ABSTRACT

Purpose: To see the potency of mixed extracts of Ginger (Zingiber officinal Rosc.), Citronella (Cymbopogon nardus (L.) Randle, Cinnamon (Cinnamomum burmanii B.) Against blood sugar levels in alloxan-induced Novergicus Rattus male rats.

Theoretical reference: Insulin resistance is necessary to maintain dysglycemia, where insulin levels are higher than usual. Insulin resistance often occurs in people who are overweight, commonly known as obesity. 2. Pancreatic B Cell Dysfunction, decreased pancreatic beta cell function, and continued increase in insulin resistance, causing chronic hyperglycemia and stage liver disease. 3. Environmental factors are individuals who consume excessive carbohydrates and sugars.

Method: Using Complete Randomized Design (RAL) consisting of 7 groups with 4 repetitions, namely K0 (Normal group), K- (Negative group), K+ (Positive group given meformin), K1, K2, K3 and K4 (mixed extracts of ginger, lemongrass, cinnamon 100, 200, 300 and 400mg/kg bw), The data obtained were analyzed using ANOVA with a sig < 0.05.

Results and conclusion: It showed that the administration of mixed extracts of Ginger (Zingiber officinal Rosc.), Citronella (Cymbopogon nardus (L.) Randle, Cinnamon (Cinnamomum burmanii B.) affected reducing blood glucose levels and improving pancreatic histology. A mixed extract dose of Ginger (Zingiber officinal Rosc.), Citronella (Cymbopogon nardus (L.) Randle, Cinnamon (Cinnamomum burmanii B.) 400 mg/kg bw was most effective in lowering blood glucose levels and improving alloxan-induced pancreatic histology.

Implications of the research: When you want to control blood sugar, you can use a mixture of Ginger (Zingiber officinal Rosc.), Citronella (Cymbopogononnardus (L.), and Cinnamon (Cinnamomum burmanii B) extracts in balanced portions).

Originality/Value: Ginger (Zingiber officinal Rosc), Citronella (Cymbopogon Nardus (L.), and Cinnamon (Cinnamomum burmanii B) have potential as herbal medicine ingredients.

Keywords: Diabetes mellitus, Alloxan, blood glucose levels, pancreatic histology.

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BIOATIVIDADE DE GENGIBRE (ZINGIBER OFFICINALE ROSC), CITRONELLA (CYMBOPOGNARDUS (L.) E CANELA (CINNAMOMUM BURMANNII B) CONTRA DIMINUIÇÃO DOS NÍVEIS DE AÇÚCAR NO SANGUE DE ALLOXAN INDUZIDO (RATTUS NOVERGICUS)

RESUMO

Propósito: Ver a potência de extratos mistos de gengibre (Zingiber officinal Rosc.), Citronela (Cymbopogon nardus (L.) Randle, canela (Cinnamomum burmanii B.) Contra os níveis de açúcar no sangue em ratos machos Novergicus Rattus induzidos por alloxano.

Referência teórica: A resistência à insulina é necessária para manter a disglicemia, onde os níveis de insulina são mais altos do que o habitual. A resistência à insulina geralmente ocorre em pessoas com excesso de peso, comumente conhecidas como obesidade. 2. Disfunção Pancreática das Células B, diminuição da função das células beta pancreáticas e aumento contínuo da resistência à insulina, causando hiperglicemia crônica e doença hepática em estágio. 3. Fatores ambientais são individuos que consomem carboidratos e açúcares em excess.

Método: Utilizando o Desenho Aleatório Completo (RAL) composto por 7 grupos com 4 repetições, nomeadamente K0 (Grupo Normal), K- (Grupo Negativo), K+ (Grupo Positivo dado meformina), K1, K2, K3 e K4 (extratos mistos de gengibre, erva-limão, canela 100, 200, 300 e 400mg/kg pc), Os dados obtidos foram analisados utilizando ANOVA com sinal < 0,05.

Resultados e conclusão: mostrou que a administração de extratos mistos de gengibre (Zingiber officinal Rosc.), Citronela (Cymbopogon nardus (L.) Randle, canela (Cinnamomum burmanii B.) afetou a redução dos níveis de glicose no sangue e a melhoria da histologia pancreática. Uma dose de extrato misto de gengibre (Zingiber officinal Rosc.), Citronella (Cymbopogon nardus (L.) Randle, Canela (Cinnamomum burmanii B.) 400 mg/kg de peso corporal foi mais eficaz na redução dos níveis de glicose no sangue e na melhoria da histologia pancreática induzida por alloxano.

