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Identification of Gc-Ms Nanoemulsion Preparation of Temulawak Extract (Curcuma Zanthorrhiza Roxb) in Improving Kidney Function and Histopathological Features of the Kidneys of Diabetes Mellitus Rats

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ABSTRACT

The kidneys are the main organs that can excrete drugs..One of the causes of kidney damage in a person is due to continuously high blood glucose conditions which can lead to diabetes mellitus.This study aims to identify the GC-MS nanoemulsion preparation of temulawak extract (Curcuma Zanthorrhiza Roxb) in improving kidney function and histopathological features of the kidneys of diabetic rats. The sample of rats used was 20 in 5 groups (positive and negative controls, treatments 1, 2 and 3) where the extract preparation in the treatment was in the form ofnanoemulsion of temulawak extractondoses of 50 mg/kgbb, 100 mg/kgbb, 150 mg/kgbb. The results areCompounds such as xanthorrhizol, germacrone, curcumin, and β-caryophyllene are the main candidates in temulawak extract that contribute to its antidiabetic effects. The extract was effective in treating diabetes in mice and improving kidney function at a dose of 150 mg/kg body weight.

Keywords: Curcuma, Nanoemulsion, Kidney, Diabetes

INTRODUCTION

Based on data from the Health Insurance Administration Agency (BPJS)(Tempo, 2024), the number of cases of kidney failure sufferers in 2019 was 1.93 million with costs incurred by the government in handling this problem amounting to IDR 2.79 trillion. From this data, it can be seen that sufferers of kidney failure are increasing day by day due to unhealthy lifestyle patterns which result in damage to kidney function.

The kidneys maintain the body's homeostasis by controlling the concentration of plasma components, especially electrolytes and water, and excreting metabolic waste.(Sherwood L., 2014)The kidneys are one of the body's most important organs, responsible for filtering various waste products from the blood before they are excreted through urine.This organ is also responsible for filtering blood before returning it to the heart.The kidneys are the primary organs responsible for drug excretion. Through urine, the kidneys excrete substances the body no longer needs.

Diabetes is a disease with the criteria of high blood sugar levels (hyperglycemia), diabetes is a disease that must be paid attention to because it is a long-lasting disease.(Piero MN, Nzaro GM, 2014)Age, in addition to genetics, can be a contributing factor to diabetes mellitus. The risk of developing type 2 diabetes increases with age. Long-term consumption of sweet and high-sugar foods can lead to diabetes.(Spătărelu & Popa, 2021).

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In adults with long-term kidney disease, antioxidants do not reduce the risk of death but can improve kidney function, thereby reducing the risk of kidney failure and cardiovascular disease. The use of herbal medicines derived from plants and herbs can benefit organ health due to their high antioxidant content. These herbs are plants that have high antioxidant content. Antioxidants are compounds that can counteract or reduce the negative effects of oxidants in the body.(Mawarni et al., 2020).

Temulawak rhizome is the primary ingredient in traditional medicine. Temulawak's benefits include maintaining health, improving health, and treating disease. The active component responsible for its antioxidant properties is curcumin.(Regitha Claudia, 2024). Temulawak in(Dr. Budiyanto, 2025)It's also been described as improving kidney function. Curcuma works by maximizing the organ's ability to cleanse the blood of toxins and metabolic waste through the consumption of the plant's essential oils. Regular consumption of this herb will provide the benefits derived from the plant's phellandrene content. Toxins are excreted from the body in urine, feces, and sweat.

In line with its presentation, identification of compounds contained in the extract can also use the GC-MS method. Gas chromatography-mass spectrometry (GC-MS) is a method that combines gas chromatography and mass spectrometry to identify different compounds in sample analysis. Gas chromatography-mass spectrometry (GC-MS) is a method that combines gas chromatography and mass spectrometry to identify different compounds in sample analysis.(Sparkman DO, Penton Z, 2011).

