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Effectiveness analysis of turmeric ethanol extract on pancreatic amylase enzyme in wistar rats

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Abstract
Turmeric (Curcuma longa Linn), a family of Zingiberaceae, contains curcuminoids that can be used for treating and preventing human diseases. This type of research is experimental, which determines the effect or relationship between the independent and dependent variables. The independent variable is turmeric ethanol extract, while the dependent variable is the decrease in fasting KGD in April 2023. Data were analyzed using the Shapiro-Wilk method to see the normality of the data. If the data were normally distributed ($P > 0.05$), continue using the Way ANOVA method to determine the average difference between groups. If there is a difference ($P < 0.05$), continue with the Post Hoc Tukey HSD test to see the real difference between treatments. However, the Kruskal-Wallis test is used if the data is not normally distributed. Curcuma longa ethanol extract doses of 100 mg / kgBB, 150 mg / kgBB, and 200 mg / kgBB have the activity of reducing blood glucose levels with significantly different ($P < 0.05$) with the negative control group, which is only given CMC-Na and doxorubicin. Curcuma longa extract doses of 100 mg/kgBB, 150 mg/kgBB, and 200 mg/kgBB have the activity of reducing HbA1c levels with significantly different ($P < 0.05$) with the negative control group, which is only given CMC-Na and doxorubicin.

Keywords: Curcuma longa linn, amylase enzyme, pancreas

Introduction

Type two diabetes mellitus is not only influenced by genetically abnormal islets, weight gain, physical activity, high-fat diet, and medication, but lifestyle factors also play a role in type two diabetes. Patients with diabetes mellitus can be at risk of chronic complications: coronary heart disease and stroke, kidney failure, retinopathy, and diabetic gangrene. Diabetes mellitus (DM) is a severe chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood glucose) or when the body cannot effectively use the insulin it does make. Risk assessment methods for diabetes mellitus have been widely applied; in clinical practice, risk models identify patients at risk of developing the disease due to using drugs such as doxorubicin [1].

Natural ingredients, such as turmeric, must be prioritized as primary prevention of kidney disease (Curcuma longa Linn). Turmeric is a family of Zingiberaceae, containing several phytoconstituents that fall into the category of alkaloids, glycosides, triterpenoids, sterols, including three curcuminoids (curcumin): diferuloylmethane (the main constituent and the one responsible for its bright yellow color), demethoxycurcumin, and also bisdemethoxycurcumin. Curcuma is popular in indigenous medicine systems, such as treating and preventing human diseases [2].

Various biological and pharmacological activities of curcumin have been investigated and used for antioxidant, antiviral, anti-inflammatory, antifungal, liver protection, gastrointestinal effects, dissolving gallstones, anticarcinogenic, antimicrobial, cardiovascular, tonic for the digestive system, to eliminate worms in the gastrointestinal tract, enhance the immune system, antifertility, menstrual disorders, anti-diabetes mellitus, hypolipidemic, urinary tract and kidney protection, anti-blood clotting, appetite enhancement, cough, rheumatism, sinusitis, and anti-HIV properties [3]. Curcuma longa is further explored for its potential as a functional food for pancreas-related diseases. Based on the background, the researcher was encouraged to test the pancreoprotective activity of turmeric ethanol extract on experimental animals by measuring biochemical parameters of ad random blood sugar levels, HbA1c, and amylase enzymes and conducting histopathological studies of the pancreas of experimental animals [4].

Research Methods

This type of research is experimental, which determines the effect or relationship between the independent and dependent variables. The independent variable is turmeric ethanol extract, while the dependent variable is the decrease in fasting KGD in April 2023. The tools used were surgical tools, microscope, 1 ml syringe, 3 ml syringe, oral sonde, centrifuge, tube, animal balance, analytical balance, beaker glass, mortar, stamper, spatula, parchment paper, volumetric flask, spectrophotometer, cuvette, micropipette, microtome, water bath, and object glass, Glucometer + stick. The materials used in this study were EEBM, Doxorubicin, NaCl, 10% formalin, chloroform, CMC-Na, Rats, Virgin coconut oil, reagents, liquid paraffin, toluene, acetone, ethanol extract of turmeric. The experimental animals were 24 healthy male Wistar rats (Rattus norvegicus) (active and able to eat). Researchers have approved ethical clearance at the Research Ethics Commission for research involving living things so that this research is feasible, and the research results can be accounted for and meet the requirements for publication in national and international journals.

Preparation of turmeric ethanol extract, phytochemical screening by examining alkaloid, flavonoid, glycoside, saponin, tannin, and steroid/triterpenoid compounds. Testing the pancreoprotective activity of turmeric ethanol extract *in vivo*. This test was conducted using male Wistar

rats as subjects. The *in vivo* test in the experiment used 24 (twenty-four) healthy rats with a body weight of approximately 170 g ± 10%, then divided into 4 (four) groups, and each group consisted of 5 (five) rats:

a. Group I (Normal Group): Na-CMC suspension
b. Group II (Negative Group): Mice injected with doxorubicin
c. Group III (Positive control): male wistar rats (Rattus norvegicus) induced by doxorubicin + Vitamin E 1% BW
d. Group IV (Treatment 1): Male Wistar rats (Rattus norvegicus) induced doxorubicin + 100 mg/kg bw turmeric ethanol extract
e. Group V (Treatment 2): Male Wistar rats (Rattus norvegicus) induced doxorubicin + 150 mg/kg bb turmeric ethanol extract
f. Group VI (Treatment 3): male Wistar rats (Rattus norvegicus) caused by doxorubicin + 200 mg/kg bb turmeric ethanol extract

