Trop J Nat Prod Res, June 2024; 8(6): 7410-7415 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of the Effectiveness of Metformin and Black Rice Ethanol Extract Combination in Reducing Blood Glucose Levels and Protecting Kidney, Liver, and Pancreatic Cells

Arifah S. Wahyuni*, Diski W. Wijianto, Dhani Iswanto, Afifah Listiadewi

Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Sukoharjo, 57162 Indonesia

ARTICLE INFO

Article history:
Received 03 January 2024
Revised 21 May 2024
Accepted 23 May 2024
Published online 01 July 2024

ABSTRACT

Metformin (MET), a first-line oral antidiabetic medicine, is known for its ability to increase insulin sensitivity. However, uncontrolled lactic acidosis, a potential side effect of MET, can cause organ injury. On the other hand, antioxidants like black rice bran (BRB), containing *cyanidin-3-glucosidase* (C3G), have demonstrated efficacy in preventing diabetic kidney injury and fibrosis due to oxidative stress. The objective of this study is to investigate the potential of combining the ethanol extract of black rice bran (EEBRB) with MET in the regulation of various parameters in hyperglycemic rats. In this study, 20 male rats were divided into different groups. They were treated for 21 days after being divided into the normal group, alloxan (ALX) group 150 mg/kg BW, MET 63 mg/kg BW, and a combination of MET 63 mg/kg BW and EEBRB 50 mg/kg BW. The results of the study indicate that both treatments properly controlled fasting blood glucose levels with no hypoglycemia over a duration of 21 days. Furthermore, histopathological examinations revealed significant protection against kidney, liver, and pancreatic injury in the group receiving the combination treatment. Although there was no statistically significant weight loss, the combination did not lead to excessive weight gain, a common concern with some antidiabetic medications. MET and EEBRB can regulate blood glucose levels and attenuate organ damage in animals with hyperglycemia. Further investigation is necessary to elucidate the processes underlying the safety and efficacy of the combination approach.

***Keywords*:** Alloxan, Antioxidant, Black Rice Bran, Diabetes Mellitus, Metformin,

Introduction

Diabetes is a significant health challenge worldwide. The estimated worldwide incidence of diabetes mellitus (DM) in 2019 was 9.3%, affecting approximately 463 million people. Estimations indicate that this prevalence will continue to rise to 10.2% (578 million) by 2030 and further increase to 10.9% (700 million) by 2045.[1] Currently, many non-insulin antidiabetic drugs are available for DM management, including metformin (MET). MET is classified as an oral antidiabetic medication belonging to the biguanide class. Its primary mechanisms of action include enhancing insulin sensitivity, promoting intracellular glycogen synthesis, inhibiting hepatic glucose production, and augmenting muscle glucose absorption.[2] Clinically, this drug is a first-line treatment in patients with type 2 diabetes mellitus (T2DM) to reduce hyperinsulinemia and body weight, effectively reducing HBA1C.[2,3] MET has also been proven to increase insulin sensitivity by balancing the intestinal microflora in T2DM mice to maintain the integrity of the intestinal structure.[4] Another advantage of MET in maintaining blood glucose levels is the prevention of weight gain by modulating the hypothalamic appetite control center.[1] Research results also show the nephroprotective[5] benefits of MET and endometriosis alleviation.[6,7]

*Corresponding author. E mail: arifah.wahyuni@ums.ac.id
Tel: +62-813-2900-8616

Citation: Wahyuni, AS, Wijianto, DW, Iswanto, D, Listiadewi A. Phytochemical and Pharmacological Investigations of Different Extracts of *Dracaena spicata* Roxb. Available in Bangladesh. Trop J Nat Prod Res. 2024; 8(6): 7410-7415. https://doi.org/10.26538/tjnpr/v8i6.11
Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

However, MET use has also been noted to trigger organ damage in several incidents, including the risk of fatty liver and fibrosis with MET use for two years.[8] Lactic acidosis is a significant adverse impact of MET usage, which, if not handled, can lead to a number of health complications, including liver and renal impairment.[7]

Various studies have found that antioxidants can protect against organ damage from oxidative stress.[9] Black rice bran (BRB) contains *anthocyanin*-type *cyanidin-3-glucosidase* (C3G)[10] and has been proven to prevent kidney dysfunction and fibrosis,[11] protecting against kidney damage.[12] As per previous studies, this *anthocyanin* content also reduces blood glucose, improving the blood chemistry profile of diabetic mice.[13]