Based on this background, the researcher is interested in conducting research with the title "GC-MS identification of nanoemulsion preparation of temulawak (Curcuma Zanthorrhiza Roxb) extract in improving kidney function and histopathological features of the kidneys of diabetic rats".

LITERATURE REVIEW

According to the American Diabetes Association in 2010, Diabetes Mellitus (DM) is a type of metabolic disease that causes hyperglycemia due to abnormalities in insulin secretion or insulin function or both (Ndraha, 2014).Here are some types of complications that may occur due to diabetes in general through the article(Makarim, 2024)such as heart disease, damage to the retina of the eye, the walls of small blood vessels, etc.

The kidneys are one of the most important organs in the body, tasked with filtering various wastes in the blood before they are excreted through urine.(Rahayu, 2024)The kidneys maintain fluid balance in the body, control and filter minerals from the blood, remove waste from food, drugs, and toxic substances, and produce hormones that help form red blood cells, promote bone health, and regulate blood pressure.(Wahyuni FS, Putri IN, 2017)**.** Glomerular disease can be caused by many different diseases, some of which are infections, some may be the result of diseases that affect the whole body, such as diabetes or lupus.(Maya Sari, 2015). High blood glucose resulting in Diabetes mellitus, known as hyperglycemia, causes damage to the walls of blood vessels, which leads to microvascular complications, including diabetic nephropathy.

Temulawak has many health benefits due to its ingredients. Besides helping improve the body's digestive processes, it also helps relieve muscle fatigue.(Dr. Budiyanto, 2025). Temulawak has many benefits, such as analgesic, anthelmintic, antibacterial and antifungal, antidiabetic, antihepatotoxic, anti-inflammatory, antioxidant, antitumor, and central nervous system depressant properties. Additionally, it is diuretic, hypolipidemic, hypothermic, insecticidal, and central nervous system depressant.(Sina, 2013).

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METHODS

This study uses an experimental quantitative research type, namely by using a true experiment or laboratory experimental design where this study was conducted to see the GC-MS identification of nanoemulsion preparations of temulawak extract (Curcuma Zanthorrhiza Roxb) in improving kidney function and histopathological features of the kidneys of diabetic rats.The research design uses a post-test with control group design.

The research sample was male Wistar rats (Rattus norvegicus), with four per group. In this study, researchers used 20 Wistar rats for each experimental group. The experimental groups were divided into positive and negative control groups, and treatment groups 1, 2, and 3. The animals were acclimatized for 7 days in the laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra.

MakingA nanoemulsion of temulawak extract using 30% temulawak extract was developed. The results were administered to mice once daily for 14 days.and the method of administering the extract to the sample using a gastric tube. ExaminationThe compound content in the extract was identified using GC-MS using a Gas Chromatography-Mass Spectroscopy tool.

Administering treatment to treatment groups 1, 2 and 3 usingnanoemulsion of temulawak extractondose 50 mg/kgbb, 100 mg/kgbb, 150 mg/kgbb. Rat kidney function was assessed by measuring urea and creatinine levels, as seen from blood sample tests of rats that had experienced increased blood glucose levels, then comparing them before and after the extract was administered. Histopathological images of rat kidneys were obtained using a microscopy microscope at 400x magnification.Normal levels of urea are 15-21 mg/dl and creatinine 0.2-0.8 mg/dl.Measuring blood glucose in mice using a blood glucose meter. ThenData from the research results were tabulated and analyzed with the help of SPSS (Statistics Package for Social Science).

RESULT

With the use of the amountThe research sample, namely 20 male rats, will be divided into 5 groups, namely positive and negative controls, treatment group 1, treatment 2 and treatment 3, where each group consists of 4 rats.In this study, mice were induced with diabetes mellitus with 55 mg/kg body weight of alloxan. for 14 days under observation every 3 days intraperitoneally to determine blood glucose in mice.

