International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 5, 2024

Original Article

EFFECTIVENESS AND SAFETY OF USE OF BIGUANIDE AND SULFONYLUREA DRUGS WITH HISTOPATHOLOGICAL ANALYSIS AND PARAMETERS OF THE KIDNEY, LIVER AND PANCREAS IN ALLOXAN-INDUCED RATS

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Received: 06 May 2024, Revised and Accepted: 28 Aug 2024

ABSTRACT

Objective: There are several drugs used as first-line in the treatment of type 2 diabetes with different mechanisms of action, such as metformin, glimepiride, and glibenclamide. The mechanism action of the drugs through stimulation and increased insulin sensitivity. This mechanism can have side effects on the organs excreting the drug. The aim of this study was to compare the effectiveness and safety of the three drugs in alloxan-induced rats.

Methods: A total of 15 rats were divided into five groups with each group consisting of three rats: Normal Control (NC), Induced Control (DC), Metformin 9 mg/kgBW, Glimepiride 1 mg/kgBW, and Glibenclamide 5 mg/KgBW. Fasting Blood Glucose (FBG) levels, Blood Urea Nitrogen (BUN), Creatinine Serum (SCr) and Serum Glutamate Pyruvate Transaminase (SGPT) were obtained. Data analysis using ANOVA and a post-hoc test.

Results: The results show that in comparison of the three anti-diabetic drugs, glibenclamide causes the highest damage to the pancreas and liver. Meanwhile, glimepiride provides the safest results for the kidneys, and metformin is safest for the liver.

Conclusion: The result showed that the drug glibenclamide caused the highest damage to pancreatic cells and glimepiride gave the greatest decrease in blood glucose levels, but the use of glimepiride for 14 d was safer for use than for glibenclamide for kidney parameters.

Keywords: Diabetes mellitus, Metformin, Glimepiride, Glibenclamide, Alloxan, Histopathology

DOI: https://dx.doi.org/10.22159/ijap.2024.v16s5.52469 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

According to the World Health Organization (WHO) 2021, approximately 422 million people worldwide suffer from diabetes mellitus and 1.5 million deaths are caused by diabetes every year. The number of cases and prevalence of diabetes has been increasing over the last few decades [1]. Diabetes is a metabolic disorder characterized by increased blood sugar caused by abnormalities in insulin production and/or insulin action, as well as impaired function of carbohydrate, lipid and protein metabolism, which results in macro and microvascular complications. Inflammation, endothelial dysfunction, and hypercoagulability correlate with each other, playing an important role in the development of vascular complications in diabetic patients [2]. Diabetes mellitus type 1 (IDDM) causes absolute insulin deficiency due to the destruction of beta cells. While diabetes mellitus type 2 (NIDDM), there is an insulin combination resistance action and an inadequate compensatory insulin secretory response [3]. In the last three decades. the prevalence of type 2 diabetes has increased in countries with all levels of income [1]. The first line of pharmacotherapy used in type 2 DM is metformin. Metformin is mainly used because it has a low risk of side effects of hypoglycemia and has no impact on body weight [4]. To achieve maximum blood glucose-lowering effect. Metformin is commonly combined with other blood glucose-lowering agents, such as thiazolidinediones [3]. Whereas, sulfonylurea is recommended as a second-line treatment in the management of NIDDM [5]. However, the drug is still commonly used as a first-line treatment compared to metformin [6]. However, according to PERKENI (2021) the main side effect of using sulfonylureas is a high risk of hypoglycemia with impaired liver and kidney function. the metformin dose in patients with renal impairment with a Glomerular Filtration Rate (GFR) of 30-60 ml/min/1.73 m2 must be decreased and should not be administered to patients with GFR less than 30 ml/min/1.73 m2. The novelty of this study is to compare the effects of reduced Fasting Blood Glucose levels (FBG) in three drugs with two different groups and to find out the safety of three drugs on liver, kidney, and pancreas organs with histopathological analysis.

MATERIALS AND METHODS

Material

2610 g triple beam balance scale (Lark. China), analytical scale (Presica A-SCS), 1.0 ml and 3.0 ml injection spuit (Terumo), 18 gauge oral spuit, cut knife, holder, vortex, centrifuge (Mini Spin), glass tools (Pyrex), semi-micro, 5-50 µl micropipette, 100-1000 µl (Accura), UV/VIS spectrophotometer (Stardust MC*15).

