin-6. This path analysis is conducted by examining the significance of the t-statistic in the bootstrapping calculation. The t-statistic is considered valid if it exceeds 1.96. Based on Figure 2, the path coefficient values and t-statistics are presented in more detail in Table 4 below.

Table 4: Path Analysis of the Relationship Between Variables

The Influence of Variables	Path Coefficient	T-statistic	Description
EB → IL-6	-0.470	3.125	Significant, inversely proportional
EB → Necrosis	-0.253	2.191	Significant, inversely proportional
IL-6 → Necrosis	0.735	7.831	Significant, directly proportional

At a confidence degree of 95% or $\alpha = 5\%$, the p-value test value is considered significant if it is less than $\alpha = 0.05$ (p-value < 0.05), while it has no significant effect if it is more than $\alpha = 0.05$ (p-value > 0.05). The path coefficient shows the closeness of the relationship between variables. The greater the path coefficient, the stronger the correlation between variables. A positive value on the path coefficient indicates a positive correlation: the higher the path coefficient of a variable, the more it increases or drives the dependent variable. Meanwhile, a negative value indicates a negative correlation, meaning that the higher the path coefficient of a variable, the more it will reduce or inhibit the dependent variable.

4. Discussion

4.1 Blood Glucose Reduction

The purpose of the post-treatment blood glucose level examination (GDP Post) was to determine how blood glucose levels had changed after the use of red onion extract. The results demonstrated a difference in average blood glucose at the end of the study. Fasting blood glucose levels above 126 mg/dL are the diagnostic criterion for DM. In Wistar rats with hyperglycemia, alloxan can increase pre-treatment blood glucose levels. In this study, the experimental animals were given hyperglycemia at a dose of 120 mg/kg BW/day from alkan. This is due to the diabetogenic effect of alloxan on pancreatic beta cells. Alloxan damages pancreatic beta cells responsible for insulin production, leading to decreased or absent insulin secretion, which in turn causes hyperglycemia (Sekiou et al., 2019). As a result, alloxan is often used in research to cause

diabetes in laboratory animals.

Alloxan damages pancreatic beta cells, the cells that produce the hormone insulin, resulting in elevated blood glucose levels. By facilitating the entry of glucose into cells for storage or use as an energy source, insulin plays a crucial role in regulating blood glucose levels (Abdulkareem et al., 2019). Alloxan enters the body through the GLUT2 transporter of pancreatic cells. Inside the cells, alloxan generates reactive oxygen species, also known as free radicals. Oxidative stress harms cell structure and function, rendering it impossible for cells to produce adequate insulin (Ajiboye et al., 2019). As a result, glucose cannot enter the cells and remains in the bloodstream, causing hyperglycemia, or high blood glucose levels.

4.2 Prevention of Liver Damage

The purpose of this study was to determine the extent to which red onion extract contributed to the reduction in hepatic necrosis induced by alloxan. The results showed a significant difference in the average change in necrosis among the treatment groups receiving red onion extract (D, E, and F) compared to the positive control group (B) given alloxan. The highest mean increase in the number of necrosis-positive cells was observed in group B (3,550), which served as the positive control group for alloxan. In contrast, the lowest value was found in group F (1,300); the treatment group received 800 mg/kg of red onion extract daily. Red onion extract dose of group F showed significantly different results from group D. It happens because the content of active compounds is more than the red onion extract dose of 200 mg/kg BW/day in group D. Similarly, group D, which was given red onion extract, showed significantly different results from group A.

This is likely since in group D, the dose of red onion extract is lower, so the decrease in necrosis is not as significant as in the normal group (group A), which received no therapy. Still, group D has been able to prevent liver damage by decreasing necrosis. All groups given red onion extract have reduced liver damage, as evidenced by the difference between group B, induced by alloxan, and group C, which shows a decrease in average necrosis. This indicates that administering red onion extract reduces the average level of necrosis. The dose of metformin (120 mg/kg BW) differs from that of the normal group (A). This is because metformin can reduce the average necrosis, but cannot heal or permanently regenerate damaged cells. Metformin only affects the muscles and liver to lower blood sugar. If no other ingredients protect or regenerate pancreatic cells, such as red onion extract or potent antioxidant herbal ingredients, cell healing does not occur.

Statistical analysis of necrosis revealed that group B, the alloxan-positive control group that received no therapy, exhibited relatively high rates of necrosis. This group was significantly different from all other groups, including the negative control group that received red onion extract and metformin therapy. The higher necrosis rate in group B was due to the induction effect of alloxan. When alloxan, a substance frequently used to generate diabetes in lab animals, is administered, it can result in liver necrosis or damage to the liver cells. Alloxan can induce the body to produce numerous free radicals, which can damage liver cells and lead to necrosis (Elderbi et al., 2025). It can decrease insulin production if alkaloids compromise the integrity of pancreatic beta cells in the Langerhans islets. This may be due to the toxic nature of alloxan to pancreatic beta-cells. Alloxan can inhibit the action of glucokinase in pancreatic cells and generate free radical species through repeated redox reactions, as its structure is similar to that of glucose. Using glutathione (GSH) reduces the accumulation of alloxan in pancreatic cells, thereby reducing the production of dialuric acid.