OnThe nanoemulsion formulation of Javanese turmeric extract showed satisfactory results based on various characterization parameters. The small particle size and uniform distribution ensure system stability and increase the possibility of active substance absorption through biological membranes. The high zeta potential value supports long-term stability without the need for additional preservatives. The higher antioxidant activity in the nanoform indicates the potential for increasing the pharmacological effectiveness of Javanese turmeric extract in the nanoemulsion system.

And on observationGC-MS (Gas Chromatography-Mass Spectrometry) on the nanoemulsion of temulawak extract, several compounds were detected, namely:

Table 1. GC-MS Identification of Nanoemulsion Compounds of Temulawak Extract

No	Compound	Category	Activity
1	Xanthorrhizol	Sesquiterpenes	Antibacterial, anti-inflammatory, anticancer

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2	α-Curcumene	Sesquiterpenes	Anti-inflammatory
3	Ar-turmerone	Turmerone (ketone)	Antioxidant, antimicrobial
4	β-Caryophyllene	Sesquiterpenes	Antioxidant, analgesic
5	Germacrone	Sesquiterpenes	Anticancer, antimicrobial
6	Curzerenone	Sesquiterpenes	Anticancer, anti-inflammatory
7	D-Limonene	Monoterpenes	Antioxidant, natural solvent

Confirmed the presence of key active compounds such as xanthorrhizol, ar-turmerone, and others, which are key markers of pharmacological activity. Compounds such as xanthorrhizol, germacrone, curcumin, and β-caryophyllene are the main candidates in temulawak extract that contribute to its antidiabetic effects.

If the rat is said to have diabetes, the extract is given to itsee successnanoemulsion of temulawak extract (Curcuma Zanthorrhiza Roxb) decreased levels of urea, creatininein mice that have experienced diabetes mellituswhich was then observed histopathologically on the rat kidneys.After the procedure, the test animals' glucose, urea, and creatinine levels were measured again to determine any reductions following administration of the temulawak extract nanoemulsion. Normal creatinine levels in mice range from 0.2 to 0.8 mg/dL. Normal urea levels in mice range from 15 to 21 mg/dL. After 14 days of treatment, the mice were euthanized under anesthesia and a laparotomy was performed to remove the kidneys. After the kidneys were removed, the mice were buried..

Reporting Research Results

In the observation of blood glucose levels in mice at the beginning of the study (day 0), after induction (day 14) and after being given the treatment of temulawak extract nanoemulsion in treatment groups 1, 2 and 3 on (day 28).Normal blood sugar levels in mice are65.97 - 97.89 mg/dl. Here's the data:

Table 2. Average Glucose (mg/dL) of Mice During the Study

Group	Beginning (D-0)	After Induction (D-14)	After Extraction (D-28)
*ControlNegative	78	82	85
Positive Control	76.5	338	175
Treatment 1 (P1)	74.8	329	109
Treatment 2 (P2)	87	358	96
Treatment 3 (P3)	85	345	93

Note: * not induced diabetes

From the table above, it can be seen that on day 0, all research groups had an average normal glucose level, while after induction of diabetes using alloxan 55 mg/kgbb. On day 14, the positive control group, treatment 1, 2, and 3 experienced diabetes mellitus with glucose levels of 338 mg/dL, 329 mg/dL, 358 mg/dL, and 345 mg/dL. And after being given treatment for 14 days, the results were seen on day 28 in the other test group, namely the positive control group that was given metformin treatment for 14 days at a dose of 200 mg/dL, experienced a decrease in blood glucose with an average of 175 mg/dL, but was still in a high glucose condition in mice. For Treatment Group 1 (P1) with the administration ofnanoemulsion of temulawak extract dose50 mg/kg body weight also experienced a decrease in glucose to an average of 109 mg/dL and was still above normal glucose levels in mice, in treatment group 2 (P2) administrationnanoemulsion of temulawak extract dose100

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mg/kg body weightalso experienced a decrease in glucose to an average of 96 mg/dL and treatment group 3 (P3) was givennanoemulsion of temulawak extract dose150 mg/kg body weightalso experienced a decrease in glucose to an average of 93 mg/dL.