The use of a combination of ethanol extract of black rice bran (EEBRB) and MET can provide an effective way to control blood glucose. It may also improve blood glucose levels by inhibiting insulin production during gluconeogenesis, affecting the amount and structure of liver glycogen.[14] Meanwhile, BRB can reduce blood glucose by inhibiting the α-glucosidase enzyme in the gastrointestinal tract, increasing insulin secretion and glucose uptake in peripheral cells.[15] This research aimed to evaluate the effectiveness of the combination of EEBRB and MET in controlling blood glucose and protecting the liver and kidneys after 21 days of use in hyperglycemic mice.

Materials and Methods

Materials

The materials used were a vacuum rotary evaporator, a water bath, a 2610-gram rat scale (Lark, China), an analytical balance (Presica A-SCS), a visible spectrophotometer (Star Dust FC*15), a sonicator, a centrifuge (Mini Spin), and a CX23 binocular microscope (Olympus®). MET was obtained from Indofarma. Additionally, alloxan (ALX, Sigma-Aldrich®), reagent kit Glucose GOD FS, distilled water, 96% ethanol, hydrochloric acid, glucose, ketamine,

xylazine, and saline solution (0.9% NaCl solution) were used for the experiment.

Plant collection and identification
The materials used were BRB, obtained from the harvest in Kingkang, Klaten, West Java, Indonesia.

Animals
The male white *Wistar* rats used in this study were obtained from the Pharmacology Laboratory, Faculty of Pharmacy, Muhammadiyah University, Surakarta. These rats had a weight range of roughly 160–180 g and were approximately 2–3 months old. Additionally, they were provided with a standard mouse feed and full access to water, and they were kept in cage enclosures at a temperature of 25 ± 1°C. Ethical approval for the study was obtained from the ethical committee of KEPK (No. 3733/A-1/KEPK-FKUMS/X/2021).

Extraction of BRB for anthocyanin enrichments
Two hundred (250) gram of dried BRB was soaked in a solvent mixture of ethanol, water, and hydrochloric acid in a volume ratio of 50:50:0.5, v/v/v, for two hours. The macerate was evaporated with a rotary evaporator for six hours, followed by a dry exhauster for 24 hours. The dry extract was obtained by adding 10:1 desiccant to the BRB extract to enrich it with C3G content.[12,16]

Modeling hyperglycemic animals
Male white rats, which had been adapted to the experimental environment and had set initial parameters as baseline values, were induced with ALX 150 mg/kg BW (0.3% in saline) intraperitoneally. Treatment was administered to animals whose blood glucose levels were above 200 mg/dL on the fourth day following induction.[17]

Treatment of experimental animals
The animals were treated with distilled water, ALX, MET, and a combination of MET and the extract for 21 days. The normal control group received distilled water; the negative control group was injected with ALX (150 mg/kg BW);[18] the MET group received 63 mg/kg BW; and the combination group received a combination of MET (63 mg/kg BW) and EEBRB (50 mg/kg BW). Colorimetry was employed to detect the levels of fasting blood glucose (FBG), blood urea nitrogen (BUN), serum creatinine (SCr), and serum glutamic pyruvic transaminase (SGPT) on days 0, 7, 14, and 21 using appropriate reagents. On day 21, all test animals were sacrificed, and their pancreas, liver, and kidneys were retrieved to be fixed in normal buffered formalin and stained with hematoxylin-eosin (HE). The normal and damaged cells in the tissue were observed using a binocular microscope with 1000x magnification. A small section of the liver was washed off with saline solution and then dehydrated in an oven in order to determine its glycogen content.[21]

Determination of rat liver glycogen levels
A total of 25 mg of liver, which had been dried at 50ºC overnight, was extracted using 1 mL of a 30% KOH solution. The mixture was then incubated in a boiling water bath for 20 minutes and left to cool at room temperature. The sample was added to 1.5 mL of cold ethanol (95%), and it was kept at 4ºC for 30 minutes. The glycogen precipitate was separated by centrifugation (2500 rpm for 20 minutes). Then, 1 mL of distilled water was added to the sample, and a 100 µL sample was taken into a test tube. After this, 3 mL of *anthrone-sulfuric acid* (0.2%) was heated, and the color changed to green. The absorbance of the sample was measured with a spectrophotometer at a wavelength of 620 nm.[19,20]

Statistical analysis
An ANOVA was carried out on the data obtained from the treatment groups, followed by a *Tukey post hoc* test. Meanwhile, non-parametric tests were employed in the analysis to quantify normal cells and cells.

