Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. 9 No. 2, April - June 2024
https://ojs.stfmuhammadiyahcirebon.ac.id/index.php/iojs

EFFECTIVITY TEST OF THE COMBINATION OF ETHANOL EXTRACT MAHKOTA DEWA FRUIT (*Phaleria macrocarpa* (Scheff.) Boerl.) WITH METFORMIN TO LOWERING BLOOD GLUCOSE LEVELS IN ALLOXAN-INDUCED WISTAR WHITE RATS STRAIN

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Submitted: April 25, 2024 ***Revised: May 30, 2024*** ***Accepted: June 24, 2024***

ABSTRACT

Metformin is a first-line biguanid drug for type 2 of diabetes mellitus. Metformin potentially causes liver fibrosis in long-term use, its decrease it in their effectiveness of antidiabetic. Combining metformin with antidiabetic α-glucosidase enzyme inhibitors can increase the effectiveness of metformin. Mahkota dewa fruit is a natural ingredient that has antidiabetic activity with the mechanism of action of inhibiting α-glucosidase enzyme. This study aims to determine the effect of the combination of mahkota dewa fruit extract (EEMF) with metformin on the effectiveness of reducing blood glucose levels in diabetic rats. A total of 20 rats were grouped into 4, namely, group I: given distilled water (negative control); group II: given metformin 45 mg/kgBW; group III: given EEMF 50 mg/kgBW; group IV: given a combination of metformin 45 mg/kgBW and EEMF 50 mg/kgBW. The study was conducted by determining blood glucose levels using the GOD-PAP method. The results showed that the combination of EEMF with metformin had an average decrease per day from after induction to day 7 of 42,9 mg/dL and from day 7 to day 14 of 0,45 mg/dL. It can be concluded that the combination of EEMF dose 50 mg/kgBW with metformin 45 mg/kgBW is not recommended to be used together, because the large decrease has the risk of hypoglycemia effect.

Keywords: antidiabetic, alloxan, metformin, fruit of mahkota dewa.

INTRODUCTION

Diabetes mellitus (DM) is a health disorder indicated by the condition of hyperglycemia, which is when the fasting glucose level is ≥126 mg/dL. As a chronic disease, diabetes mellitus requires long-term medical care to minimize the risk of long-term disability, complications and mortality. Metformin is a antihyperglycemic agent that works by improving insulin sensitivity through increased peripheral glucose utilization (Zhou *et al.*, 2018). Metformin also inhibits gluconeogenesis in liver perfusion, mainly through inhibition of lactate uptake in the liver (Hundal *et al.*, 2000). The use of metformin in type 2 DM patients for two years has the potential to cause liver fibrosis which results in decreased effectiveness in reducing blood glucose levels, because metformin works in the liver (Lee *et al.*, 2021). Which can be done to overcome this condition is by combining drugs with different mechanisms of action between drugs so that they can cause a potentiation effect, namely the two drugs increase the effectiveness of each other.

The 70% ethanol extract of mahkota dewa fruit (*Phaleria macrocarpa* (scheff.) Boerl.) has antidiabetic activity. According to the study, EEMF in doses of 250 and 500 mg/kgBW significantly reduced blood glucose levels after 7 days of administration. The mechanism of action is to prevent an increase in blood glucose levels by inhibiting the

enzyme alpha-glucosidase, which is responsible for converting disaccharides to glucose (Dede et al., 2019).

Metformin and α-glucosidase inhibitors are recommended as first-line therapy, either as monotherapy or combination therapy, if one agent fails to control glycemia (Joshi *et al.*, 2014). The role of EEMF as an antidiabetic agent by α-glucosidase inhibitors is a consideration for testing the combination of drugs with metformin in the hope of increasing the effectiveness of lowering blood glucose levels, since the effectiveness of metformin is reduced with long-term use due to the onset of side effects of liver fibrosis (Lee *et al.*, 2021). Therefore, this study was conducted to determine the efficacy of reducing blood glucose levels by the combination of EEMF and metformin in alloxan-induced Wistar white rats.