Implicações da pesquisa: Quando você quer controlar o açúcar no sangue, você pode usar uma mistura de gengibre (Zingiber officinale Rosc), Citronella (Cymbopogonnardus (L.), e canela (Cinnamomum burmannii B) extratos em porções equilibradas).

Originalidade / Valor: Gengibre (Zingiber officinale Rosc), Citronela (Cymbopogon Nardus (L.), e canela (Cinnamomum burmannii B) têm potencial como ingredientes de medicamentos à base de plantas.

Palavras-chave: Diabetes Mellitus, Aloxano, níveis de glicose no sangue, histologia pancreática.

1 INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by increased glucose levels in the blood, lack of insulin, or ineffectiveness of insulin (Bulu et al., 2019). The estimated prevalence of diabetes will increase in line with population growth to 19.9% or 111.2
million people in the age range of 65-79 years. It is estimated to reach 578 million in 2030 and 700 million in 2045 (IDF, 2019). Hyperglycemia for a long time can cause complications of various diseases, disability, and damage to multiple organs of the body that are dangerous, such as heart disease, nerve problems, kidney lag, and disorders of the eye that cause retinopathy and vision loss.

Diabetes mellitus has two types. Type 1 is caused by a lack of insulin production caused by damage to pancreatic cells. In contrast, type 2 is a condition characterized by insulin resistance and relative insulin deficiency that co-occurs. (Adeoye et al., 2017). Diabetes mellitus occurs due to damage to beta cells in the pancreas, so these cells cannot produce the hormone insulin needed by the body. As a result, insulin deficiency occurs (American, 2017). Histological damage to the pancreas is characterized by changes in the shape of the pancreas, such as shrinking the size of the Langerhans islets. The symptoms often experienced by people with diabetes mellitus include polyuria, polyphagia, drastic weight loss, weakness, and blurred vision (Musfiroh et al., 2022).

Factors causing diabetes mellitus (Fig. 1) include: 1. Insulin resistance is necessary to maintain dysglycemia, where insulin levels are higher than usual. Insulin resistance often occurs in people who are overweight, commonly known as obesity. 2. Pancreatic B Cell Dysfunction, decreased pancreatic beta cell function, and continued increase in insulin resistance, causing chronic hyperglycemia and stage liver disease. 3. Environmental factors are individuals who consume excessive carbohydrates and sugars (Nurmalasari, 2021).

Alloxane as much as 150mg/kg body weight given by intraperitoneal injection, is a diabetogenic that is often used in trials because it can quickly cause permanent hyperglycemia within 2-3 days (Al-awar et al., 2016). Treatment for people with diabetes mellitus can be done by adjusting glucose levels to remain in a normal state, namely by taking oral hypoglycemic drugs or insulin therapy. However, both ways can cause uncontrolled hypoglycemia, allergic reactions, and insulin resistance (Widiana, 2022).
In addition, there is also another way to deal with diabetes, namely by taking chemical drugs such as metformin. However, the use of this drug can cause side effects such as diarrhea, nausea, vomiting, and potentially dangerous lactic acidosis (Neal, 2006). To avoid the chemical effects of drugs, using plants that can potentially lower blood sugar levels helps treat diabetes (Riris, 2018).

Some plants also have antidiabetic activity that can regulate insulin release, insulin sensitivity in cells, and glucose absorption (Choudhury, 2018). It is known that herbal plants such as ginger, lemongrass, and cinnamon have secondary metabolite compounds containing active compounds such as flavonoids, gingerol, shogaol, and oleoresins (Suharto et al., 2019). The lemongrass plant (Cymbopogon citratus) is an herbal plant that contains saponins, alkaloids, phenols, steroids, tannins, and flavonoids that act as antidiabetic drugs (Hijramayasari et al., 2019). Bioactive components in essential oils produced from lemongrass include geranial, neral, limonene, geraniol, and β-myrcene (Widaryanti et al., 2021). The citral compound content contained in lemongrass leaf essential oil can reduce glucose concentration by blocking the activity of the enzyme β-glucosidase (Mirghani et al., 2012).

Cinnamon (Cinnamomum Burmanii) is a plant that can be used as herbal medicine (Arrafi & Amanatie, 2018). It contains secondary metabolites of flavonoids, polyphenols, saponins, tannins, alkaloids, cinnamate, and cinnamaldehyde (Emilda, 2018). It can treat gout, hypertension, ulcers, asthma, canker sores, constipation It, it and diabetes It (Syarif et al., 2015). In this study, we reported that mixed extracts of Ginger (Zingeber officinale Rosc.), Lemongrass (Cymbopogon nardus (L.) Randle and Cinnamon (Cinnamomum
burmanii B.) can affect lowering blood sugar levels in alloxan-induced male norvegicus rats and on pancreatic histology.