Results and Discussion

Hyperglycemia was induced in the rats with intraperitoneal ALX (150 mg/kg BW). This dose was 2–3 times higher than the intravenous dose. ALX reacts with two (-SH) groups in the binding of glucokinase glucose, resulting in the formation of disulfide bonds and the inactivation of the enzyme. This reaction produces hyperglycemia after exceeding the four phases of increasing and decreasing FBG levels. The first stage after an ALX injection will be hypoglycemia, which lasts about 30 minutes. The second stage, one hour after the ALX injection, causes an increase in blood glucose concentration, which is the first hyperglycemic phase after the interaction between the pancreatic *β*-cells and the toxin. This occurs for 2–4 hours and causes the plasma insulin concentration to decrease due to the inhibition of insulin secretion in the pancreatic *β*-cells—caused by the induction of toxicity to *β*-cells. The third stage will be a second hypoglycemia, which lasts around 4–8 hours after the ALX injection. Insulin deficiency occurs due to ALX induction and the rupture of cell membranes, resulting in a hypoglycemic transition. The last part of the blood glucose response is a permanent diabetic phase with high blood sugar during degranulation and *β*-cell damage between 24 and 48 hours after ALX administration. Specifically, in the current study, hyperglycemia was observed on the fifth day after administration, with blood glucose levels exceeding 400 mg/dL (Table 1). This elevated blood glucose level persisted until the end of the 21st day of the experiment. The elevation occurred due to the inhibition of insulin production in pancreatic beta cells stimulated by glucose.[21]

Treatment with MET 63 mg/kg BW reduced FBG levels to an average level of 72.67 ± 30.238 (Table 1) on day 21. The MET reduces FBG levels by inhibiting excessive hepatic glucose production through reduced gluconeogenesis and increased peripheral glucose utilization, which brings down food intake and glucose absorption in the intestine.[22] Glycogenolysis releases glucose into the bloodstream for uptake by other cells, so suppressing glycogenolysis reduces liver glucose output as a practical approach to controlling hyperglycemia conditions.[23] While reducing FBG levels, MET does not stimulate endogenous insulin secretion, so it does not cause hypoglycemia or hyperinsulinemia, common side effects associated with other antidiabetic drugs.

Several ingredients support the ability of EEBRB to reduce FBG levels, including C3G. Research has demonstrated that C3G exhibits the ability to enhance insulin resistance in adipocyte cells and modulate insulin signaling through the regulation of insulin receptor-related pathways and the augmentation of glucose transporter 4 (GLUT4) translocation,[9,10] which inhibits glucose absorption. In the current study, the combination of EEBRB and MET controlled FBG from day 7 to 21, reducing FBG levels to 83.0 ± 33.0 (Table 1). The reduction in levels from this combination was a combined effect of the two ingredients.

Similarly, the body weights of the animals were monitored throughout the experiment. The final body weight of mice decreased in each group. The final body weight of the mice treated with MET decreased to 185.80 g ± 18.074, while that of the mice in the group combining MET with EEBRB also reduced to 160.60 g ± 43.517, which was statistically insignificant (Figure 1). A higher final body weight was noted in the normal group in comparison to the MET group alone, but overall, MET administration did not cause excess body weight. MET rarely causes hypoglycemia and excess body weight,[24] so MET is the first choice for diabetes treatment in overweight patients who are unable to control diabetes through diets.[25]

Anthocyanin is one of the antioxidants in BRB, which contains a pharmacological activity, among others, capable of lowering FBG[26] by inhibiting the *alpha-glucosidase enzyme*, resulting in delayed blood glucose absorption.[27] MET acts by reducing hepatic glucose production (gluconeogenesis), bringing down glucose absorption in the large intestine, and increasing insulin receptor sensitivity.[23] The administration of MET and the combination of MET and EEBRB for 14 days showed relatively normal FBG until the 21st day and maintained stable FBG levels (< 126 mg/dL). EEBRB has been proven to be potent in reducing FBG by inhibiting glucose absorption through *alpha-glucosidase enzyme* inhibition[27] and increasing insulin

secretion.[15] The MET and EEBRB combination slowly decreased FBG levels without a hypoglycemic effect (Table 1). damaged by histopathological tests.