Then p is doneMeasurement of urea levels after mice were given treatment in each groupAs is known, normal urea levels in mice range from 15 to 21 mg/dL. Here are the results of the mice's urea levels:

Table 3. Data on the Results of Urea Level Tests in Rats

	Group					Sig
	K- n=4 Mean±SD	K+ n=4 Mean±SD	P1 n=4 Mean±SD	P2 n=4 Mean±SD	P3 n=4 Mean±SD	
urea	18.5±1.25	24.9±1.16	20.3±0.10	18.21±0.13	17.3 ± 0.47	
Shapiro Wilk	0.860	0.057	0.957	0.124	0.806	
Homogeneity						0.065
One-Way ANOVA						0.00

Then, we also conducted similar observations of kidney function through the rats' creatinine levels. As is known, normal creatinine levels in rats range from 0.2 to 0.8 mg/dL. The following are the rats' creatinine levels:

Table 4. Data on the Results of the Rat Creatinine Level Test

	Group					Sig
	K- n=4 Mean±SD	K+ n=4 Mean±SD	P1 n=4 Mean±SD	P2 n=4 Mean±SD	P3 n=4 Mean±SD	
Creatinine	0.74±0.37	1.02±0.95	0.77±0.28	0.60±0.26	0.59 ± 0.40	
Shapiro Wilk	0.976	0.420	0.962	0.369	0.304	
Homogeneity						0.084
One-Way ANOVA						0.00

After observing kidney function through urea and creatinine levels, the next step is to look at kidney function and describe it through histopathological images of kidney function to determine the impact of kidney function that has experienced diabetes mellitus and provide a score for these observations, the following are the results of the observations:

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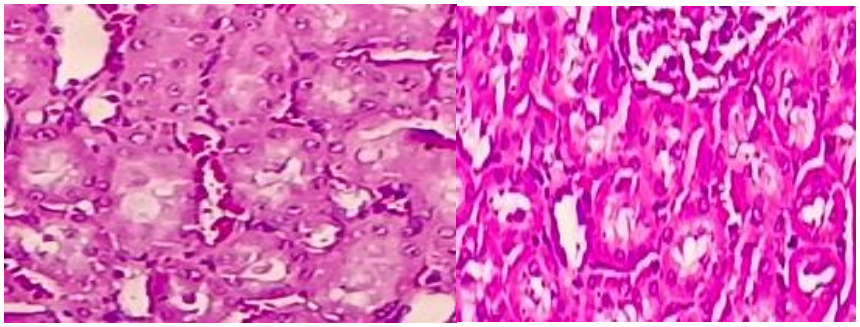

Figure 1. Kidney Histopathology of Negative Control Group (K-)

In the image of the kidney organs in the negative group (K-), the histopathology of the kidneys shows a normal structure.

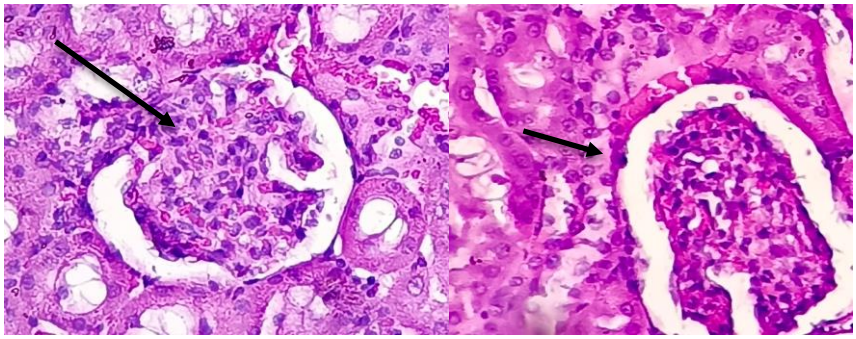

Figure 2. Kidney Histopathology Positive control group (K+)

In the image of the kidney organs in the positive group (K+), necrosis and bleeding are seen in the kidney cells (Bowman's Spatium Edema or bleeding), so this group gets a score of 3 for necrosis.

