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GC-MS Identification of Lime Peel Extract Nanoemulsion (Citrus Aurantifolia) Against the LevelsGDP,HbA1c and MDA And Histopathological Image of Pancreas of Male Wistar Rats Induced by Streptozotocin

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ABSTRACT

Some people may not realize they have diabetes because they do not experience any symptoms..In some cases, the disease can be detected early with routine blood tests before symptoms appear.This study aims to identify the GC-MS of lime peel extract nanoemulsion (Citrus aurantifolia) against the levels ofGDP,HbA1c and MDAand histopathological picture of the pancreas of rats. Male Wistar rats induced by streptozotocin. A sample of 24 rats in 4 groups (control, treatment 1, 2, and 3) where the extract preparation in the treatment was in the form ofNanoemulsion of lime peel extract (Citrus aurantifolia) at concentrations F1 (1%), F2 (1.5%) and F3 (2%). The results showed that the compounds contained in lime extract (Citrus aurantifolia) through GC-MS analysis werelimonene, hesperidin, and limonin. Fasting blood sugar levels (GDP), glycated hemoglobin levels(HbA1c) and levelsMalondialdehyde (MDA) levels improved in the group given lime extract nanoemulsion.(Citrus aurantifolia), and the most effective is given at a concentration of 2%. In the observation of the pancreas organs of mice through histopathology of the pancreas of mice, it was seen that the administration of lime extract nanoemulsioneffective in healing pancreatic cells that suffer from diabetes mellitus.

Keywords: Lime Peel, Nanoemulsion, GC-MS, Pancreas, GDP, HbA1c, MDA

INTRODUCTION

Diabetes mellitus is classified into 2 types, namely type 1 diabetes which is thought to be caused by a combination of genetic susceptibility and environmental factors, while in type 2 diabetes mellitus the body's cells experience resistance to insulin action. Some of the causes include age, obesity, high cholesterol, high blood sugar levels and so on.(Sunu, 2022) Diabetes is a common chronic condition, especially type 2, that can be diagnosed at any age. Some people may not realize they have diabetes because they don't experience any symptoms. Elevated blood glucose levels above normal are the primary sign of the disease.(Herman, 2014)Diabetes may not show any symptoms at first. In some cases, the disease can be detected early with routine blood tests before symptoms appear.

In some cases, the disease can be detected early with routine blood tests before symptoms appear.(Drg. Widyawati, 2021)Patients with type 2 diabetes are susceptible to both short-term and long-term complications, which can lead to death. The HbA1c test is the initial step to measure glycemic levels related to complications and the risk of diabetes.(Anita Rosari

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Dalimunthe, Livinda Christy Wijaya, 2023)An HbA1c level of 6.5% or higher indicates diabetes. Routine HbA1c testing can be performed to measure glycemic levels and correlate with the risk of diabetes complications. Oxidative stress plays a key role in the pathogenesis and complications of diabetes.(Yang H, Jin X, Lam CWK, 2011)One biomarker of lipid peroxidation is malondialdehyde (MDA), the levels of which are found to be increased in diabetic patients.

The hyperglycemia of type 2 diabetes mellitus can cause cell death in both pancreatic β cells and other cells. Preventing this cell death is necessary to prevent complications in type 2 diabetes mellitus. One way to prevent this is through consuming substances containing antioxidants. The two main functions of the pancreas are exocrine, which aids digestion, and endocrine, which maintains blood sugar balance. The exocrine function of the pancreas produces digestive enzymes to break down food, facilitating its absorption.

The endocrine function of the pancreas is to produce the hormones glucagon and insulin to control blood sugar and release it into the bloodstream.(Alex, 2024)Diabetes can be caused by damage to pancreatic cells caused by chronic pancreatitis. However, if the pancreas is not functioning properly, this gland will not be able to produce digestive enzymes or the hormone insulin properly. This can lead to diseases such as diabetes and food intolerances.(Karpińska, M., & Czauderna, 2022). Therefore, it is very important to make every effort to avoid damage to the function of the pancreas caused by high blood sugar which causes diabetes.