RESEARCH METHODS

Tools and Materials

The tools used in this study are centrifuge (mini spin), visible spectrophotometer (DiaSys Star Dust MC15), triple beam balance scale capacity 2610 g (China), 1 mL and 3 mL syringe, micropipette, and a set of surgical tools. Materials used included ripe red crown of God fruit from Magelang, Central Java and harvested in August 2023, 70% ethanol (technical), alloxan monohydrate (Sigma-Aldrich), NaCl solution, metformin (Indofarma), Glucose GOD FS reagent kit (DiaSys), urine test strips (URiSCAN®), yellow tip, blue tip, 1.5 mL and 5 mL Eppendorf microtubes, capillary tubes.

Research Procedure

1. Collection and simplification of materials

   The mahkota dewa (*Phaleria macrocarpa* (Scheff.) Boerl.) samples were separated from the seeds. The samples were rinsed with running water to remove the dirt and then thinly sliced. Dried symplisia were obtained by drying in a drying oven at 50°C for 1-2 days. The dried symplisia was weighed as dry weight and pulverized by mixing. The sample was subjected to phytosanitary examination in the Biological Laboratory, Faculty of Teacher Training and Education, Muhammadiyah University of Surakarta with letter number : 022/A.E-I/LAB.BIO/VIII/2023. Proof that the examined sample is real Mahkota Dewa fruit of the species *Phaleria macrocarpa* (Scheff.) Boerl.
2. Preparation of ethanol extract of Mahkota Dewa Fruit (EEMF) (*Phaleria macrocarpa* (Scheff.) Boerl.)

   A total of 300 g of Simplisia was macerated with 70% ethanol in a ratio of 1:10. The vessel was tightly closed, stored at room temperature and without exposure to sunlight for 5 × 24 hours, with stirring several times. The macerate was then filtered through filter paper. The juice was kept in a closed vessel, allowed to settle for 1 night, and the filtrate (filtrate 1) was collected. The dregs were then macerated with 70% ethanol up to 1:10 by weight of Simplisia, then covered and left out of the sun for 2 days with stirring several times a day. The macerate was filtered and pressed, the resulting was precipitated for 1 night and mixed (filtrate 2). Filtrates 1 and 2 were combined to a concentrated ethanol extract on a rotary evaporator and evaporated again on a water bath until a thick extract was obtained. (Sutrisna *et al.*, 2010).
3. Phytochemical Screening Test
   a. Flavonoids

      The sample solution is prepared by heating 0,5 g of extract with 10 mL of methanol in a water bath for up to 10 minutes. Mixed while hot and filtrated with 10 mL of distilled water. Wait until cold, then add 5 mL of wash benzene, shake carefully and let stand. The methanol layer (bottom layer) was removed, then evaporated and the results were diluted with 5 mL of ethyl acetate and then mixed. Taubeck test, which is evaporated sample solution to dry, the residue is dissolved with acetone, put a little oxalic acid powder and boric acid powder. Heated in water, but avoid overheating. The residue is mixed with 2 mL of chloroform. The sample is

observed under UV light at 366 nm, the appearance of intense yellow fluorescence indicates the presence of flavonoids (Widyaningrum *et al.*, 2020)

b. Saponins

In a test tube, 0,5 g of extract was dissolved in 10 mL of hot water, shaken vigorously for 10 seconds and 1 drop of HCl 2 N was added. The tube was allowed to stand and the formation of a stable foam was observed. The sample is declared to contain saponins if a stable foam with a height of 1-3 cm is formed for 30 minutes (Dede *et al.*, 2019).

c. Alkaloids

A 0,5 gram amount of the extract was evaporated. To the residue was added 5 mL of 2N HCl and then divided into two test tubes. Three drops of Dragendorff's reagent were added to the first tube and three drops of Mayer's reagent were added to the second tube. The presence of alkaloids is indicated by an orange precipitate in the first tube and a yellow precipitate in the second tube (Wahid and Safwan, 2020).