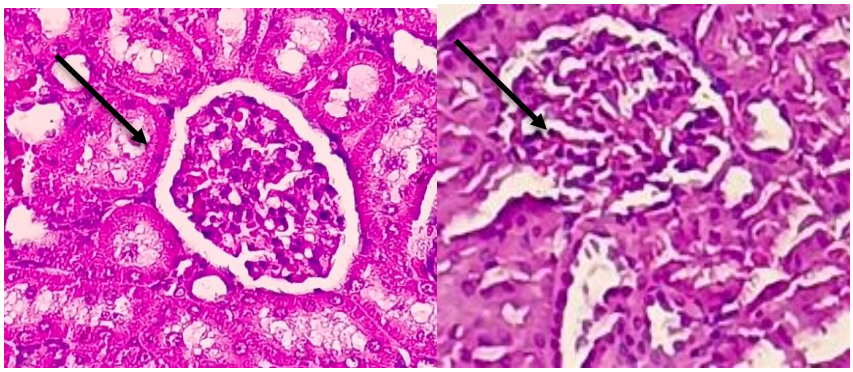

Figure 3. Kidney Histopathology of Treatment Group 1

In the image of the kidney organs in treatment group 1, the histopathological image of the rat kidneys shows necrosis and bleeding (Bowman's Spatium Edema or) so this group gets a score of 3.

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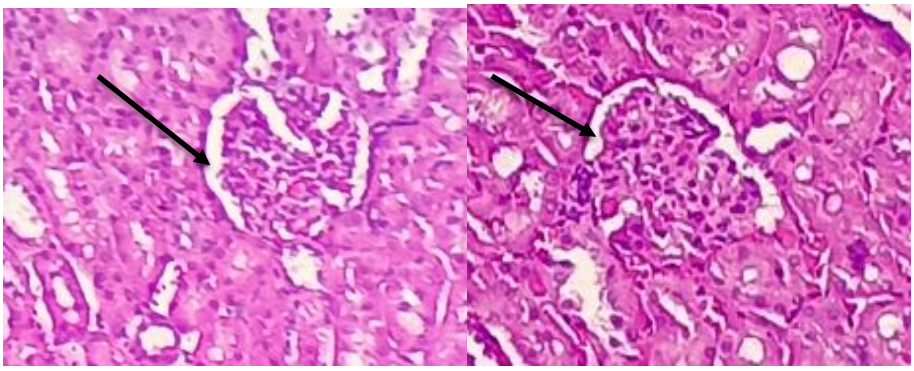

Figure 4. Kidney Histopathology of Treatment Group 2

In the image of the kidney organs in treatment group 2, there was an improvement in the kidney structure, although there was still visible Bowman's spat edema or bleeding, so treatment group 2 received a score of 2.

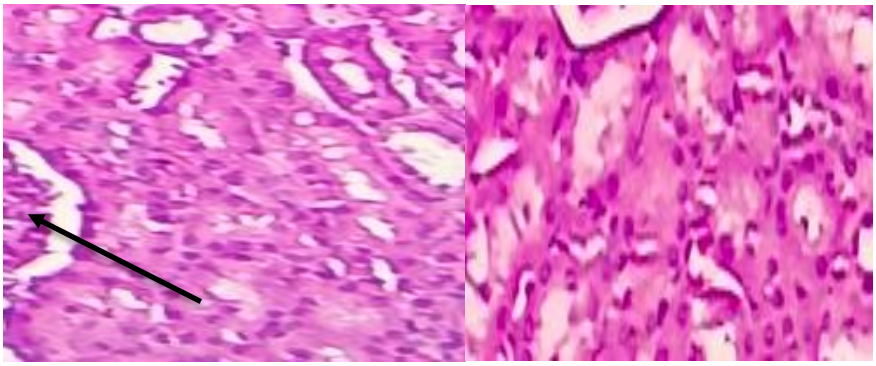

Figure 5. Kidney Histopathology of Treatment Group 3

In the image of the kidney organs in treatment group 3, improvements in the kidney structure were seen, although there was still an infection in the kidney organs, so treatment group 3 received a score of 1.