Autooxidation will occur in the products, reducing them to alloxan. These redox cycles lead to decreased antioxidant levels and increased free radicals, both of which can harm pancreatic cells. The steadily rising oxidative stress significantly influences the development of diabetic problems in various tissues in diabetics. The body uses the liver as the primary organ for detoxification and oxidative processes; in diabetes, hepatic oxidative stress biomarkers increase. Diabetes, hepatocellular carcinoma, and nonalcoholic steatohepatitis are caused by oxidative stress-mediated hepatic damage. Oxidative stress is an imbalance between the generation of ROS and cells' ability to detoxify them, leading to cell damage. One of the many causes of increased ROS in DM is the chain reaction involving enzymatic and non-enzymatic processes and mitochondrial respiration. Conversely, in diabetic conditions, antioxidant activity is suppressed, leading to increased oxidative stress. Lipid peroxidation and damage to cell membranes and organelles, including mitochondrial membranes, are caused by increased oxidative stress. All of this prevents hepatocytes from oxidising free fatty acids, resulting in fatty liver or excessive triglyceride deposition in the liver.

The findings of this investigation are consistent with the research carried out by Emamat et al. (2015), who found that the histopathological condition of the liver improved when onion extract was administered. Fancy onions, which contain numerous flavonoids, particularly quercetin, exhibit hypoglycemic effects and may benefit individuals with diabetes. Hyperglycemia, which causes oxidative stress, can be improved in the presence of antioxidants. In another study, DALIA et al. (2019) discovered that red onion extract decreased severe liver damage-induced necrosis and elevated liver enzyme levels. In animal studies, Quecan et al. (2019) have shown that red onion extract increases antioxidant activity in liver tissue. As a major flavonoid antioxidant, onion extract contains quercetin, which can reduce oxidative stress by preventing oxidative damage to liver cell membranes and eliminating free radicals. In addition, it can reduce inflammation and programmed cell death in the liver.

The organic sulfur compounds, such as allicin and diallyl disulfide, may support liver detoxification (by assisting phase I and II enzymes) and act as hepatoprotective agents (protecting liver tissue from toxins) by promoting liver cell regeneration. Saponins and tannins may also function as anti-inflammatory and antioxidant agents, protecting the liver from fibrosis and necrosis induced by toxic stress (Nugraha et al., 2023). Thus, it can be concluded that quercetin and sulfur compounds in shallots play a crucial role in preventing hepatic necrosis by reducing oxidative stress and inflammation and facilitating faster liver detoxification. Red onion extract may also have hepatoprotective effects, which can minimize necrosis in tested animals with diabetes mellitus.

4.3 Reduction of Interleukin-6

Group B, the untreated diabetic control group, showed relatively high IL-6 expression results. This group was different from all control groups, both treated diabetic and non-diabetic, based on statistical analysis of IL-6 expression. This suggests that IL-6, a cytokine, plays a role in the formation of diabetes mellitus. In their study, Fadaei et al. found that people with type 2 diabetes had high serum concentrations of IL-6 (Fadaei et al., 2020). This could worsen type 2 diabetes and be an indication of the disease. Patients with type 1 diabetes have higher levels of IL-6, and type 1 diabetes research also benefits from IL-6. Compared to those without diabetes, patients with type 1 diabetes have stronger artery walls and greater serum levels of IL-6. According to Fadaei et al. (2020), in non-obese diabetic (NOD) mice, IL-6 production in pancreatic beta cells increases inflammation, and IL-6 is required for the development of diabetes in NOD mice.

IL-6 plays a role in both type 1 diabetes and type 2 diabetes, according to some studies (Zhao et al., 2021). IL-6 can affect the body's metabolism, including its role in diabetes mellitus and other metabolic diseases. Examined how IL-6 contributes to type 2 diabetes. The results suggest that a small proportion of type 2 diabetes mellitus is caused by an autoimmune reaction to IL-6. If pancreatic beta cells produce high levels of IL-6, this can lead to hyperglycemia and alter GLUT-2 transporter expression. A transmembrane protein called GLUT-2 is often present in high concentrations in pancreatic beta cells and is necessary for the cellular uptake and metabolism of glucose. Inflammatory factors, such as prostaglandins, can be generated due to local IL-6 expression. This may affect GLUT2 or insulin expression and worsen the severity of diabetes mellitus.