4. Selection and adaptation of experimental animals

A total of 20 male white Wistar rats, aged 2-3 months and of weight 150-200 g, were selected according to healthy criteria, i.e. clean white hair, clear red eyes, active and normal behavior. Rats were acclimated to laboratory conditions for at least 1 week prior to the study (Sutrisna *et al.*, 2010). The purpose of acclimation is to allow the experimental animals to adapt to the environment in a way that will help them survive. Plastic cages measuring 39 cm × 42 cm × 15 cm contain a maximum of 5 rats. The top is covered with barbed wire. Each cage is equipped with sufficient bowls and a water dish. The recommended cage temperature is 25°C ± 2°C. Cage lighting was alternated between 12 hours of light and 12 hours of darkness (Rejeki *et al.*, 2018). This study received ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine, Muhammadiyah University of Surakarta with letter number: 5033/A.1/KEPK-FKUMS/IX/2023.

5. Dose Determination and Preparation

The dose was determined based on previous studies, and an orientation was performed to determine the effective dose. Orientation was performed at a dose of 200mg/kgBW, which was referenced from previous studies, but the dose provided a decrease in blood glucose levels too large, so it was reoriented using a lower dose. The effective dose obtained was 50 mg/kgBW. Administered orally with a volume of administration of 1mL/200gBW. EEMF dose of 10mg/200BW, prepared a stock solution of 100 mL by dissolving 1000 mg of EEMF in 100 mL of distilled water. Dissolution is assisted by a sonicator for 20-30 minutes.

The dose of metformin for rats is 45 mg/kgBW/day (Kosnayani *et al.*, 2021). The dose for 200 gBW rats is 9 mg. Metformin is administered orally with an administration volume of 1 mL/200gBW. The stock solution is then prepared by weighing 900 mg of metformin, dissolving to 100 mL with distilled water, and stirring until homogeneous.

The dose of alloxan for diabetes induction is 150 mg/kgBW (Oshkondali *et al.*, 2019). Then the alloxan is weighed at 30 mg/200gBW. Alloxan induction is done intraperitoneally with a volume of 1 mL/200gBW. Then alloxan monohydrate powder is weighed 150 mg, dissolved with 0.9% NaCl solution up to 5 mL. NaCl 0.9% as a solvent of alloxan to maintain the pH stability of alloxan so as not to irritate the body of rats when induction.

6. Generation the diabetic rat model

Diabetes was induced in rats after adaptation by the use of alloxan. Prior to induction, the rats were fasted for ± 18 hours, i.e. they were deprived of food but still given sufficient water. The induction of diabetes mellitus in experimental animals with alloxan is performed intraperitoneally. After induction, the rats were fed and 5 hours later they were given 30% glucose orally in a volume of 2 mL (Lenzen, 2008; Firdaus *et al.*, 2016). Glucose is given 5 hours after induction so that the animals do not die if they experience hypoglycemia after alloxan induction. 30% glucose loading is given daily to

keep the animals in a state of hyperglycemia. Normal blood glucose levels in rats are in the range of 50-135 mg/dL, and diabetic is defined as blood glucose levels >200 mg/dL (BPOM, 2021). If the rat does not experience hyperglycemia, the rat is included in the termination criteria and is replaced with a new rat.

7. Treatment of Experimental Animals

   The treatment of the experimental animals with the test materials is carried out for 14 days, starting after they are declared diabetic. A total of 20 male white rats of Wistar strain were weighed and then randomly divided into 4 groups of each 5 rats. Each group received oral treatment as follows:

   a. Group I as a negative control, received aquadest.
   b. Group II as a single metformin group, given metformin 45 mg/kgBW.
   c. Group III as a single EEMF group, given a dose of 50 mg/KgBW EEMF (oriented).
   d. Group IV as a combination group, given a dose of EEMF 50 mg/kgBW and metformin 45 mg/kgBW.

8. Blood Glucose Determination

   Rats were first fed for ± 18 h before blood samples were collected for determination of blood glucose levels (Amir *et al.*, 2020). Determination of baseline blood glucose levels was performed on day 7 after adaptation before alloxan induction, then on day 5 after alloxan induction to observe an increase in blood glucose levels, and on day 7 and day 14 after test material administration to observe a decrease in blood glucose levels (Dede *et al.*, 2019).