Apart from consuming medicines and taking medical action to lower blood sugar levels, according to several studies on the properties of plants that can lower glucose levels in the blood, such asplantLime plant (Citrus Aurantifolia). Limes are a good fruit for people with diabetes. This is because they have a low glycemic index, so they won't cause blood sugar spikes. Furthermore, the vitamin C in limes can help lower blood sugar levels. Not only the fruit, but the peel also contains many compounds beneficial in medicine, especially for treating cuts, such as flavonoids.In limes, especially in the skin, it is also known that Phytochemical results show the presence of various secondary metabolic contents, namely flavonoids, alkaloids, and saponins, where these compounds act as antihyperglycemics.(Loizzo, 2012).So this plant alsoIt is thought to contain antioxidant compounds that are able to break down damage to pancreatic β cells caused by exposure to free radicals that enter the body.

In identifying the compounds contained in the extract, researchers used the gas chromatography-mass spectrometry (GC-MS) tool and method, which is a method that combines gas chromatography and mass spectrometry to identify different compounds in sample analysis.(Sparkman DO, Penton Z, 2011)This technique is commonly used to identify a compound in a gas mixture and also to determine the concentration of a compound in the gas phase.

Based on this background, the researcher is interested in conducting research with the title "Identification ofGC-MS of lime peel extract nanoemulsion (Citrus aurantifolia) on GDP levels,HbA1cand MDA and histopathological features of the pancreas of male Wistar rats induced by streptozotocin"

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 examine the GC-MS identification of lime peel extract nanoemulsion (Citrus aurantifolia) on GDP,

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HbA1c and MDA levels and histopathological features of the pancreas of male Wistar rats induced by streptozotocin. The ethics clearance letter number is 171/KEPK/UNPRI/VI/2025. The research design uses a post-test with control group design or controls the sample based on the treatment group.*post test*was carried out to see the success of the extract in reducing blood glucose levels in mice that have been inducedstreptozotocin which was then carried out histopathological observations on the pancreas of mice in each treatment group.

The sample of this study was male Wistar rats (Rattus norvegicus) weighing 160-250 grams and aged 2-3 months. The number of test animals was 6 per group. In this study, researchers used 24 Wistar rats for each experimental group. The test animals were acclimatized for 7 days in the laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. The preparation of lime peel extract nanoemulsion using the steam distillation method and the results will be given to the rat samples once a day for 14 days. Treatment was given to treatment groups 1, 2, and 3 using the concentration of lime peel extract nanoemulsion preparation.F1 (1%), F2 (1.5%), and F3 (2%).Measurement of blood glucose in mice using a blood glucose meter with Easy Touch GCU which is taken through the mouse's tail and the comparison is seen before and after being given the extract that is measured.GDP, HbA1c, and MDA. For histopathological description of the pancreas of rats using pancreatic observations using a mycopsis magnification of 400x. Diabetes mellitus rat model with streptozoticin induction within 14 days of induction. Then the data from the research results were tabulated and analyzed using SPSS (Statistics of Package for Social Science).

RESULT

Results of nanoemulsion characterization testsLime peel extract (Citrus Aurantifolia) with organoleptic test results, namely pale yellow color, distinctive lime aroma, thin viscosity (thickness) and no lumps, homogeneous phase indicating a stable emulsion. The results of the particle test of 120.5 nm using a particle size analyzer, pH 5.6 which is suitable for topical use, a speed centrifugation test with 3000 rpm in 30 minutes and the result is no phase separation and a viscosity test with a result of 15.4 cP.

In the identification of compounds using GC-MS (Gas Chromatography-Mass Spectrometry) analysis, lime peel extract contained compounds such as flavonoids in significant amounts, limonoids (limonin) andLimonene.

This study was conducted to investigate the GC-MS identification of lime peel (Citrus aurantifolia) nanoemulsion extract on GDP, HbA1c and MDA levels and histopathological features of the pancreas of male Wistar rats induced by streptozotocin. In this study, researchers used 24 Wistar rats for each experimental group. The test animals were randomly assigned into 4 test groups, namely the control group, treatment group 1, treatment group 2 and treatment group 3.

Previously, all groups of mice were acclimatized for 7 days and before the study was carried out, the mice were fasted for 12 hours and then their blood glucose was measured before streptozotocin induction was carried out. At a dose of 55 mg/kg body weight to induce diabetes mellitus, the treatment was administered by intraperitoneal injection three times a week. After 14 days, the mice's glucose levels were measured again. Mice exhibited hyperglycemia if their glucose levels were greater than 200 mg/dL.

The following are the results of the initial glucose measurements of the mice.

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Table 1. Average Initial Glucose Levels in Mice

Group	Initial Glucose Level	Information
Control	80.8	Normal
Treatment 1	85	Normal
Treatment 2	85	Normal
Treatment 3	81.5	Normal

The table above shows that all experimental groups had normal glucose levels. Then, after the mice were given an induction, their blood glucose levels were checked again.