Glycogen is usually formed through glycogenesis, which involves insulin, leading to glucose storage in the liver. In this study, MET administration for 21 days increased liver glycogen levels by stimulating glucose production and lactate release (Figure 2). MET, together with EEBRB, increases glycogen levels. The *anthocyanin* compound C3G increases glucose absorption and reduces glycolysis activity by increasing glucose 6-phosphate (G6P) and reducing phosphoenolpyruvate and lactic acid. Increasing G6P to pentose phosphate increases *nicotinamide adenine dinucleotide phosphate* (NADPH) production. NADPH produced from the pentose phosphate pathway may be used to maintain the cellular oxidative capacity of the liver, which increases *glutathione* (GSH) levels, implying that C3G increases the antioxidant capacity in the liver[28] by forming glycogen for glucose storage in the liver.

The kidney function was studied by monitoring the SCr and BUN parameters. Urea is the final product of amino acid and protein catabolism, which is filtered by the glomerulus, and some of it is reabsorbed if kidney function is disrupted. Serum urea measurements can be used as a reference for evaluating kidney function. Creatinine is a waste product of muscle breakdown, excreted through the kidneys.[29] The ALX and MET groups showed higher BUN and SCR values than the others (Table 2). Renal dysfunction in the ALX and MET groups was verified using histological analysis.

In the current study, the results of the kidney histopathology showed that the ALX group had the most cell damage compared to the others ($p < 0.05$, Figure 3). Similar damage was observed in the group given MET 63 mg/kg BW ($p < 0.05$, Figure 3). Chronic exposure to relatively high reactive oxygen species (ROS) causes cell function disorders[30] in prolonged hyperglycemic conditions, including kidney cells, endothelial cells, smooth muscle cells, mesangial cells, podocytes, and tubular cells.[31] The MET and EEBRB combination groups did not differ in the number of damaged kidney cells from the normal group ($p > 0.05$, Figure 3). The *anthocyanins* in EEBRB, which have antioxidant activity, can reduce kidney cell damage in hyperglycemic mice, reduce ROS production, and increase antioxidant enzyme activity.[10] C3G, an *anthocyanin* in EEBRB, is rapidly distributed to several organs, including the kidneys, and eliminated intact,[32] thus possibly protecting these organs.

Liver histopathology showed that the ALX group exhibited more liver cell damage than the other groups ($p < 0.05$, Figure 3). This may have occurred due to hyperglycemia induced by ALX triggering morphological and ultrastructural changes in the liver similar to those in human diseases, ranging from steatosis to steatohepatitis and liver fibrosis with unclear mechanisms.[33] *Anthocyanins*, which also act as antioxidants, can prevent and repair liver cell damage. C3G increases glucose absorption and reduces glycolytic activity. The increase in 6-phosphogluconate as a substitute for ribose 5-phosphate and ribose 1-phosphate indicates that C3G increases the pathway conversion of G6P to pentose phosphate only slightly, thereby increasing NADPH production and can be used to increase GSH levels, which increases the antioxidant capacity of the liver.[28] This can also be seen from the liver function test results. The MET and EEBRB combination group showed higher levels of normal cells than the group given only MET (Figure 3).

The examination of pancreatic damage in the MET and EEBRB combination group showed that the number of normal pancreatic cells in this group was more significant than that in the group given MET alone, with the number of normal cells not significantly differing from the normal group ($p > 0.05$, Figure 3). In hyperglycemic modeling, the main factor for pancreatic cell damage is the use of ALX as a diabetogenic agent, which can damage pancreatic beta cells by forming ROS. However, if ROS production is excessive or continuous, it will have a negative correlation because ROS can damage pancreatic β-cells.[12,34,35] *Anthocyanins*, which also act as antioxidants, can prevent and repair damage to pancreatic, kidney, and liver cells.[12] The results of this study showed the ability of a MET and EEBRB combination to protect the kidneys, liver, and pancreas, which can be seen in the histopathology results with HE staining (**Figure 4**). Furthermore, the number of normal cells in the group treated with MET and EEBRB did not differ significantly from that of the normal group, according to the statistical analysis (Figure 3).