Metformin, Glimepiride and Glibenclamide were purchased from PT. Indofarma, Jakarta; alloxan monohydrate (Sigma-Aldrich, NSW, Australia), Formalin, ethanol 96%, aquadest. Hematoxylin-eosin (HE), GOD FS KIT reagens (Diasys), GPT reagent (Diasys), Urea FS reagent (Diasys), and Creatinine FS reagent (DiaSys) [8].

Animal test

Fifteen male wistar rats weighing 150-200 g and 2–3 mo old were obtained from the Pharmacology Laboratory of the Universitas Muhammadiyah Surakarta [9]. Rats were given ad libitum standard balanced food and water and kept in a room at a temperature (25±1 °C) with lighting on a 12 h cycle of dark/12 h light/dark. The rats were allowed to adjust to the environment for 1 w before being used in this study [8]. The experimental procedure used in this study was approved by the Health Research Ethics Commission (KEPK) of the Faculty of Medicine of Muhammadiyah University of Surakarta under letter number 4529/A.1/Kepk-FKUMS/X/2022.

Hyperglycemic animal modeling

Alloxan 150 mg/kg body weight (0.3% saline solution) was administered intraperitoneally to 15 rats. For the next 2 h, 20% glucose was given orally to avoid hypoglycemic condition. The animal was used as a model for diabetic rats with blood sugar levels>200 mg/dl [8]. Blood glucose levels were monitored on day 0 as baseline and on day 5 of induction. Determination of FBG samples were taken before treatment and after alloxan induction on 4th d [5]. Determination FBG levels were carried out using the spectrophotometric method with Glucose Oxsidase– Aminoantypyrine Peroxidase reagent (GOD FS KIT) [10].

Analysis of blood glucose concentration were performed on the 4th, 7th and 14th d [21]. A blood sample of about 0.5 ml was taken from the test animal through the conjunctive vein of the eye. The sample was centrifuged at a speed of 12.000 rpm for 20 min to obtain a blood serum that would be used as a sample for analysis [11].

Treatment of test animals

The animals consisting of 15 rats were divided into five groups so that each group contained three animals:

- The normal group (I) 3 rats was treated aquadest and pellets [12]
- The negative control group (II) treated with induced alloxan [12]
- The group III was treated Metformin (9 mg/kgBW) orally [13]
- The group VI was treated Glimepiride (1 mg/kgBW) orally [14]
- The group V was treated Glibenclamide (5 mg/KgBW) orally [15]

Alloxan 150 mg/kgBW in (0.3% saline solution) intraperitoneally were induced to all control except normal control [12]. To avoid hypoglycemia in the blood of the negative control group. every day rats were given 20% oral glucose. Each treatment group consisting of 3 rats with confirmed diabetes was treated with metformin, glimepiride, and glibenclamide.

Sampling was done on day 0, 7, and 14 [16]. The collected serum is stored at a temperature of-21 °C [17]. All control are determined FBG levels in the serum by spectroscopic photometry using the Glucose gxidase-peroxidase reagent Aminoantyoirine (GOD-PAP) [11]

Determination of glucose levels of metformin glimepiride and glibenclamide in serum with a Stardust MC*15 spectrophotometer

Supernatant, standart, and blank of 10 µl is taken using a micropipette into the cuvvete and mixed with 1000 µl GOD FS kit reagent. Then, incubated for 10 min with 37 °C. The visible spectrophotometer read at λ 500 nm [11].

Clinical blood chemistry of metformin, glimepiride, and glibenclamide in serum with the Stardust MC*15 spectrophotometer

Determination of SGPT levels (Serum glutamic pyruvic transaminase)

Serum of 100 µl is added 1000 µl SGPT monoreagent with an operating time of 1 min. and using a blank aquadest. The reading was done at a wavelength of 340 nm, instrument temperature of 37 °C using the Stardust MC*15 spectrophotometer [18].

Determination of SCr levels (Creatinine Serum)

A sample of 50 µl plus 1000 µl of urea monoreagent with a blank aqua dest. Incubated at 37 °C for 2 min using the Stardust MC*15 spectrophotometer.

Determination of BUN levels

A sample of 50 µl plus 1000 µl of creatinine monoreagen with an aquadest blank. Incubated at 37 °C for 3 min using the Stardust MC*15 spectrophotometer.

Laboratory animal testing termination

All animals were sacrificed with cervical dislocation and were previously given a combination of zoolytic (50 mg/kgBW) and acepromazine (2.5 mg/kgBW) intraperitoneally. The experimental animals who died were then histopathology with organ parts of the pancreas, liver, and kidneys. After taking the necessary organs then the test animals are buried [8].