DISCUSSION

Diabetes is a disease with the criteria of high blood sugar levels (hyperglycemia), diabetes is a disease that must be paid attention to because it is a long-lasting disease.(Piero MN, Nzaro GM, 2014)The risk of developing type 2 diabetes increases with age. Long-term consumption of sweet and high-sugar foods can lead to diabetes.(Spătărelu & Popa, 2021). Temulawak rhizome is the primary ingredient in traditional medicine. Temulawak's benefits include maintaining health, improving health, and treating disease. The active component responsible for its antioxidant properties is curcumin.(Regitha Claudia, 2024).

With the use of the amountThe research sample, namely 20 male rats, will be divided into 5 groups, namely positive and negative controls, treatment group 1, treatment 2 and treatment 3, where each group consists of 4 rats.Before the data was analyzed, the administration of diabetes mellitus treatment using a nanoemulsion preparation at doses for treatment groups 1, 2 and 3, namely55 mg/kg, 100 mg/kg, and 150 mg/kg. The negative control group (K-) was not given any treatment, while the positive control group was given metformin 200 mg/kg. The extraction was administered for 14 days.

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In the observation of blood glucose levels of mice at the beginning of the study (day 0), after induction (day 14) and after being given treatment with temulawak extract nanoemulsion in treatment groups 1, 2 and 3 on (day 28). With the results on day 0 all research groups had an average normal glucose level, while after induction of diabetes using alloxan 55 mg / kg body weight. On day 14 the positive control group, treatments 1, 2 and 3 experienced diabetes mellitus with glucose levels of 338 mg / dL, 329 mg / dL, 358 mg / dL, and 345 mg / dL. And after being given treatment for 14 days, the results were seen on day 28 in the other test group, namely the positive control group (K +) experienced a decrease in blood glucose with an average of 175 mg / dL, but was still in a high glucose condition in mice. For Treatment group 1 (P1) also experienced a decrease in glucose to an average of 109 mg/dL and still above the normal glucose level in mice, in treatment group 2 (P2) also experienced a decrease in glucose to an average of 96 mg/dL and treatment group 3 (P3) also experienced a decrease in glucose to an average of 93 mg/dL. Thus in treatment groups 2 and 3, the examination of blood glucose levels on the 28th day of observation showed normal results. So that the administration ofadministration of nanomelution extractJavanese ginger (Curcuma zanthorrhiza Roxb) considered effective in reducing blood glucose levels in mice at effective doses.100 mg/kgbb and 150 mg/kgbb.

In observing urea and creatinine levels on day 28, it was seen thatnegative control group (K-)get the average and standard deviation results18.5±1.25 and the average urea levels were still within normal limits. In the positive control group (K+)get the average and standard deviation results24.9±1.16, and the average urea levels were above the normal range in mice, indicating that metformin did not significantly reduce urea levels in mice with diabetes mellitus. In treatment group 1 (P1),get the average and standard deviation results20.3±0.10, and the results showed that the rats' urea levels were within the normal range. For treatment group 2 (P2),get the average and standard deviation results18.21±0.13 indicates the normal urea level in the rats. Then, in treatment group 3 (P3), the rats were induced with alloxan and given temulawak nanoemulsion at a dose of 150 mg/kg body weight.get the average and standard deviation results17.3 ± 0.47 indicates the condition of the rat's urea in the normal category. So in treatment groups 1, 2 and 3 the urea levels have returned to normal in rats with diabetes mellitus and given treatment with nanoemulsion of temulawak extract (Curcuma zanthorrhiza Roxb). And the results of data analysis in the normality test show that all data have been normally distributed, this can be seen from the results of the Shapiro Wilk normality test data showing significance data >0.05. In the results of the homogeneity test using Levene's Test the resulting data is p = 0.065 where >0.05 so that the data is homogeneous or comes from the same variant. And in the One-way ANOVA test at sig 0.00 means that the administration of nanoemulsion of temulawak extract (Curcuma zanthorrhiza Roxb) has an effect on kidney function (ureum) in rats with diabetes mellitus.