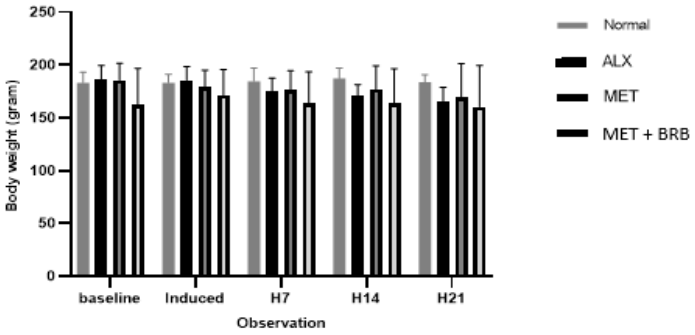

Figure 1: Average body weight before and after treatment for 21 days in normal, alloxan (ALX), metformin (MET), and combination metformin (MET) and ethanol extract of black rice bran (EEBRB) groups (n = 5).

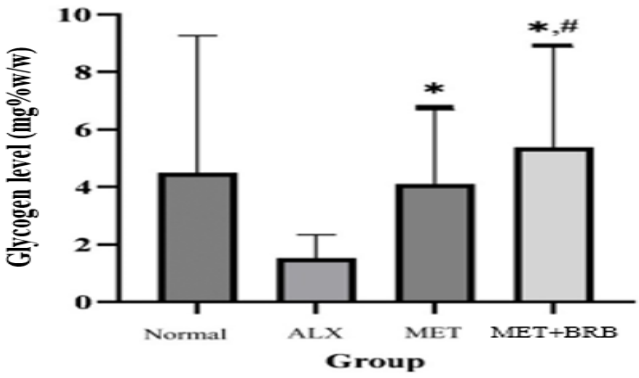

Figure 2. Liver glycogen levels (%b/b) after 21 days of treatment in various groups (n = 5).
*Significant ALX-group ($p < 0.05$); # not significant with MET 63 mg/kg BW ($p < 0.05$).

Table 1: Average blood glucose levels before and after treatment with distilled water, MET 63 mg/kg BW alone, and joint administration with EEBRB 50 mg/kg BW (n = 5)

Parameter	Fasting Blood Glucose (mg/dL)				
	Baseline	**Induksi**	**Day 7**	**Day 14**	**Day 21**
Normal	80.0 ± 28.4	93.0 ± 31.6	99.6 ± 17.1	84.4 ± 35.8	100.2 ± 22.2
ALX	91.4 ± 4.4	412.2 ± 94.5	450.0 ± 91.4	480.2 ± 80.9	542.4 ± 83.5
MET	91.8 ± 5.1	435.6 ± 63.1	174.0 ± 139.8	102.6 ± 39.2	79.2 ± 24.4
MET + BRB	93.4 ± 8.9	453.4 ± 129.7	288.4 ± 146.2	183.6 ± 44.3	83.0 ± 33.0

Table 2: Data on BUN, SCr, and SGPT levels in various treatment groups specified at baseline, induction, and days 14 and 21 after treatment (n = 5)

Parameter	SGPT Level (IU/L) After Treatment			
	Baseline	**Induced**	**Day 14**	**Day 21**
Normal	20.8 ± 7.5	21.2 ± 6.4	39.4 ± 4.5	31.20 ± 4.9
ALX	28.0 ± 8.0	44.6 ± 9.5	57.0 ± 8.3	83.40 ± 34.0
MET	27.40 ± 3.5	45.6 ± 18.3	43.0 ± 9.4	47.00 ± 10.5
MET+ BRB	38.20 ± 28.0	35.40 ± 9.2	40.8 ± 10.5	31.40 ± 6.0
Parameter	**SCr**			
	Baseline	**Induced**	**Day 14**	**Day 21**
Normal	0.90 ± 0.2	0.86 ± 0.1	0.94 ± 0.1	0.96 ± 0.0
ALX	0.74 ± 0.2	0.88 ± 0.2	0.92 ± 0.2	1.30 ± 0.3
MET	0.76 ± 0.1	0.94 ± 0.2	0.86 ± 0.1	1.06 ± 0.1
MET+ BRB	0.76 ± 0.1	1.02 ± 0.1	0.86 ± 0.2	0.82 ± 0.1
Parameter	**BUN**			
	Baseline	**Induced**	**Day 14**	**Day 21**
Normal	36.6 ± 6.6	32.80 ± 6.9	39.0 ± 7.6	42.2 ± 9.7
ALX	40.2 ± 9.3	95.2 ± 48.2	103.0 ± 68.1	135.2 ± 63.6
MET	42.4 ± 10.4	74.0 ± 51.8	60.6 ± 29.1	69.6 ± 30.9
MET+ BRB	33.4 ± 10.1	91.6 ± 25.0	64.2 ± 21.2	45.0 ± 10.4