   Blood samples were collected from the retroorbital vein of the rats. Up to 1 mL of blood was collected using a 1,5 mL Eppendorf tube. The centrifugation method was performed to separate serum and blood cells at a speed of 13.400 rpm for 10 minutes. Serum samples were assayed for blood glucose using the GOD PAP method. Serum was collected in 5 µL and placed in a cuvette to which 500 µL of GOD FS glucose reagent was added. Incubation was performed for up to 10 minutes. Blood glucose levels were measured using a Stardust spectrometer. The results displayed on the monitor screen are in mg/dL (Lestari *et al.*, 2021; Subiyono *et al.*, 2016)

9. Body weight measurement

   Body weight was measured on the same day as blood sampling, i.e., before and after alloxan induction, as well as on day 7 and day 14 of test material administration. Body weight measurements are used to determine the effect of treatment on changes in body weight. In addition, body weight measurements are used to determine or adjust the next dose to be administered.

10. Urine Chemical Profile Measurement

    Urine chemical profile measurement is performed on the same day as blood sampling, i.e., before and after alloxan induction, as well as on day 7 and day 14 of test substance administration. Measurement of urine chemical profile is used as supporting data. The instrument used was a urine strip test (URiSCAN®). Parameters measured in the urine chemical profile include urine glucose, urine protein, urine specific gravity, urobilinogen and bilirubin.

11. Measurement of pancreas and liver weight

    On the 14^{th} day of treatment, the test animals were subjected to organ dissection. Organ dissection was performed to measure the weight of pancreas and liver. The weight of the pancreas is used as supporting data to determine the effect of treatment on the pancreas and liver organs of the animals test. Rats that are to be dissected are first sacrificed by cervical dislocation, which is by squeezing the rat's head with the thumb and forefinger, and then pulling hard and suddenly on the tail (BPOM, 2021).

Data Analysis

Blood sugar level data is presented as mean ± standard deviation. The normality of data distribution was tested using the Shapiro-Wilk test, and data homogeneity was tested using

the Levene test. It was found that the data were not normally distributed, so the Kruskal-Wallis nonparametric test and the Mann-Whitney post hoc test were performed.

RESULTS AND DISCUSSION

Preparation of Mahkota Dewa Fruit Extract (*Phaleria macrocarpa* (scheff.) Boerl.)

Extraction was carried out using the maceration method with 70% ethanol as a solvent. The use of 70% ethanol, which contains a polar hydroxyl group, was chosen for the extraction process. Therefore, 70% ethanol can attract secondary metabolite compounds in the form of saponins, flavonoids, and alkaloids from the mahkota dewa fruit. The maceration method offers several advantages, such as simple process, relatively high yield, and no potential damage to the compounds contained in the natural materials, as the process does not involve heating and can be re-macerated twice to increase efficiency. The EEMF yield is 30,81%, calculated based on the weight of dry simplisia (300 grams) and the weight of thick extract (92,45 grams).

Phytochemical Screening of Ethanol Extract Mahkota Dewa Fruit (*Phaleria macrocarpa* (Scheff.) Boerl.)

Phytochemical screening test is to determine the content of secondary metabolite compounds contained in ethanol extract of mahkota dewa fruit, so that it can determine compounds that have an antidiabetic role. Identification of saponin, flavonoid, and alkaloid compounds is done because it is suspected that these compounds have an antidiabetic role. The results of phytochemical screening in this study showed that EEMF contained saponins and alkaloids **Table I**. According to previous studies, flavonoid compounds were found in mahkota dewa fruit, but in this study no flavonoid compounds were found. This condition may be due to flavonoids which are thermolabile compounds degraded during the evaporation process with a rotary evaporator and or with a waterbath for 2 days, because the extract is constantly exposed to hot temperatures (Rahma *et al.*, 2017). Nevertheless, the content of saponins and alkaloids in EEMF still has activity in reducing blood sugar levels, it is known that saponins and alkaloids are inhibitors of the α-glucosidase enzyme (Arjadi and Mustofa, 2017).