Reporting Research Results

After being induced and re-examined, the glucose levels (FBG) of the mice were first fasted for 12 hours before blood samples were taken and re-examined for FBG 1 (after streptozotocin induction) and FBG 2 (after being given treatment) nanoemulsion of lime peel extract (Citrus aurantifolia).

As for the provisionnanoemulsion of lime peel extract (Citrus aurantifolia)was given for 14 days, so the total duration of this study was 28 days. The dosage of the extract given to each group was different, namely the control group of mice induced by streptozotocin but not given any treatment, treatment group 1 (P1) was given nanoemulsionlime peel extract (Citrus aurantifolia)with a concentration of 1%, Treatment 2 (P2) with a concentration of 1.5%, and Treatment 3 (P3) with a concentration of 2%.

As forNormal GDP levels in mice are 80-120 mg/dL, normal valueHbA1c is 3.5 – 5.5%, and the normal value of serum MDA is 1.5 – 3.5 nmol/mL. The following are the research results and data analysis:

Table 2. Results of GDP Level Observations (mg/dL),HbA1c (%) and MDA (nmol/mL)

	Group (n=4) Mean ± Standard Deviation				Normality	Homogeneity	P Value
Variables	**P0**	**P1**	**P2**	**P3**	***Shapiro Wilk***	***Levene Test***	
GDP 1	272.2±12.4	271±13.08	262.5±13.6	271.3±5.9	All Groups p>0.05	0.200	0.472
GDP 2	281±9.6	134.3±7.03	95.8±3.06	83.8±3.4	All Groups p>0.05	0.087	0,000
HbA1c	11.4±0.3	6.3±0.24	4.9±0.10	4.5±0.12	All Groups p>0.05	0.087	0,000
MDA	8.9±0.17	3.6±0.32	2.6±0.17	2.2±0.12	All Groups p>0.05	0.109	0,000

Note: GDP1 = after Alloxan Induction, GDP 2 = After Extraction Treatment

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GDP level variable data after inductionStreptozoticin (GDP 1) obtained an average GDP result in all groups > 200 mg / dL, where all groups have been declared hyperglycemia and suffering from diabetes mellitus, while the group with the highest average is in the control group (P0) with GDP results (mg / dL) 275.2 ± 12.4. So that streptozoticin induction successfully caused diabetes mellitus in mice. After data analysis, the normality test using the Shapiro Wilk test showed a p value > 0.05, the homogeneity test using the Levene test produced p > 0.05. It can be concluded that GDP 1 data is normally distributed and homogeneous. Continued one-way ANOVA testing showed a p value = 0.472 where p > 0.05 so it can be concluded that there is no significant difference between treatment groups.

GDP variable data afterfor 14 days providing treatment nanoemulsi mangosteen peel extract (GDP 2) showedThe average GDP results in the control (P0) with GDP results (mg/dL) of 281±9.6, then the mice in this group were declared to still have diabetes mellitus with glucose results > 200 mg/dL and far from the normal GDP level limits in mice. In treatment group 1 (P1) with results of 134.3±7.03, treatment 2 (P2) with results of 95.8±3.06 and treatment group 3 (P3) with results of 83.8±3.4, from these data it can be seen that there was a decrease in glucose levels so that the administration ofnanoemulsilime peel extract (Citrus aurantifolia) at concentrations of 1%, 1.5% and 2%able to lower blood glucose levels in mice and glucose levels returned to normal. After data analysis, the normality test using the Shapiro Wilk test showed a p value > 0.05, while the homogeneity test using the Levene test produced a p value > 0.05. It can be concluded that the GDP 2 data is normally distributed and homogeneous. Continued one-way ANOVA testing showed a p value = 0.000 where p < 0.05 so it can be concluded that there is a significant difference between the treatment groups. The best order of effectiveness of administeringnanoemulsilime peel extract (Citrus aurantifolia) on reducing glucose levels in rats in groups P3, P2 and P1 with concentrations of 2%, 1.5% and 1%.

Level Glycated hemoglobin or HbA1c (%) in each research group showed the highest average in the control group (P0) of11.4±0.3 while the lowest average was in treatment group 3 (P3) which was 4.5±0.13. P. So the best order of effectiveness of administeringlime peel extract (Citrus aurantifolia)the group that lowersHbA1c and reached normal values in treatment groups P3, P2 and P1 with concentrations of 2%, 1.5% and 1%.