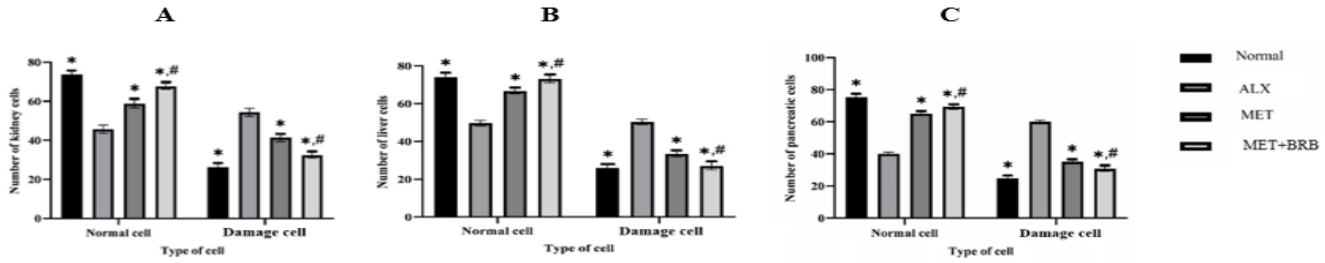

Figure 3: Results of the number of normal and damaged (A) kidney cells, (B) liver cells, and (C) pancreatic cells in the various treatment groups (n = 5)

* marks the parameters that showed a significant difference with the alloxan group ($p < 0.05$); # marks the parameters that did not significantly differ with the normal group ($p > 0.05$)

Conclusion

In this study, it was observed that the combination of MET and EEBRB successfully lowered blood glucose levels in hyperglycemic rats and protected them against damage to the kidneys, liver, and pancreatic cells. The mechanism of this effect may be unclear, but it may have occurred because of the synergistic effects of the two components. Future studies are suggested to elucidate the possible cellular mechanism of the antihyperglycemic effects of the MET and EEBRB combination.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors thank Muhammadiyah University of Surakarta for individual donations from lecturers who have funded this research and publication. The implementation agreement for PID (Individual Lecturer Research) is associated with contract No. 34/FF/A.3-III/2019.

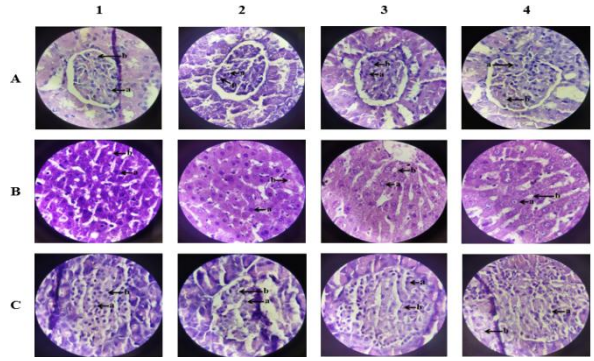

Figure 4: Image (1) normal control, (2) alloxan 150 mg/kg BW, (3) MET 63 mg/kg BW, and (4) MET 63 mg/kg BW + EEBRB 50 mg/kg BW with (a) normal cell and (b) damage cell of the tissue seen from the histopathology results of the (A) kidney, (B) liver, and (C) pancreas organ stained with HE (hematoxylin-eosin) and seen under a microscope with 1000x magnification.