2 METHOD

2.1 MATERIAL PREPARATION

The ingredients used in this study include Ginger (Zingiber officinale Rosc.), Lemongrass (Cymbopogon nardus L.), Cinnamon (Cinnamomum burmannii B) obtained from Siantar Simalungun district. 11.5 kg of ginger, lemongrass, and cinnamon plants weighing 5 kg, 5 kg, and 1.5 kg were thoroughly washed, dried, aerated, and used in a drying oven. Each ingredient is mashed with a blender: ginger powder 200 gr, lemongrass 200 gr, and sweet skin 50 gr stirred evenly, then boiled with 5 liters of water until boiling and concentrated to a third, then concentrated with a rotary evaporator.

2.2 EXPERIMENTAL ANIMALS AND OTHER MATERIALS

White rats, as many as 28 males (Rattus norvegicus Wistar strains) aged 8-12 weeks with a body weight of 100-200 g were obtained from the Ello Science Laboratory. Other additional ingredients are alloxane, which induces diabetes mellitus, and 789-S-type mouse feed pellets produced by PT. Charoen Phokpahan Medan-Indonesia, 96% ethanol, metformin, equates, filter paper to filter Maserati, alcohol, xylol, and chloroform.

2.3 INVESTIGATION

Experimental animals were grouped into seven groups and acclimatized for seven days before treatment. Experimental animals from 7 groups were acclimatized for seven days and then treated. All experimental animals were given standard feed. Group 1 was only given feed (K0), Group 2 inducted alloxane (K+), Group 3 was given alloxane and metformin (K-), Group 4, 5, 6, and 7 (K1, 2, 3 and 4) namely rats given standard feed, alloxane and plant extracts of Ginger, Citronella, Cinnamon, at doses of 100, 200,300 and 400 mg/kg body weight. The treatment was given orally for 14 days, starting day 8. Extracts of plant mixtures were given to rats and carried out with a sonde through their mouths. The dose of ginger, citronella, and cinnamon extract is based on research (Widyasti, 2019) and is performed daily for 14 days. Rat blood sugar levels were measured three times, namely measurement of average blood sugar levels a week after
acclimatization, blood sugar levels after induction, and blood sugar levels after administration of extracts for 14 days.

2.4 RATS WEIGHT MEASUREMENT

A mixture of plant extracts occurs after acclimatization, namely day 8. Body weight was measured thrice on days 7, 14, and 21. Rat-weight measurements were carried out before the treatment, and the tool used to weigh rats was an analytical scale.

2.5 OBSERVATION OF THE PANCREAS OF RATS

After 14 days of treatment, the rats were anesthetized with chloroform by putting the animals in jars that had been mixed with cotton and chloroform. Then, the pancreas was dissected, and the pancreas was taken. Anesthetized animals are characterized by the absence of response to pain stimuli and are then placed on a paraffin table. Surgery is performed by making incisions on the skin and abdominal muscles of the mouse until the abdominal cavity opens. At that time, blood is taken until the heartbeat stops, and pancreatic organs are taken.

2.6 MANUFACTURE OF PANCREATIC PREPARATIONS BY THE PARAFFIN METHOD

2.6.1 Preparation

Making histological preparations through the paraffin method consists of several stages: material selection, fixation, drying, dissolving, paraffin penetration, rolling, pasting, deparafinization, returning to its original state, staining, closing, and labeling. To take samples of the rats' pancreas, the rats were narcotized using chloroform and then dislocated their necks after surgery on the abdominal area. Once the abdominal cavity is open, the pancreas is removed and inserted into a vial containing 0.9% NaCl until it is clear of residual blood.

2.6.2 Fixation

The pancreatic organ is fixed by immersing it in Bouin's solution. It is recommended to use a fixative volume of 15-20 times the volume of the network, which is about 10-20 mL for networks with a size of 1-2 cm. The fixation process takes 3 hours. Washing: After the fixation process, the pancreas is washed repeatedly using 70% alcohol...
until the washing color is no longer yellow. Then, the organ is soaked in 70% alcohol.

Dehydration: The tissue is immersed in a solution of alcohol with stratified concentrations such as 70%, 80%, 96% alcohol, and pure alcohol sequentially for 60 minutes.