Table I. Result of Phytochemical Screening Ethanol Extract Mahkota Dewa Fruit (*Phaleria macrocarpa* (Scheff.) Boerl.)

Identification	Result	Marks
Saponins	+	Formed stable foam of 1-10 cm for 10 minutes
Flavonoids	-	Taubeck test: no intensive yellow fluorosity formed under UV 366 light
Alkaloids	+	+ Dragendorff formed orange precipitate, + Mayer formed yellow precipitate

Description:
(+) : contains the compound (positive)
(-) : does not contain the compound (negative)

Blood glucose levels and body weight determination

Alloxan, a diabetogenic agent, was administered at a dose of 150 mg/kgBW to induce type II diabetes in animals. Alloxan induction in experimental animals produces pancreatic lesions, where the size of the lesion is proportional to the dose of alloxan used and correlates with pancreatic insulin content (Oshkondali *et al.*, 2019). Alloxan enters pancreatic beta cells through the GLUT2 glucose transporter. In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Autooxidation of dialuric acid generates superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These hydroxyl radicals trigger beta cell death, causing beta cell toxicity and selective beta cell necrosis. Beta cell damage leads to

reduced insulin secretion, causing an increase in blood sugar levels. Following alloxan induction, a hypoglycemic phase occurs approximately 4-8 hours later, which can last for several hours and may result in fatal seizures in test animals. To prevent this, oral administration of 30% glucose is necessary (Lenzen, 2008).

Table II. Result of Blood Glucose Levels Determination

Group	Mean Blood Glucose Level (mg/dL) ± SD				Decrease per Induced – D7	Decrease per day D7 – D14
	Baseline	Induced	Day 7	Day 14		
I	99,4±15,32	345,4±83,73	323,4±111,1	270,4±41,25	-4,43±10,3	-7,57±16,11
II	124,8±16,18	265,8±55,41	156,8±106,46[a]	109,80±37,06[a]	-15,57±9,23	-6,7±10,7
III	100,6±16,20	239,6±23,78	138,8±31,35[a]	100,6±12,22[a]	-14,4±5,01[a]	-5,45±5,67
IV	103±10,37	394,6±142,67	94,2±29,08[c]	97,4±22,38	-42,9±20,49[abc]	-0,45±4,28

Description:

Mean of decreased blood glucose level per day, Induced – H7= $\frac{(H7 - Induced)}{7}$

Mean of decreased blood glucose level per day, H7 – H14= $\frac{(H14-H7)}{7}$

a: significant difference to group I
b : significant difference to group II
c: significant difference between group III and IV
Class I (K-): given aquadest
Class II (single met.): given met. 45 mg/KgBW
Class III (single EEMF): given a dose of EEMF 50 mg / kgBW
Class IV (combination): given a dose of EEMF 50 mg / kgBW and met. 45 mg/KgBW

The statistical analysis with post hoc Mann Whitney showed a significant difference ($p<0.05$) between the group given the test material and the negative control group **Table II**. However, the negative control group also experienced a decrease, which may be attributed to the normalization of endocrine function without treatment after alloxan administration. This process involves the regeneration and neogenesis of pancreatic β-cells from either ductal or acinar cells (De Haro-Hernández *et al.*, 2004). The results indicate that groups II (met.), III (EEMF), and IV (EEMF + met.) all have the ability to reduce blood sugar levels. Notably, group IV (EEMF + met.) exhibited a significant decrease on day 7 compared to groups II (met.) and III (EEMF). The combination group's significant decrease may be a risk for hypoglycemia, so it is less effective when combined.

Metformin is a first-line antidiabetic drug that has a mechanism of action that inhibits hepatic gluconeogenesis by inhibiting lactate uptake (Hundal *et al.*, 2000). Meanwhile, EEMF has a mechanism of action that reduces glucose levels by inhibiting the α-glucosidase enzyme, which inhibits carbohydrates in the small intestine. This is justified by PERKENI 2021, hat the combination of antidiabetic drugs can be done provided that the two drugs have different mechanisms of action. According to Joshi *et al.*, 2014, metformin and α-glucosidase inhibitors are recommended as first-line combination therapy when one agent fails to control hyperglycemia.