Histopathologycal examination

The organs are kept in the neutral buffered formalin (NBF) for histopathological examination. Then, the tissue is dyed with Harris Hematoxylin-Eosin (HE). Each tissue slide is then counted for normal cells and damaged cells [8].

Statistical analysis

The data obtained were blood sugar and blood chemical data such as SGPT, SCr, and BUN. which were processed using normality tests and data homogeneity tests first to determine the distribution of the data. Then the data was processed with ANOVA if it was normal distribution and Kruskal-Wallis if it wasn't normally distributed. If the results of the ANOVA or Kruscal-Wallis tests showed significant differences between the groups, the next step was to perform a post-hoc test to determine between which groups the differences were located.

RESULTS AND DISCUSSION

Negative controls after administering alloxan on day 4 had hyperglycemia with blood sugar levels above 400 mg/dl (table 1). Negative control remained in a state of hyperglycemia for 14 d with the 14th d average glucose level of 260.6±45.6 mg/dl (tabel 1). These controls are used as a baseline to compare the decrease in blood glucose levels. Alloxan is very quickly absorbed by beta cells through the secretion of insulin that determines the diabetogenesis of alloxan. Therefore, control alloxan is very prone to hyperglycemia condition.

The result of blood glucose measurement is that glimepiride can produce the greatest reduction in blood sugar levels between metformin and glibenclamide. Metformin can lower blood sugar levels for 14 d by an average of 86.6±8.5 mg/dl (table 1). However, a sudden drop in blood sugar can increase the secretion of endogenous insulin, which can lead to hypoglycemia [6].

Table 1: Average blood sugar levels on various treatment groups specified at baseline, induction, day 7 and 14 after treatment (n=3)

Groups	Fasting blood glucose (mg/dl)			
	Baseline	Induced	Day 7	Day 14
Normal Control (NC)	87.0±1.7	103.6±8.0	107.3±17.2	102.3±0.5
Induced Control (DC)	104.6±3.0	426.0±84.8	260.0±25.5	260.6±45.6
Metformin	119.0±11.5	243.3±39.9	112.0±3.6	86.6±8.5
Glimepiride	109.6±8.1	337.0± 108.2	139.3±11.2	90.3±2.5
Glibenclamide	122.3±2.0	214.3±17.0	114.6±19.4	80.6±18.7

Histopathology of pancreatic, kidney, and liver cells

In kidney organs, cell damage can include cell degeneration and cell necrosis. The rate of damage to the kidney is usually divided into four degrees, ranging from normal condition to severe damage with cell damage reaching 25% or more [19]. The results of the histopathological tests of the kidney organs showed that the treatment control of glibenclamide had the most significant difference with the normal control ($P<0.05$) and significant with the control of alloxan ($P<0.05$). It means that glibenclamide has effect that toxic with kidney because based on the result glibenclamide has the highest rupture cell compared other control.

For pancreatic organs, normal cells are usually round-shaped with a nucleus in the middle. Pancreas cells that are degenerated usually show changes in shape and structure. In addition, damage to pancreatic cells can also cause a decrease in the secretion of the hormone insulin [20, 19]. The results of the histopathological tests of the pancreas organs showed that the pancreas treatment control of glibenclamide had the most significant difference from the normal control ($P<0.05$) and significant with the control of alloxan ($P<0.05$). It means that glibenclamide has effect that dangerous with pancreas because based on the result glibenclamide has the highest rupture cell compared to other control.

Meanwhile, in liver organs, damaged liver cells usually grow in size and can cause impaired blood flow. The results of the liver organ histopathology test showed that the treatment controls of glibenclamide had the most significant difference with the normal control (P<0.05) and significant with the alloxan controls (P<0.05). It means that glibenclamide has effect that toxic with liver because based on the result glibenclamide has the highest rupture cell compared other control.