Level Malondialdehydeor MDA (nmol/mL)in each research group showed the highest average in the control group (P0) of8.9±0.17 while the lowest average was in treatment group 3 (P3), namely 2.2±0.12. So the best order of effectiveness of administeringlime peel extract (Citrus aurantifolia)the group that lowersMDA levels and reached normal values in treatment groups P3, P2, P1 with concentrations of %, 1.5% and 1%.

Then histopathological observations were carried out on the mouse pancreas.Histopathological observations were conducted using a light microscope at 400x magnification. The purpose of this observation was to observe the structure and morphology of the cells in each pancreatic tissue specimen.

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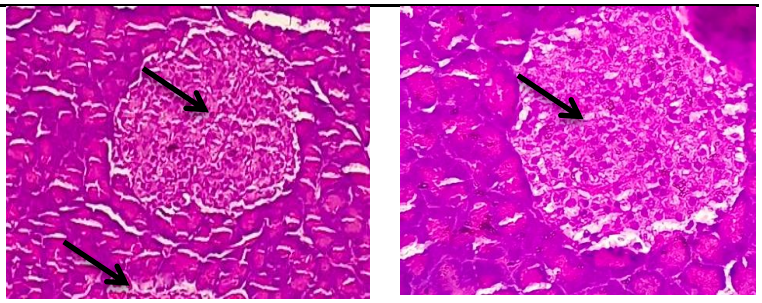
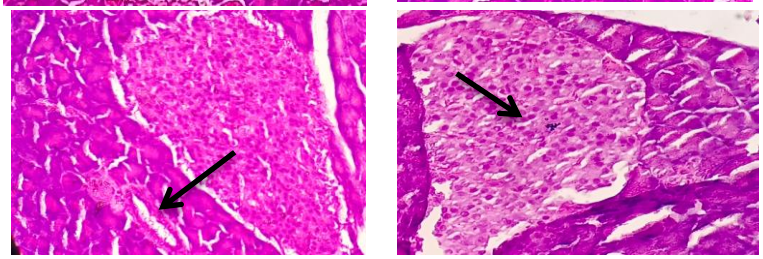
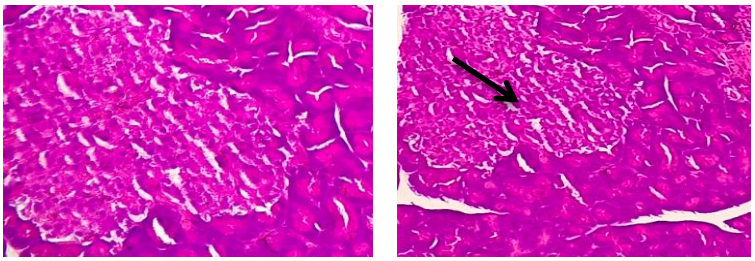
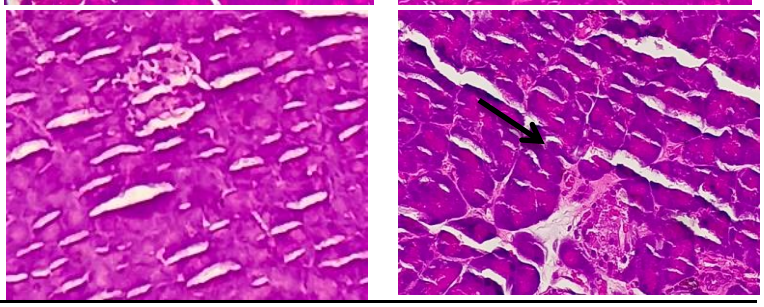
Table 3. Histopathological Description of Pancreatic Tissue

No	Group	Histopathological Image of Pancreatic Tissue
1	Control	
2	Treatment 1 (F1=1%)	
3	Treatment 2 (F2= 1.2%)	
4	Treatment 3 (F3 = 2%)	

Based on the cell morphology data above, there are differences in each group. The control group (P0) received a score of 4, which is necrosis of all pancreatic cells because this group was induced.streptozotocin (STZ) were not given extract treatment. In treatment group 1 (P1) which was inducedstreptozotocinand given lime peel extract nanoemulsion treatment with a concentration of F1 = 1% had pancreatic histopathology in the form of interstitial edema found between serous acini, leukocyte infiltration, mild vacuolization and pancreatic necrosis so that this group received a score of 3, namely there was ¾ of total pancreatic cell necrosis. In treatment group 2 (P2) which was inducedstreptozotocinand given lime peel extract nanoemulsion treatment with a concentration of F2=1.5%, a significant difference was seen due to moderate leukocyte infiltration and necrosis in pancreatic cells, so this group was given a score of 2, which is ½ total pancreatic cell necrosis. In treatment group 3 (P3) which was inducedstreptozotocinand given lime peel extract nanoemulsion treatment with a concentration of F3=2%, there was faint necrosis and slight leukocyte infiltration was still visible in the pancreatic cells so that this treatment group received a score of 1, namely ¼ pancreatic cell necrosis.