However, in this study, the results of the combination showed a large decrease, which may pose a risk of hypoglycemia. This is not consistent with the statement that metformin and α-glucosidase inhibitors are able to lower blood glucose levels without hypoglycemic

effects (Akmal and Wadhwa, 2022; PERKENI, 2021). Therefore, the combination of EEMF 50 mg/kgBW with metformin 45 mg/kgBW is not effective. The large decrease in blood glucose levels by EEMF is possible due to several mechanisms of action it has, namely inhibition of the enzyme α-glucosidase, increase in insulin secretion and regeneration of pancreatic Langerhans cells. (Arjadi and Mustofa, 2017).

Table III. Results of Rat Body Weight Analysis

Group	Rerata Berat Badan (gram) ± SD				Rerata penurunan per/hari *Induced* – H7	Rerata penurunan per/hari H7 – H14
	Baseline	***Induced***	**Hari ke-7**	**Hari ke-14**		
I	180,6±34,62	158±23,36	159,2±15,19	155,2±10,92	+0,17±1,43	-0,57±2,27
II	188,4±27,76	178,6±26,38	192±32,59[a]	164,8±50,78	+1,9±1,26	-3,89±3,98
III	163±16,54	138,6±9,61	143,8±15,35[b]	136±12,71	+0,74±1,34	-1,11±0,91
IV	172,2±11,61	157,6±17,44	141,6±14,74[b]	144±19,79	-2,28±1,73[abc]	+0,34±0,91[c]

Description:

Mean of decreased blood glucose level per day, Induced – H7= $\frac{(H7 - Induced)}{7}$

Mean of decreased blood glucose level per day, H7 – H14= $\frac{(H14-H7)}{7}$

a: significant difference to group I
b : significant difference to group II
c: significant difference between group III and IV
Class I (K-): given aquadest
Class II (single met.): given met. 45 mg/kgBW
Class III (single EEMF): given a dose of EEMF 50 mg / kgBW
Class IV (combination): given a dose of EEMF 50 mg / kgBW and met. 45 mg/kgBW

Weight loss after diabetes is possible due to insulin deficiency. Where insulin deficiency causes disruption of protein and fat metabolism. So that the amount of calorie storage decreases and results in weight loss (Rias and Sutikno, 2017). In this study **(Arjadi** and Mustofa, 2017).

Table *III*, he body weight of group I (K-), group II (met.) and group III (EEMF) experienced improvement on day 7, but decreased again after day 14. While in group IV (EEMF + met.), weight improvement began on day 14.

Metformin is one of the antidiabetic drugs that is neutral towards increasing body weight PERKENI, 2021. However, in this case, metformin actually decreased body weight on day 14. According to Yerevanian and Soukas, 2019, weight loss with metformin use may be due to a decrease in caloric intake rather than an increase in energy expenditure. Metformin appears to affect appetite regulation either directly or indirectly through its gastrointestinal side effects. Similarly, EEMF, which is an α-glucosidase enzyme inhibitor, has a weight loss effect that may also be the result of its gastrointestinal side effects. (Akmal and Wadhwa, 2022)

Results of Urine Chemistry Analysis

Table IV. Results of Urine Chemistry Analysis

Measurements	Group	*Baseline*	*Induced*	D7	D14
Urine Glucose	I	-	++++	+++	++
	II	-	++	-	-
	III	-	++	-	-
	IV	-	++	-	-
Urine Protein	I	+	+++	+++	++++
	II	-	++	+++	+++
	III	+++	+++	++	+
	IV	++	++	+++	+
Specific gravity of urine	I	1.005	1.010	1.020	1.030
	II	1.020	1.025	1.030	1.020
	III	1.030	1.030	1.025	1.025
	IV	1.025	1.010	1.010	1.030
Urobilinogen	I	-	-	-	-
	II	-	-	-	-
	III	-	-	-	-
	IV	-	-	-	-
Bilirubin	I	-	-	-	-
	II	-	-	-	-
	III	-	-	-	-
	IV	-	-	-	-