Table 2: Data BUN, SCr, SGPT on various treatment groups specified at baseline, induction, day 14 and 21 after treatment (N=3)

Groups	GPT (IU/l)			
	Baseline	**Induced**	**Day 7**	**Day 14**
Normal Control (NC)	21.6±1.5	22.0±1.0	42.3±3.0	28.0±1.0
Induced Control (DC)	26.6±10.9	39.0±5.2	54.0±8.6	60.3±15.9
Metformin	29.3±2.8	52.6±22.0	47.3±9.7	47.3±8.1
Glimepiride	31.3±7.5	51.6±12.6	46.6±16.9	45.6±6.2
Glibenclamide	59.3±19.1	53.3±15.0	39.3±7.3	50.6±6.3
Groups	BUN (mg/dl)			
	Baseline	Induced	Day 7	Day 14
Normal Control (NC)	33.0±5.5	29.6±7.5	36.3±3.0	41.0±6.2
Induced Control (DC)	39.3±10.6	99.3±67.3	87.6±56.7	110.0±70.1
Metformin	45.3±13.4	92.6±63.5	68.0±38.3	76.3±41.4
Glimepiride	39.3±813.5	105.6±90.5	45.3±8.7	67.6±10.6
Glibenclamide	40.0±1.7	67.3±35.5	54.3±20.5	49.6±12.7
Groups	SCr (mg/dl)			
	Baseline	Induced	Day 7	Day 14
Normal Control (NC)	0.77±0.15	0.77±0.05	0.90±0.17	0.93±0.05
Induced Control (DC)	0.63±0.05	0.77±0.11	0.80±0.17	1.20±0.20
Metformin	0.77±0.15	1.03±0.32	0.80±0.1	1.13±0.15
Glimepiride	0.67±0.11	0.97±0.25	0.60±1.0	1.0±0.0
Glibenclamide	0.57±0.25	0.77±0.15	0.80±0.1	0.67±0.11

The results of an average examination of the functional parameters of organs such as liver and kidneys comparing the levels of GPT, BUN, SCr on day 14 showed that the GPT value of Glibenclamide had the highest value of 50.6±6.3 IU/l (table 2). The BUN value of Metformin had the maximum value of 76.3±41.4 mg/dl (tables 2).

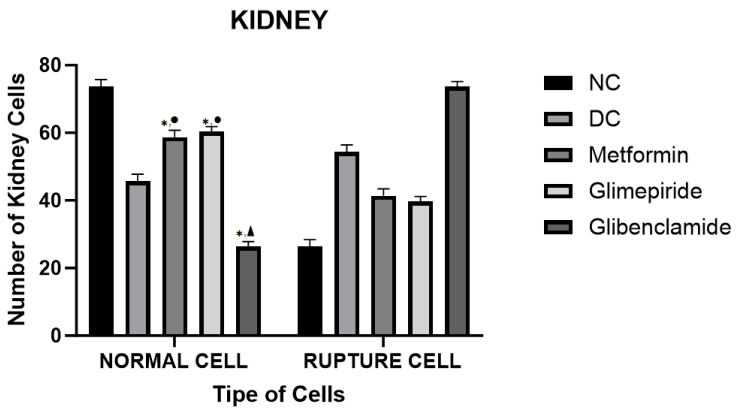

Fig. 1: Graph of normal and rupture kidney cells in various treatment groups (n=3), *a significant difference with the alloxan group (P<0.05), ▲ a significant difference with with normal group (P<0.05), ● is not significant different with alloxan group (P>0.05)

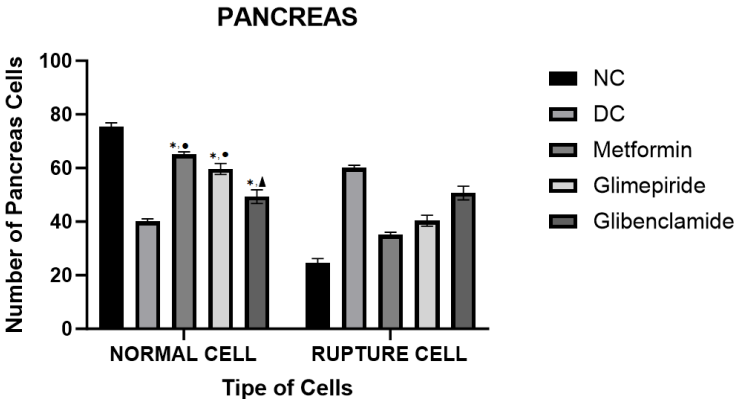

Fig. 2: Graph of normal and rupture pancreatic cells in various treatment groups (n=3), *a significant difference with the alloxan group (P<0.05), ▲ a significant difference with with normal group (P<0.05), ● is not significant different with alloxan group (P>0.05)