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843.
2. Yerevanian A, Soukas AA. Metformin: Mechanisms in human obesity and weight loss. Curr Obes Rep. 2020;8(2):156–64.
3. Wu M, Xu H, Liu J, Tan X, Wan S, Guo M, Long Y, Xu Y. Metformin and fibrosis: A review of existing evidence and mechanisms. J Diabetes Res. 2021;2021:1–11.
4. Aggarwal N, Singla A, Mathieu C, Montanya E, Pfeiffer AFH, Johnsson E, Zhao J, Iqbal N, Bailey C. Metformin extended-release versus immediate-release: An international, randomized, double-blind, head-to-head trial in pharmacotherapy-naïve patients with type 2 diabetes. Diabetes, Obes Metab. 2018;20(2):463–7.
5. Peng Z, Zheng Y. Ganjiang Huangqin Huanglian Renshen Decoction improves insulin sensitivity by regulating intestinal flora in rats with type 2 diabetes mellitus. Trop J Pharm Res. 2023;22(April):833–9.
6. Yerevanian A, Soukas AA. Metformin: Mechanisms in human obesity and weight loss. Curr Obes Rep. 2019;8(2):156–64.
7. Sepahi MA, Mehrabi S, Valizadeh R, Kellner SJ, Mirzazadeh A, Ebrahimi S. Nephroprotection and chemotherapy sensitivity impact of metformin during cisplatin therapy; an updated review. J Nephropathol. 2019;8(4).
8. Zhang H, Liu F, Huang Y, Liu W. The role of metformin in the management of endometriosis: A systematic review and meta-analysis. Trop J Pharm Res. 2023;22(5):1115–20.
9. Reeker W, Schneider G, Felgenhauer N, Tempel G, Kochs E. Metformin-induced lactacidosis. Dtsch Medizinische Wochenschrift. 2000;125(9):249–51.
10. Lee HW, Lee JS, Kim BK, Park JY, Kim DY, Ahn SH, Kim SU. Evolution of liver fibrosis and steatosis markers in patients with type 2 diabetes after metformin treatment for 2 years. J Diabetes Complications [Internet]. 2021;35(1):107747. Available from: https://doi.org/10.1016/j.jdiacomp.2020.107747
11. Agustini R, Sanjaya GM, Herdyastuti N. In vivo assessment of nanocapsules of black rice (*Zizania aquatica*) yeast extract in diabetes mellitus type 2-induced mice (*Mus musculus*). Trop J Nat Prod Res. 2023;7(1):2237–43.
12. Les F, Cásedas G, Gómez C, Moliner C, Valero MS, López V. The role of anthocyanins as antidiabetic agents: From molecular mechanisms to in vivo and human studies. J Physiol Biochem. 2020;77(1):109–31.
13. Alnamshan MM. Antioxidant extract of black rice prevents renal dysfunction and renal fibrosis caused by ethanol-induced toxicity. Brazilian J Biol. 2022;82:1–10.
14. 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. Int J Pharm Res. 2020;12(1):509–17.
15. Watanabe M. Effects of black rice containing anthocyanins on plasma and hepatic parameters in type 2 diabetic db/db mice. Food Sci Technol Res. 2016;22(5):719–25.
16. Ailanen L, Bezborodkina NN, Virtanen L, Ruohonen ST, Malova AV, Okovityi SV, Chistyakova EY, Savontaus E. Metformin normalizes the structural changes in glycogen preceding prediabetes in mice overexpressing neuropeptide Y in noradrenergic neurons. Pharmacol Res Perspect. 2018;6(2).
17. Wahyuni AS, Munawaroh R, Da'i M. Antidiabetic mechanism of ethanol extract of black rice bran on diabetic rats. Natl J Physiol Pharm Pharmacol. 2016;6(2):106–10.
18. Hou Z, Qin P, Zhang Y, Cui S, Ren G. Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. Food Res Int. 2013;50(2):691–7.
19. Sutrisna E, Hervian L, Sahadewa FA. Hypoglicemic effect of 70% ethanolic extract of *Tinosporacrispa* L. (Bratawali) stem from Indonesia in Wistar rat induced by alloxan. J Clin Diag Res. 2018;12(9):FF01–3.