2.6.3 Dealcoholization

The tissue is put into a bottle filled with toluene. If the toluene solution turns milky, it indicates that the dehydration poses are not complete. If so, put the organ back into pure alcohol. The tissue is immersed in toluene to replace the alcohol in the tissue completely.

Infiltration: Infiltration was carried out at 60°C, and the filter was sequentially inserted in paraffin toluene for 30 minutes pure paraffin I, II, and III for 60 minutes.

Embedding: Paraffin heated in the oven is poured over the prepared block, after which the tissue is inserted into the desired position. Then, paraffin blocks are manufactured (planting of tissue in cassettes) and stored in the refrigerator. Microtomy and Affixing: The paraffin block is then cut 2 mm thick using microtomes. The resulting cut is immersed in hot water at 60°C to prevent the tissue from folding. After that, the sample is taken and placed on the glass of the object smeared with glycerin. Deparaffinization: Deparaffinization using xylol for 3 minutes. Hydrate with alcohol for 3 minutes and wash off under running water (2 minutes). Staining: Staining using Hematoxylin and Eosin (HE). The glass of the object is immersed in a solution of hematoxylin for 8 minutes, then the glass of the thing is washed under running water. The glass is soaked in 1% alcohol and cleaned by five immersions using running water. Then, dry using 80%, 90%, and pure alcohol twice for 3 minutes each and finally dry. After that, the slide is infused in a 1% eosin solution for 3 minutes. The fall is then put into a 96% alcohol liquid and pure alcohol sequentially for 1 minute and drained. Then, the slide is put in a solution of xylol for 3 minutes.

Mounting: The glass of the object is covered with a cover glass and given a Canada balm, then labeled. After that, it is observed under a microscope with a magnification of 400x.

2.7 MEASUREMENT OF LANGERHANS ISLANDS AND NUMBER OF BETA CELLS

Measurements of the island of Langerhans, namely the preparation, were observed using a Nikon Eclipse e200 microscope with a magnification of 400x. To see the number
of cells, use 400x magnification with HE staining to clarify the shape and location of the island of Langerhans.

2.7.1 measurement of rat blood sugar levels

The rats used were 28 male rats (Novergicus) into seven groups. One group of rats, namely K0 as a control group, and six other groups of alloxan-induced rats, namely in groups K+, K-, K1, K2, K3, and K4: 100, 200, 300, and 400 mg / KgBW. The dose of alloxan used is 150mg / KgBW. It has been proven that the dose of alloxan as much as 150mg / KgBW has rats affected by diabetes (Putra et al., 2020)

3 RESULTS AND DISCUSSION

3.1 EFFECT OF ALLOXAN ON BLOOD SUGAR LEVELS OF RATS

A total of 28 male rats (Novergicus) aged ten weeks with a weight of ± 200g were given standard feed, divided into seven groups, namely: K0 as a control group and six other groups of rats, all rats induced alloxan at a dose of 150 mg/kg BW (Putra et al., 2020). namely in the K+ group and given metformin 33 mg/kg BW; K- (only alloxan); K1, K2, K3, and K4 were given a mixture of ginger, lemongrass, cinnamon extracts at doses of 100, 200, 300, and 400 mg / KgBW (Table 1).

Alloxan induction gave the impact of a significant increase in blood sugar in rats. Exposure to diabetogenic compounds such as alloxan lowers insulin levels and disrupts blood glucose homeostasis (Zhang et al., 2002; Li et al., 2009). Alloxan induction in test animals damages pancreatic tissue, decreasing insulin production by pancreatic islet cells (Szkuldeski, 2001; Nugroho, 2006). (Table 2) The results of data analysis using the ANOVA test obtained a sig value of 0.000, which is smaller than 0.05. This shows that it can be concluded that the administration of an alloxan dose at 150mg/kgBW in male white rats affects the increase in glucose levels of rats.