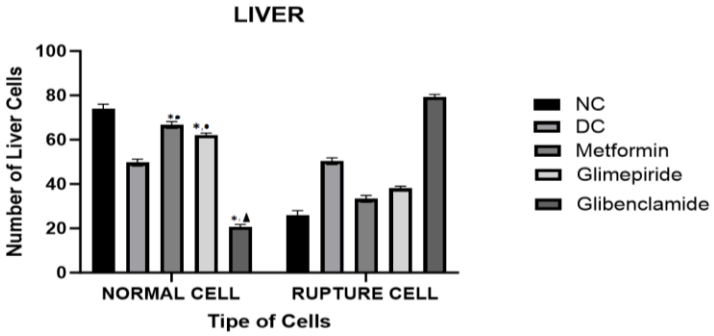

Fig. 3: Graph of normal and rupture liver cells in various treatment groups (n=3), *a significant difference with the alloxan group (P<0.05), ▲ a significant difference with with normal group (P<0.05), ● is not significant different with alloxan group (P>0.05)

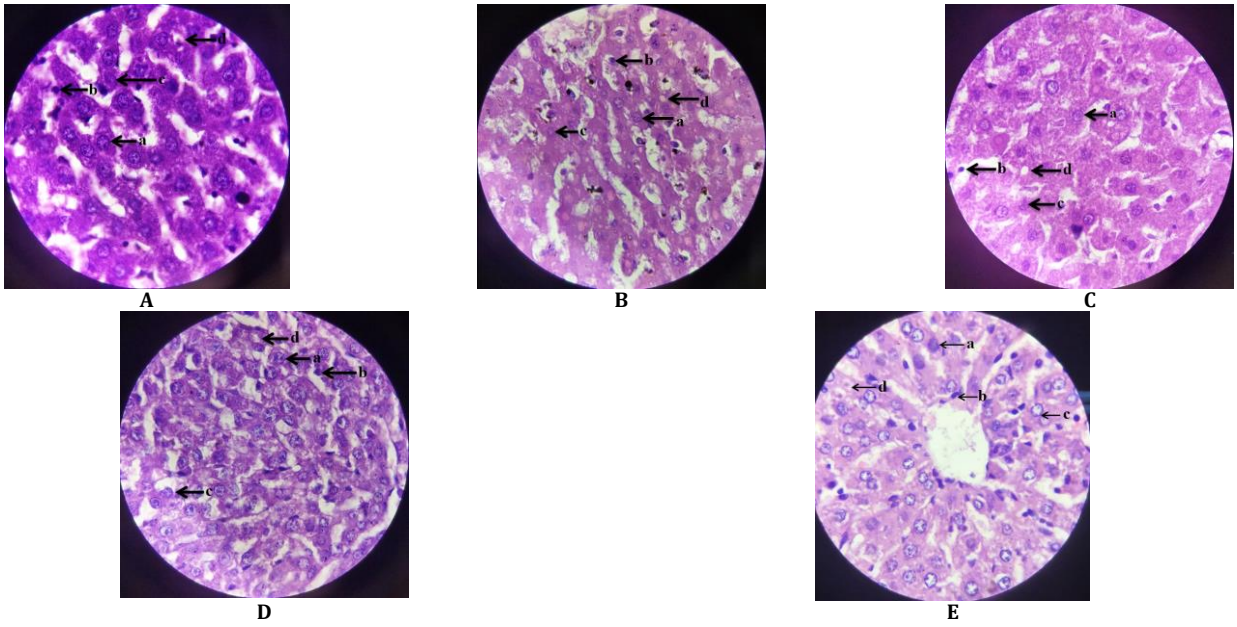

Fig. 4: Image of A. Normal Control, B. Alloxan Control, C. Metformin 9 mg/kgBW, D. Glimepiride 1 mg/kgBW, E. Glibenclamide 5 mg/kgBW with (a) Normal cells (b) Pyknosis (c) Karyorrhexis (d) Karyolysis of the tissue seen from the histopathology results of the pancreatic organ stained with HE (Haematoxilin-Eosin) and seen under a microscope with 1000x magnification