Description:
Measurement of each group = 1-3 animals
Sign (-): indicates a negative value
Group I (K-): given aquadest
Group II (met.): given met. 45 mg/KgBW
Class III (EEMF): given a dose of EEMF 50 mg / kgBW
Class IV (combination): given a dose of EEMF 50 mg / kgBW and met. 45 mg/KgBW

Chemical analysis of urine glucose parameters **Table IV**, group I showed positive values after induction until day 14, and the other groups showed negative values after treatment. This is because in diabetic conditions there is an increase in plasma glucose so that glucose absorbed by tubular transport exceeds the capacity of the kidneys and results in glucosuria, which is the presence of glucose in the urine (Liman and Jialal, 2023). The results of the urine glucose control correlated with the blood glucose control **Table II**Error! Reference source not found.. Groups I, II, and III showed that the rats were no longer hyperglycemic.

The rats have normal protein levels, which are <35 mg/dL (Haryoto et al., 2015). Proteinuria is one of the manifestations of damage to the glomerular membrane, resulting in the leakage of protein molecules into the urine. The amount of proteinuria in 24 hours is used as an indicator to assess kidney function. This condition can also be a marker of diabetic nephropathy (Aitekenov et al., 2021). The results of this study, the signs (+++) and (++++) indicate the value of protein levels above normal. The treatment in group I (K-) and group II (met.) induced proteinuria, while the treatment in group III (EEMF) and group IV (EEMF + met.) showed an improvement of the previously occurring proteinuria.

The condition of diabetes can affect the increase in specific gravity of urine. The presence of large molecule substances in urine affects the specific gravity of urine, such as glucose and protein. Glucose has a large sugar molecule, so the glucose content in urine causes an increase in urine specific gravity (Azizah *et al.*, 2021). In patients without renal

disease, glucose in plasma is absorbed by the tubular transport system. However, absorption is not optimal if the nephron is abnormal. Thus, at the threshold of the nephron's ability, glucose is excreted in the urine, which causes an increase in urine osmolarity and increases the specific gravity of urine in diabetic conditions to 1.045 to 1.050 (Akarsu *et al*., 2006). In this study, there were no experimental animals with positive specific gravity values, so it is possible that the renal function of the experimental animals was not damaged.

The detection of bilirubin in the urine at higher than normal levels indicates the presence of liver disease. Bilirubin is normally absent (negative) in rat urine. Bilirubin is formed from the breakdown of hemoglobin, which is transported to the liver and excreted as bile. There are two types of bilirubin, conjugated (direct) and unconjugated (indirect). Bilirubin is transported to the liver as unconjugated bilirubin, which is bound to albumin. Upon entering the liver, the unconjugated bilirubin bond is released and then binds to glucoronic acid to form conjugated bilirubin. Conjugated bilirubin is water soluble, allowing bilirubin to appear in the urine (Haryoto et al., 2015). In this study, there was no positive result for bilirubin in urine.

Urobilinogen is formed from bilirubin that is conjugated by intestinal bacteria in the duodenum. Most of the urobilinogen returns through the bloodstream to the liver for processing into bile, and 1% is excreted by the kidneys in the form of urine. The pathological condition associated with elevated urobilinogen is excessive hemoglobin breakdown. Urobilinogen is used to assess hepatic excretory function or the presence of hepatic excretory abnormalities. The normal level of urinary urobilinogen is <40 mg/mL (Haryoto et al., 2015). In this study, neither urobilinogen nor bilirubin was found in urine. This may be due to the short duration of the study, in which the possibility of liver damage occurs in the long term.

Results of Pancreas and Liver Measurements

Table V. Results of Pancreas Measurements

Group	Rats Weight (g) ($\bar{x}$ ± SD)	Pancreas Weight (g) ($\bar{x}$ ± SD)	% Relative Weight of Organs
Group I	155,5 ± 10,92	0,52 ± 0,08	0,34 ± 0,57
Group II	164,8 ± 50,79	0,66 ± 0,74[a]	0,44 ± 0,13
Group III	136 ± 12,71	0,55 ± 0,09	0,41 ± 0,03[a]
Group IV	144 ± 19,79	0,67 ± 0,14	0,47 ± 0,49[a]

Description:

% Relative organ weight = $\frac{\text{Bobot pankreas (g)}}{\text{Bobot tikus (g)}} \times 100$

a: significant difference to group I.