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DISCUSSION

The endocrine function of the pancreas is to produce the hormones glucagon and insulin to control blood sugar and release it into the bloodstream.(Alex, 2024)Maintaining blood sugar balance is essential for ensuring proper functioning of organs such as the kidneys, liver, and brain. Diabetes can be caused by damage to pancreatic cells from chronic pancreatitis. However, if the pancreas is not functioning properly, it cannot produce digestive enzymes or the hormone insulin properly. This can lead to conditions such as diabetes and food intolerances.(Karpińska, M., & Czauderna, 2022)Therefore, it is very important to make every effort to avoid damage to the pancreas function caused by high HbA1c examination is the first step that can be taken to measure glycemic levels related to complications and risk of diabetes.(Anita Rosari Dalimunthe, Livinda Christy Wijaya, 2023). the blood sugar that causes diabetes.

In this study, streptozotocin was used to induce diabetes in experimental animals. Streptozotocin (STZ) is a chemical with diabetogenic properties.(Haghani et al., 2022). Streptozotocin (STZ) is a compound often used to increase blood sugar levels in test mice to obtain mice with diabetes mellitus.

GC-MS (Gas Chromatography-Mass Spectrometry) analysis of lime peel extractcan be concludedLime peel extract (Citrus Aurantifolia) has potential as an antidiabetic agent based on bioactive compounds such as limonene, hesperidin, and limonin, which show antioxidant and anti-inflammatory activities, as well as the potential to regulate blood glucose levels.

This was proven through experiments on mice, by observing the fasting blood glucose (FBG) levels in mice after being induced by streptozoticin (FBG 1) increased, namely with an average in all groups > 200 mg / dL where all groups have been declared hyperglycemia. And after observing the administration of lime extract nanoemulsion for 14 days, re-examination was carried out on the 28th day with the results of a decrease in FBG levels (FBG 2) and it was seen that treatment groups 1, 2 and 3 experienced a decrease until blood glucose levels returned to normal. In line with that, it can also be seen that the levels of HbA1c (%) and MDA (nmol / mL) are different from the control group that was not given any treatment, so it can be concluded that the administration of lime peel extract (Citrus aurantifolia) is effective in reducing blood glucose levels in mice that were declared hyperglycemic by observing the levels of FBG, HbA1c and MDA. The administration at the best concentration is in the formulation of 2% and 1.5%.

In histopathological observations of the pancreas of mice, it can be seenadministration of nanoemulsion of lime peel extract (Citrus aurantifolia)effective in healing pancreatic cells that suffer from diabetes mellitus andadministration at a concentration of 2% extract showed that pancreatic cells experienced slight necrosis so that the score was 1. This is inseparable from the efficacy of lime peel extract (Citrus aurantifolia) which contains bioactive compounds such as limonene, hesperidin, and limonin, which show antioxidant, anti-inflammatory activity, and the potential to regulate blood glucose levels that can repair damaged cell tissue due to diabetes mellitus experienced by white rats (Rattus norvegicus) Wistar strain.

This is in line with research(Loizzo, 2012)who saidin limes, especially in the skin, it is also knownPhytochemical results show the presence of various secondary metabolic contents, namely flavonoids, alkaloids, and saponins, where these compounds act as antihyperglycemics.

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CONCLUSION

1. The compounds found in lime extract (Citrus aurantifolia) through GC-MS analysis are:limonene, hesperidin, and limonin.
2. Fasting blood sugar (FBS) level, Glycated hemoglobin level(HbA1c) and levelsMalondialdehyde (MDA) levels improved in the group given lime extract nanoemulsion.(Citrus aurantifolia), and the most effective is given at a concentration of 2%, 1.5% and finally 1%.
3. In observations of the pancreas organs of mice through histopathology of the pancreas of mice, it was seen that the administration of lime extract nanoemulsioneffective in healing pancreatic cells that suffer from diabetes mellitus

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