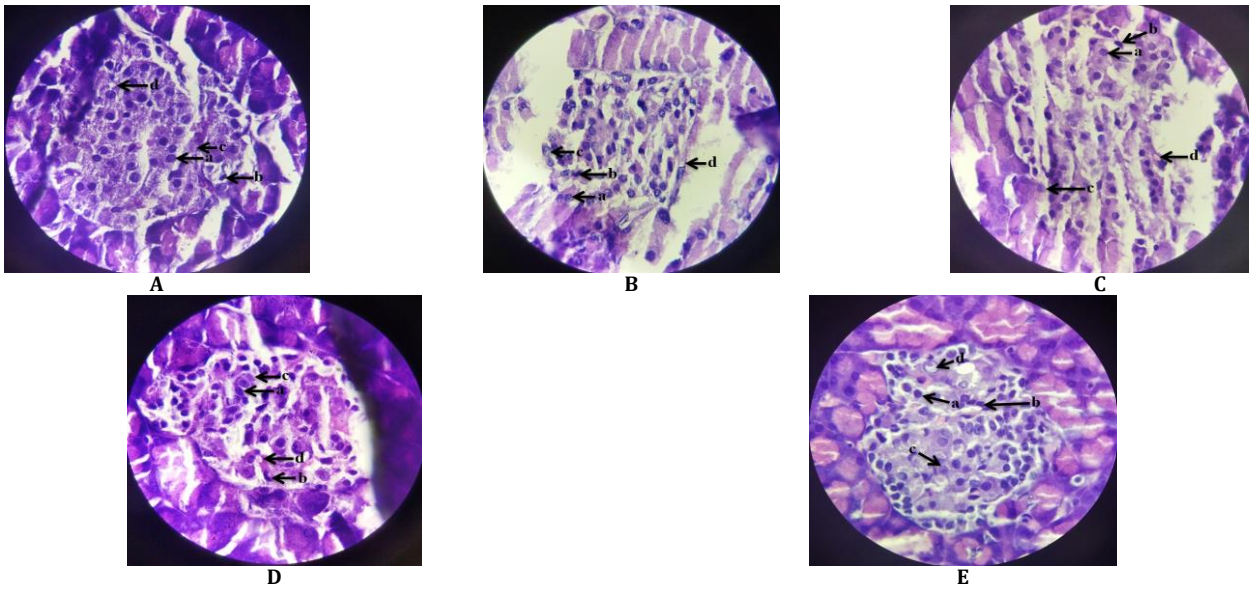

Fig. 5: Image of A. Normal Control, B. Alloxan Control, C. Metformin 9 mg/kgBW, D. Glimepiride 1 mg/kgBW, E. Glibenclamide 5 mg/kgBW with (a) Normal cells (b) Pyknosis (c) Karyorrhexis (d) Karyolysis of the tissue seen from the histopathology results of the liver organ stained with HE (Haematoxilin-Eosin) and seen under a microscope with 1000x magnification

DISCUSSION

This study aims to compare the effects of blood glucose reduction (FGB) in 3 drugs from 2 different groups and to find out the safety of the treatment of 3 drugs against liver and kidney organs. In the results of low blood sugar (table 1), glimepiride has the greatest effectiveness in lowering FGB compared to the other two drugs, while glimepiride is the second therapy of NIDDM. The mechanism of glimepiride increases the secretion of endogenous insulin. However, a significant decrease in the use of glimepiride can lead to hypoglycemia. In patients with long-lasting diabetes mellitus, hypoglycemic loss of consciousness may manifest when the symptoms of autonomous hypoglycemia are not experienced by the patient, thereby causing neuroglycopenia symptoms and potential hypoglicemia [20].

Diabetes is the most important risk factor for CKD. Many diabetics and practitioners are afraid to use metformin in patients with kidney problems even though they only suffer from albuminuria [19]. In addition to parameters in the kidneys, often drugs that are metabolized in the liver can also affect the function of the livers themselves. Based on the results of the average examination of the function parameters of organs such as liver and kidneys comparing the drug levels of GPT, BUN, SCr on the 14th d shows that the GPT value of Glibenclamide has the highest value of 50.6±6.3 IU/l (table 2). Metformin is not metabolized in the liver, but metformin excreted unchanged through the urine. Glibenclamide has significant liver metabolism [6] with cytochrome P-450 (CYP) 3A4 to be the main enzyme involved in glibenclamide metabolism. The enzymes CYP2C9 and 2C19 are also partly involved. As for glimepiride, the drug is mainly metabolized in the liver by CYP2C9 into the active M1 (hydroxyl) metabolite and then into the inactive M2 (carboxide) metabolites [21]. From the results obtained in accordance with the theory of glibenclamide there was an increase in Glutamic Pyruvic Transaminase (GPT) values until the 14th d has the highest GPT value of 50.6±6.3 (table 2) where glibenclamide metabolism is very significant at heart [22].

The histopathological results of this study showed that metformin did not cause the greatest damage to the kidneys. Renal histopathology results from glibenclamide drug control have the greatest cell damage, almost close to aloxan control ($P<0.05$) (fig. 1). The primary excretion pathway of glimepiride is through the kidneys. Approximately 60% of the metabolites are excreted through the urine (mostly M1) and the rest through the stools (especially M2) [21].