20. Muhtadi M, Haryoto H, Sujono TA, Suhendi A. Antidiabetic and antihypercholesterolemia activities of rambutan (*Nephelium lappaceum* L.) and durian (*Durio zibethinus Murr*.) fruit peel extracts. J Appl Pharm Sci. 2016;6(4):2231–3354.
21. Mojibi N, Rasouli M. Comparison of methods to assay liver glycogen fractions: The effects of starvation. J Clin Diagnostic Res. 2017;11(3):BC17–20.
22. Suarsana IN, Priosoeryanto BP, Wresdiyati T, Bintang M. Synthesis of liver and muscle glycogen on diabetic rats by administrated of extract tempe. J Vet. 2010;11(3):190–5.
23. Carvalho CDDE, Augusto C, Filho K, Luiz A, Rocha DA, Sanchez A, Silva R. Glycogen kinetics of Wistar rats: Different exercise intensities and tissue analyzed influence. Int J Exerc Sci. 2022;15(2):289–99.
24. Elkotby D, Hassan AK, Emad R, Bahgat I. Histological changes in islets of Langerhans of pancreas in alloxan-induced diabetic rats following Egyptian honey bee venom treatments. Int J Pure Appl Zool. 2018;6(1):1–6.
25. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways. Pharmacogenet Genomics. 2012;22(11):820–7.
26. Liu X, Wang K, Zhou J, Sullivan MA, Liu Y, Gilbert RG, Deng B. Metformin and Berberine suppress glycogenolysis by inhibiting glycogen phosphorylase and stabilizing the molecular structure of glycogen in db/db mice. Carbohydr Polym. 2020;243.
27. Olamoyegun MA, Omisore NO, Yusuf AO, Odewale TE. Assessment of pharmacodynamic interactions and toxicological effects of *Vernonia amygydalina* –Metformin co-administration on streptozotocin-induced diabetic Wistar rats. Trop J Nat Prod Res. 2022;6(12):2073–80.
28. Baker C, Retzik-Stahr C, Singh V, Plomondon R, Anderson V, Rasouli N. Should metformin remain the first-line therapy for treatment of type 2 diabetes? Therapeutic Adv Endocrinol Metab. 2020;12:1–13.
29. Tantipaiboonwong P, Pintha K, Chaiwangyen W, Chewonarin T, Pangjit K, Chumphukam O, Kangwan N, Suttajit M. Anti-hyperglycaemic and anti-hyperlipidaemic effects of black and red rice in streptozotocin-induced diabetic rats. SciAsia. 2017;43(5):281–8.
30. Bae IY, An JS, Oh IK, Lee HG. Optimized preparation of anthocyanin-rich extract from black rice and its effects on in vitro digestibility. Food Sci Biotechnol. 2017;26(5):1415–22.
31. Jia Y, Wu C, Kim YS, Yang SO, Kim Y, Kim JS, Jeong MY, Lee JH, Kim B, Lee S, Oh HS, Kim J, So MY, Yoon YE, Thach TT, Park TH, Lee SJ. A dietary anthocyanin cyanidin-3-O-glucoside binds to PPARs to regulate glucose metabolism and insulin sensitivity in mice. Commun Biol. 2020;3(1):2–11.
32. Vaidyanathan K. Textbook of biochemistry for medical students. 9th ed. Vasudevan DM, editor. Textbook of Biochemistry for Medical Students. New Delhi: Jaypee Brothers Medical Publishers; 2016.
33. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013;93(1):137–88.
34. Fornasaro S, Ziberna L, Gasperotti M, Tramer F, Vrhovšek U, Mattivi F, Passamonti S. Determination of cyanidin 3-glucoside in rat brain, liver and kidneys by UPLC/MS-MS and its application to a short-term pharmacokinetic study. Sci Rep. 2016;6(22815):1–11.

35. Lucchesi AN, Cassettari LL, Spadella CT. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. J Diabetes Res. 2015;2015(5):1–11.
36. Patley C, Srivastava DN, Patley R, Kohli S. Alloxan induced oxidative stress and impairment of oxidative defense system in rats. Asian J Biomed Pharm Sci. 2012;2(15):58–61.
37. Marin DP, Bolin AP, MacEdo RDCS, Sampaio SC, Otton R. ROS production in neutrophils from alloxan-induced diabetic rats treated in vivo with astaxanthin. Int Immunopharmacol. 2011;11(1):103–9.