Group I (K-): given aquadest

Group II (single meth.): given meth. 45 mg/KgBW

Group III (single EEMF): given a dose of EEMF 50 mg / kgBW

Class IV (combination): given a dose of EEMF 50 mg / kgBW and met. 45 mg/KgBW

Alloxan induction in rats causes pancreatic injury characterized by shrinkage of the pancreatic organ. Shrinkage of pancreatic organs due to damage correlates with a decrease in the number of β-cells in the islets of Langerhans (Rahayu *et al.*, 2006). From the results of the calculation of the % relative organ weight it can be concluded that the administration of the test material can influence the improvement of the pancreas size. According to the research of Arjadi and Mustofa 2017, EEMF provides the effectiveness of Langerhans cell repair according to the dose of administration. Langerhans cell repair by EEMF is an intra-pancreatic mechanism produced by the content of chemical compounds, namely alkaloids, which act to repair (regenerate) damaged pancreatic β-cells and protect β-cells from damage. The combination of EEMF with metformin resulted in better pancreatic organ repair than

metformin alone and EEMF alone. This correlates with the blood glucose measurements in **Table II**. Which is the improvement in blood glucose levels is proportional to the improvement in pancreatic organs.

Table VI. Results of Liver Measurements

Group	Rat Weight (g) ($\bar{x}$ ± SD)	Liver Weight (g) ($\bar{x}$ ± SD)	%Relative Weight of Organs
Group I	155,5 ± 10,92	5,88 ± 0,58	3,81 ± 0,51
Group II	164,8 ± 50,79	6,62 ± 0,99	4,45 ± 2,07
Group III	136 ± 12,71	5,72± 0,14	4,24 ± 0,41
Group IV	144 ± 19,79	5,85± 0,94	4,06 ± 0,40

Description:

% Relative organ weight = $\frac{\text{Bobot pankreas (g)}}{\text{Bobot tikus (g)}} \times 100$

a: significant difference to group I.

Group I (K-): given aquadest

Group II (single meth.): given meth. 45 mg/KgBW

Group III (single EEMF): given a dose of EEMF 50 mg / kgBW

Class IV (combination): given a dose of EEMF 50 mg / kgBW and met. 45 mg/KgBW

An indication of liver damage is the occurrence of swelling and fatty liver conditions (Ghazali and Arsito, 2012). This can be observed by measuring the difference in liver weight. According to Huang *et al.*, 2022, short-term use of metformin is relatively safe, while long-term use is associated with a high risk of NAFLD (non-alcoholic fatty liver disease). This may be due to the fact that metformin works in the liver, and the workload in the liver has an adverse effect on the organ. Based on the data in **Error! Reference source not found.** it is known that there was a high increase in liver weight in group II (metformin), but this value did not show a significant difference because the study was conducted only in the short term. Meanwhile, the combination of EEMF with metformin showed a smaller liver weight than single metformin, as well as single EEMF showed smaller liver weights compared to the group given metformin. This is possible because EEBM has antioxidant activity that counteracts free radicals, the ability to suppress the inflammatory cytokine TNF-α, and inhibits HSC activation by decreasing the fibrogenic cytokine TGF-β1, thus preventing liver fibrosis (Sundari and Soetikno, 2014).

CONCLUSION

The combination of ethanol extract of mahkota dewa fruit (EEMF) dose of 50mg/kgBW with metformin 45 mg/kgBW has a greater reduction in blood glucose levels, rather than single metformin or single EEMF. This large reduction has the risk of causing hypoglycemic effects. Therefore, the combination of EEMF 50 mg/kgBW and metformin 45 mg/kgBW is not recommended. Combination testing by lowering the dose of EEMF may be possible to determine a more effective dose as a combination.

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