In a pancreatic organ, the pancreas showed that the control treatment of glibenclamide produced significant cell damage ($P<0.05$) (fig. 2). This is because the mechanism of glibenclamide itself is to stimulate the release of insulin by the pancreas. This cell damage is caused by free radicals released in mitochondria during insulin secretion. In hyperglycemic conditions that last too long, chronic exposure to relatively high levels of reactive oxygen species (ROS) causes cell function disorders, including kidney cells endothelial cells [23, 24].

In a liver organ, the liver showed a controlled treatment of glibenclamide giving significant cell damage ($P<0.05$) (fig. 3) [6]. Glibenclamide has a plasma half-life (t1/2) of about 4-6 h with the first cross-metabolism in the liver as much as 50% [2, 25]. With cytochrome P-450 (CYP) 3A4 is to be a major enzyme involved in the metabolism of glibenclamide. Whereas, based on result from statistic metformin has not significant difference with normal control. It means that metformin is not toxic to liver. This is appropriate with other study that metformin does not appear cause or exacerbate liver injury [18].

CONCLUSION

The results showed that the drug glibenclamide gave the highest damage to the pancreatic cells and glimepiride gave the greatest reduction in blood glucose levels, but the use of glimépiride for 14 d was safer to use than using glibenclamide for kidney parameters. The use metformin is safer for liver compared glimepiride and glibenclamide.

FUNDING

Universitas Muhammadiyah Surakarta

AUTHORS CONTRIBUTIONS

Data curation: Firda Ayu Fadila Rahmawati, Diski Wahyu Wijianto, Formal analysis: Arifah Sri Wahyuni, Diski Wahyu Wijianto, Investigation: Arifah Sri Wahyuni, Firda Ayu Fadila Rahmawati, Methodology: Arifah Sri Wahyuni, Retno Azzahra Umniyyah, Project administration: Arifah Sri Wahyuni, Diski Wahyu Wijianto, Resource: Firda Ayu Fadila Rahmawati, Retno Azzahra Umniyyah, Software: Arifah Sri Wahyuni, Writing original draft: all authors, Writing-review and editing: all authors

CONFLICTS OF INTERESTS

All authors have none to declare

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 Suppl 1:S62-9. doi: 10.2337/dc10-S062, PMID 20042775.
2. Idacahyati K, Wulandari WT, Gustaman F, Nurdianti IL. Activity nanokirinyuh (Chromolaena odorata) leaves extract in alloxan induced diabetic rats. Int J App Pharm. 2021;13(4):220-2. doi: 10.22159/ijap.2021.v13s4.43863.
3. Darusman F, Rusdiana T, Sopyan I, Yuliar NF, Aryani R. Bioequivalence of metformin as an oral antidiabetic: a systematic review. Int J App Pharm. 2023;15(6):76-81. doi: 10.22159/ijap.2023v15i6.49142.
4. Lipscombe L, Booth G, Butalia S, Eurich Bsp DT, Goldenberg R, Khan N. Diabetes Canada 2018 clinical practice guidelines for the prevention and management of diabetes in canada: pharmacologic glycemic management of type 2 diabetes in adults. Can J Diabetes 2018;42:S88-103.
5. Filion KB, Douros A, Azoulay L, Yin H, YU OH, Suissa S. Sulfonylureas as initial treatment for type 2 diabetes and the risk of adverse cardiovascular events: a population-based cohort study. Br J Clin Pharmacol. 2019;85(10):2378-89. doi: 10.1111/bcp.14056, PMID 31276600.
6. Basit A, Riaz M, Fawwad A. Glimepiride: evidence-based facts trends and observations (GIFTS). Vasc Health Risk Manag. 2012;8:463-72. doi: 10.2147/HIV.S33194, PMID 23028231.
7. Soelistijo S. Pedoman pengelolaan dan pencegahan diabetes melitus tipe 2 dewasa di indonesia 2021. Glob Initiat Asthma. 2021;46.
8. Wahyuni AS, Hakim L, Nurrochmad A, Astuti P. The sinergistic effect of black rice bran extract and glibenclamide on protecting renal hepatic and pancreatic cells in alloxan-induced rats. IJPR. 2020;12(1):509-17. doi: 10.31838/ijpr/2020.12.01.113.
9. Sujono TA, Kusumowati ITD. Antidiabetic effect of cashew (Anacardium occidentale l.) leaves ethanol extract in diabetic rats induced by alloxan pros 1st Annu Pharm Conf. FMIPA UNS; 2020.
10. Ambade VN, Sharma YV, Somani BL. Methods for estimation of blood glucose: a comparative evaluation. Med J Armed Forces India. 1998;54(2):131-3. doi: 10.1016/S0377-1237(17)30502-6, PMID 28775446.
11. Muhtadi PAU, Primarianti AU. Antidiabetic activity of durian (Durio Zibethinus murr.) and rambutan (Nephelium lappaceum L.) fruit peels in alloxan diabetic rats. Procedia Food Sci. 2015;3:255-61. doi: 10.1016/j.profoo.2015.01.028.
12. Sari N, Wahyuni AS. Effect of black rice bran extract to decrease glucose level of diabetic rats. Pharmacon. 2017;18:8-12.
13. Rompas IZ, Bodhi W, Lebang JS. Antihyperglycemic effectiveness test of ethanol extract of red gedi leaves (abelmoschus manihot l. medik) against white rats (rattus norvegicus) wistar strain induced by alloxan. Pharmacon. 2021;10:940-7.
14. Mondol D, Islam MN, Biswas S, Jodder P, Sana S, Saleh MA. Investigation of the synergistic effect of glimepiride and rosuvastatin on alloxan-induced diabetic rat. J Diabetes Metab Disord. 2020;19(2):1415-22. doi: 10.1007/s40200-020-00662-6, PMID 33553033.
15. Rai A, Eapen C, Prasanth VG. Interaction of herbs and glibenclamide: a review. ISRN Pharmacol. 2012;2012:659478. doi: 10.5402/2012/659478, PMID 22844612.
16. Singh S, Verma N, Karwasra R, Kalra P, Kumar R, Gupta YK. Safety and efficacy of hydroalcoholic extract from Lawsonia