Although inflammation can also cause localized damage to tissues, it serves as a defense against infection and facilitates tissue repair. The inflammatory response is associated with a range of plasma protein types and proinflammatory cytokines. Both chronic and acute inflammation result in the production of proinflammatory cytokines, such as IL-6. Diabetes mellitus is an example of a condition associated with proinflammatory cytokines. The pathophysiology of pancreatic damage is caused by interleukin-6, which is present in the early phases of the inflammatory response. However, IL-6 expression was lower in the diabetic group that received treatment. There was a notable difference between the P3 group, which received 800 mg/kg of onion extract daily, and the K2 group, which received metformin. This implies that metformin and shallot extract are nearly equally effective in reducing IL-6 expression, a proinflammatory cytokine that exacerbates diabetes mellitus. Flavonoids and saponins were found in the ethanol extract of Dayak onion bulbs. By inhibiting the release of inflammatory mediators, saponins are known to improve anti-inflammatory efficacy and increase membrane permeability (Wijesekara et al., 2024). Flavonoids have anti-inflammatory effects by inhibiting the inducible cyclooxygenase-2 (COX-2) enzyme, which is found in inflammatory tissues. The use of alloxan can lead to an increase in free radicals, resulting in inflammation and the release of proinflammatory cytokines (Malik et al., 2022). An increase in the number of IL-6-positive cells indicates increased proinflammatory cytokine levels. The administration of red onion extract can also halt the progression of inflammation by reducing inflammation and cell damage in the pancreas, as indicated by a decrease in the number of IL-6-positive cells. Only when free radicals are present in excess does IL-6 increase, as this triggers the production of proinflammatory cytokines. Consequently, the results of this study suggest that shallot extract can function as an anti-inflammatory agent by reducing IL-6 expression in experimental animals with hyperglycemia.

4.4 Analyze the Mechanism by which Red Onion Extract Prevents Liver Damage and Reduces the Level of IL-6

Based on Table 4 and Figures 1 and 2, the administration of shallot extract significantly reduces IL-6 expression and liver necrosis. The decrease in IL-6 expression also significantly reduces necrosis in *Rattus norvegicus* Strain Wistar. The greater the path coefficient, the stronger the correlation. The closest correlation, with a path coefficient ranging from 0.253 to 0.735, is between red onion extract treatment and IL-6 (negative), followed by necrosis (also negative). At the same time, the correlation of IL-6 with necrosis is positive. The purpose of this study was to investigate and analyze the potential of onion extract in preventing liver damage and reducing interleukin-6 levels. Different variables showed direct and indirect relationships. By reducing various cytokines, including IL-6, the inflammatory process in cells was decreased. Administration of red onion extract also directly reduced necrosis by 78%, thereby preventing liver damage. In response to trauma or tissue necrosis, macrophages and other cells release IL-6.

In onion extracts, a variety of bioactive compounds can be found that act as anti-inflammatory and antioxidant agents. These include phenolic acids and anthocyanins, as well as organic sulfur compounds such as allyl propyl disulfide and S-methylcysteine sulfoxide. The mechanism of action of these compounds is by stopping the activity of the NF- κ B signaling pathway, which regulates the expression of IL-6 and other inflammatory cytokines, decreasing the oxidative stress that causes IL-6 production and reducing the number of immune cells that enter damaged tissues, such as the liver (Khan et al., 2020). Previous studies have demonstrated that administering onion extract reduces tissue or serum IL-6 levels. Additionally, other studies have shown that administering onion extract can also decrease tissue and serum IL-6 levels. Bioactive compounds found in shallot extracts, including flavonoids (such as quercetin), sulfur compounds, and anthocyanins, which function to inhibit the activation of inflammatory pathways, have been linked to this reduction in IL-6 levels (Zhao et al., 2021). As a result, shallot extract may be used as a phytotherapy to reduce the inflammatory response in diseases such as diabetes mellitus.

Red onion extract has been shown to reduce the rate of necrosis and protect liver tissue. The content of active compounds, such as flavonoids (including quercetin), sulfur compounds, and anthocyanins, which possess antioxidant and anti-inflammatory properties, is believed to be responsible for this effect. In the study conducted by Elderbi et al. (2025), alloxan-induced experimental animals experienced liver cell necrosis. However, the group given onion extract (250-750 mg/kg BW) experienced lower liver cell necrosis than the negative control group, approaching the negative control group's level of necrosis. This is because these compounds can neutralise free radicals and prevent lipid peroxidation. In another study, Wistar rats had increased histopathology scores of liver cell damage due to diazinon (Elshafae et al., 2023). However, after treatment with red onion skin extract at 1200 mg/kg BW for 7 days, the damage scores returned to normal levels. This indicates that the ethanol extract of shallot skin can repair liver damage in Wistar rats. This study was limited to Wistar rats with a short intervention duration. Therefore, it needs to be developed in humans in the future. However, this study has demonstrated the potential of red onion extract as a hepatoprotective and anti-necrotic agent, which can prevent liver damage and reduce inflammation by decreasing IL-6 levels, thanks to its antioxidant compounds that combat oxidative stress and inflammation.

5. Conclusion

This study found that the dose of red onion extract varied in its ability to reduce IL-6 expression and necrosis, with an effective dose of 800 mg/kg BW per day. The mechanism of red onion extract directly reduces necrosis and IL-6 levels. This study can provide the public with information about the potential benefits of red onions as an alternative treatment for individuals with diabetes mellitus. To benefit from a more direct link to broader public health impact. Emphasize the potential clinical implications and relevance to diabetes management.

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7. References

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