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<th>Table 1. Rats’ average blood glucose level test</th>
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Source: Test Lab at Directorate of animal husbandry and animal health Medan Vertener Hall
It can be understood because alloxan can quickly reach the pancreas because insulin receptors are found in the pancreas, its action is initiated by rapid uptake by Langerhans β-cells which damages insulin receptors accompanied by damage from pancreatic Langerhans islet β-cells. Reactive oxygen formation is a major factor in cell damage (Nugroho, 2006). As a result of insulin receptor damage and pancreatic β-cell damage, insulin cannot be produced normally, this can cause blood glucose cannot be taken and utilized to be converted into energy, so blood glucose levels become high (Putri et al., 2014).

| Source: Statistical test results of rat blood glucose levels, using IBM SPSS 24 |

### 3.2 ANTIDIABETIC ACTIVITY TEST RESULTS

This antidiabetic test of Ginger, Lemongrass, and Cinnamon extracts uses alloxan induction. Alloxan was chosen as a diabetogenic agent because alloxan undergoes oxidation-reduction metabolic processes in the body that produce alloxan radicals and free radicals. These radicals can damage beta cells in the pancreas. The dose of alloxan used in this study is 150 mg/kg BW to ensure that Langerhans beta cells can still produce insulin. Experimental animals can be considered to have diabetes if they experience hyperglycemia, which is the level of glucose in the blood >200 mg/dl after alloxan induction.

![Figure 2 - The rats’ blood sugar levels before alloxan induction and after treatment](source: The Table 1)
The diagram above shows the T0 blood sugar levels of rats before treatment, T1 blood sugar levels after alloxan induction, and T2 blood sugar levels after treatment (Fig. 2). It is known that average blood glucose levels remain within the normal range. At the same time, the negative control group has increased blood sugar levels (hyperglycemia). This is due to the administration of alloxan in increasing blood glucose levels (hyperglycemia) in both groups, namely the treatment group and the positive group (metformin).

From the results of statistical analysis using the Shapiro-Wilk test on rats, it can be seen that the percentage reduction in blood glucose levels is normally distributed because the significant value of each group is > 0.05 (H0 is accepted), so it can be continued with homogeneity using Levene statistics to test whether the data obtained from each group is homogeneous. The data obtained from the homogeneity test results show that the significant value of each group is > 0.05 (H0 is accepted). Furthermore, the test continued to the One-Way ANOVA test. The sig value was found based on the ANOVA results 0.000 < 0.05 (H0 rejected) (Table 2), so it can be concluded that there is a significant difference in the blood sugar levels of rats in each group. The difference is found in (T0), where the rats have not been given any treatment with a value of 0.322 > 0.05 then (H0 accepted). In this study, ginger, lemongrass, and cinnamon extracts contain gingerol, eugenol, and polyphenols, which help increase insulin receptor protein in cells to increase insulin sensitivity (Azmaina, 2021; Rusli, 2022).

3.3 HISTOPATHOLOGY EXAMINATION RESULTS

Histology of male rat pancreas: (a) Normal control; (b) Negative control; (c) Positive control; (d) 100 mg/kgBW dose; (e) 200 mg/kgBW dose; (f) 300 mg/kgBW dose; (g) 400 mg/kgBW dose. Description: Arrows indicate islets of Langerhans in diabetic rats (Fig. 3).

Based on the image of normal control rat pancreas histology (a) without being treated, the condition of pancreatic cells looks standard with an orderly arrangement of cells scattered in the islets of Langerhans and has a uniform cell shape. In the positive group (b), induced with an alloxan dose of 150 mg/kgBW and given metformin 33 mg/kgBW, the structure of the islets of Langerhans underwent changes where the islets of Langerhans looked smaller. In the negative group (c) induced with alloxan, the islands of Langerhans appear transparent, and the presence of Langerhans is wide. Histological
picture of the pancreas of rats in the alloxan induction group and given a mixture of ginger, lemongrass, and cinnamon extract at a dose of 100mg / kgBW (c); 200mg / kgBW (d); 300mg / kgBW (e) and 400mg / kgBW (f) showed a clear structure of Langerhans islets, indicating an improvement in pancreatic cells. This proves that mixed ginger, lemongrass, and cinnamon extracts can reduce blood sugar levels and repair pancreatic tissue in test animals.

Figure 3. Histopathology of Pancreas Examination Results

(a) (b) (c) 
(d) (e) (f) 
(g)

Source: Test Lab at Directorate of animal husbandry and animal health Medan Vertenier Hall

4 CONCLUSION

Induction of alloxan in rats with a dose of 150mg/kgBW can significantly impact blood sugar levels in rats, so the impact on rats experiencing diabetic rats. The results of this research indicate that the mixture of ginger, lemongrass, and sweet bark extracts given to alloxan-induced rats has the potential to reduce blood sugar levels in these rats. The mixture of plants can be used as a medicinal plant that can potentially lower blood sugar levels; however, it still needs to be studied again to see whether the plant does not affect the kidneys if consumed for a longer time and whether it has other effects on rats.
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