inermis leaves on lipid profile in alloxan-induced diabetic rats. AYU. 2015;36(1):107-12. doi: 10.4103/0974-8520.168999, PMID 26730149.

17. Kale VP, Patel SG, Gunjal PS, Wakchaure SU, Sundar RS, Ranvir RK. Effect of repeated freezing and thawing on 18 clinical chemistry analytes in rat serum. J Am Assoc Lab Anim Sci. 2012;51(4):475-8. PMID 23043814.
18. Brackett CC. Clarifying metformins role and risks in liver dysfunction. J Am Pharm Assoc. 2010;50(3):407-10. doi: 10.1331/JAPhA.2010.08090, PMID 20452916.
19. Nuralifah N, Muhammad Fitrawan LO, Parawansah P, Trisetya M. Histopathology of the pancreatic organ of type 2 Dm rats given ethanol extract of red gedi leaves (Abelmoscus Manihot L Medik). J Syifa Sci Clin Res. 2022;4(1):141-51. doi: 10.37311/jsscr.v4i1.13566.
20. Ioannidis I. Diabetes treatment in patients with renal disease: is the landscape clear enough. World J Diabetes. 2014;5(5):651-8. doi: 10.4239/wjd.v5.i5.651, PMID 25317242.
21. Kleen M, Kangas H, Laine C. Chemical characterization of mechanical pulp fines and fiber surface layers. Nord Pulp Pap Res J. 2003;18(4):361-8. doi: 10.3183/npprj-2003-18-04-p361-368.
22. Rambiritch V, Maharaj B, Naidoo P. Glibenclamide in patients with poorly controlled type 2 diabetes: a 12 w, prospective single center open label dose-escalation study. Clin Pharmacol. 2014;6:63-9. doi: 10.2147/CPAA.S54809, PMID 24741335.
23. Candasamy M, Murthy TE, Gubiyappa KS, Chellappan DK, Gupta G. Alteration of glucose-lowering effect of glibenclamide on single and multiple treatments with fenofibrate in experimental rats and rabbit models. J Basic Clin Pharm. 2014;5(3):62-7. doi: 10.4103/0976-0105.139728, PMID 25278668.
24. Cicih A, Aligita W, Susilawati E. A review: the pharmacokinetics and pharmacodynamics of metformin herb interactions. JIF. 2022;18(1):13-25. doi: 10.20885/jif.vol18.iss1.art2.
25. Yusuf NA, Abdassah M, Sopyan I, Mauludin R, Joni IM, Chaerunisaa AY. Improved characteristics of glibenclamide as transethosome vesicular system: physicochemical solubility and *in vitro* permeation study. Int J App Pharm. 2024;16(1):172-85. doi: 10.22159/ijap.2024v16i1.49245.