Results of observations of creatinine levels in the negative control group (K-)get the average and standard deviation results0.74 ± 0.37 and the average creatinine level results still showed normal limits, so this group became the reference group in the study. In the positive control group (K +) obtained average results and standard deviations of 1.02 ± 0.95 and the average creatinine levels showed results above the normal limits of rat creatinine levels, so metformin did not reduce creatinine levels in rats with diabetes mellitus. In treatment group 1 (P1) obtained average results and standard deviations of 0.77 ± 0.28 and the results showed that the condition of rat creatinine had entered the normal category. For treatment group 2 (P2) obtained average results and standard deviations of 0.60 ± 0.26 indicating the condition of rat creatinine in the normal category. Then in treatment group 3 (P3) obtained average

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results and standard deviations of 0.59 ± 0.40 indicating the condition of rat creatinine in the normal category. So that in treatment groups 1, 2 and 3 creatinine levels have returned to normal in mice with diabetes mellitus and given extraction treatment. For the best results at a dose of 150 mg / kg body weight of temulawak extract nanoemulsion. Data normality test using Shapiro Wilk with the results of the overall data has been normally distributed, this can be seen from the results of the Shapiro Wilk normality test data showing significance data > 0.05. In the results of homogeneity testing using Levene's Test the resulting data is p = 0.084 where > 0.05 so that the data is homogeneous or comes from the same variant. And the results of One way ANOVA at sig 0.00 mean that the administration of temulawak extract (Curcuma zanthorrhiza Roxb) has an effect on kidney function (creatinine) in mice with diabetes mellitus.

And in the histopathological image of the kidneys of Wistar rats that have been induced by alloxan, kidney tissue damage was observed through histopathological images, and this image observation was carried out on the glomerulus and renal tubules. The glomerulus is a very small blood vessel node located in the nephron of the kidney and its function is as a filter or filtration tool for water, amino acid salts, glucose and also urea in producing urine.(Dallman, 2006).

This is in line with research (David, 2014), in reducing blood glucose in diabetic rats by using temulawak extract at an effective dose given to test animals, namely 200 mg / kg body weight. And also answered the efficacy inturmeric in(Dr. Budiyanto, 2025)which can improve kidney function, temulawak can work by maximizing the organ's ability to clean the blood of toxins and metabolic waste from the body.

CONCLUSION

1. Through GC-MS identification, the active compounds in temulawak (Curcuma zanthorrhiza Roxb) extract include xanthorrhizol, ar-turmerone, and others, which are key markers of pharmacological activity. Compounds such as xanthorrhizol, germacrone, curcumin, and β-caryophyllene are the main candidates in temulawak extract that contribute to its antidiabetic effects.
2. The effectiveness of administering a nanoemulsion of temulawak extract (Curcuma zanthorrhiza Roxb) was at a dose of 150 mg/kgbb, namely in treatment group 3 (P3).
3. The histopathological picture of the kidneys in the negative control group (K-) shows that the kidney histopathology depicts a normal structure. The positive control group (K+) shows necrosis in the kidney cells and bleeding (Bowman's Spatium Edema or bleeding), treatment group 1 shows histopathological picture of the rat kidneys showing necrosis and bleeding (Bowman's Spatium Edema). Treatment group 2 shows improvement in the kidney structure although Bowman's spatium edema or bleeding is still visible. And treatment group 3 shows improvement in the kidney structure although there is still infection in the kidney organ.

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