Induction of pancreatic damage using doxorubicin 5 mg/kg bw intraperitoneally on days 1, 7, 14, and 20, then EEK suspension was given daily at a dose of 100 mg/kg bw, 150 mg/kg bw, and 200 mg/kg bw. KGD ad random analysis, HbA1c, blood alpha-amylase enzyme analysis. Preparation of pancreatic tissue by organ procedures was fixed with 10% formalin solution for 3-4 hours, then with acetone three times (each for 2 hours). After that, cleaning was done using toluene three times (1-2 hours each). The embedding process was carried out in liquid paraffin at 60-70°C 3 times (each for 2 hours), then the paraffin block molding process was carried out. The cutting stage of the paraffin block was carried out using a microtome to obtain a sheet with a thickness of 5 µm. The sheet was placed in a water bath with a temperature of 30°C, then attached to an object glass and heated in the oven for 2-3 minutes. The resulting sheets were observed under a light microscope with a magnification of 10x40, and the number of necrosis and normal cells were observed. The SPSS (Statistical Product and Service Solution) program version 25 analyzed the data. The data were analyzed using the Shapiro-Wilk method to see the normality of the data. If the data were normally distributed ($P > 0.05$), continue using the Way ANOVA method to determine the average difference between groups. If there is a difference ($P < 0.05$), continue with the Post Hoc Tukey HSD test to see the real difference between treatments. However, the Kruskal-Wallis test is used if the data is not normally distributed.

Results and Discussion

Table 1: Blood Sugar Measurement Data on days 5, 10, 15, and 20

No.	Treatment group	Blood sugar levels (mg/dl)				
		0	5	10	15	20
1.	Normal Group (Not induced by DOX)	71,30 ± 0,41	74,89 ± 1,99	74,77 ± 0,24	74,68 ± 1,85	72,27 ± 1,23
2.	Negative Group (DOX + CMC)	74,50 ± 1,12	244,41 ± 4,14	272,82 ± 3,51	277,05 ± 0,93	140,51 ±12,80
3.	Positive Group (DOX + Vitamin E)	74,50 ±0,04	182,3 ± 4,44	177,21 ± 1,11	169,82 ± 0,94	85,19 ± 11,57
4.	Treatment group I (DOX + 100 mg/kg body weight)	74,50 ± 0,87	236,22 ± 4,609	222,97 ± 2,22	204,11 ± 1,763	132,16 ± 8,12
5.	Treatment group II (DOX + 150 mg/kg body weight)	72,21 ± 0,07	192,03 ± 4,22	182,522 ± 2,22	165,70 ± 4,31	99,55 ± 4,40
6.	Treatment group III (DOX + 200 mg/kg body weight)	71,42 ± 0,34	184,42 ± 4,89	176,66 ± 3,66	144,807 ± 3,24	74,20 ± 3,78

Table 1. shows the administration of turmeric ethanol extract to doxorubicin-induced rats. The average group had the lowest blood sugar levels at the end of the study on day 20, namely 70.18 ± 1.14 mg/ml, which had a significant difference ($P <0.05$) to the negative control group, treatment group I, treatment group II, and had no significant difference ($P>0.05$) with the positive control group and treatment group III. The negative control group had the highest blood sugar level of 284.22 ± 15.12 mg/ml and had a significant difference ($P < 0.05$) from the standard group, positive control group, treatment group I, treatment group II, and treatment group III. In the extract treatment group, treatment group III has a blood sugar level of 74.20 ± 3.78 mg/ml, which has a significant difference ($P <0.05$) from the negative control group and has no significant difference ($P>0.05$) from the positive control group. The group given turmeric ethanol extract showed a decrease in blood sugar levels inversely proportional to the increase in the dose of quiet ethanol extract.

Based on a literature search, turmeric (Tumeric/ Curcuma longa Linn.) is one of the Curcuma species (Zingiberaceae) and is a spice plant rich in bioactive compounds with antioxidant properties that are very important and thrive in Indonesia, both as ingredients for spices, food coloring, and as ingredients for herbal medicine or traditional medicine. In the field of conventional medicine, turmeric is widely used as an ingredient in herbal medicine, and the efficacy of turmeric has been scientifically proven as an antidiabetic agent, anti-Alzheimer, anti-inflammatory, anti-bacterial and viral, antioxidant, antimicrobial, indigestion, hepatitis, jaundice, anti-atherosclerosis, and anticancer effects. Curcumin is a yellow-colored compound found in turmeric rhizomes, commonly seen as curcuminoids, a mixture of curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. The properties and engaging physical and chemical properties of curcumin compounds make it a leading combination for developing new medicinal compounds [5].

Table 2: Percent reduction in KGD (%) on days 0 and 20

No.	Treatment group	Blood sugar levels (mg)				(%) Elevated glucose levels
		0		20		
1.	Standard Group (Not induced by DOX)	71,3	± 0,41	72,27	± 1,23	0,99
2.	Negative Group (DOX + CMC)	74,5	±0,04	140,51	± 12,80	66,01
3.	Positive Group (DOX + Vitamin E)	74,5	±0,04	85,19	± 11,57	10,69
4.	Treatment group I (DOX + 100 mg/kg body weight)	74,5	±0,04	132,16	± 8,12	57,66
5.	Treatment group II (DOX + 150 mg/kg body weight)	72,2	± 0,07	99,55	± 4,40	27,39
6.	Treatment group III (DOX + 200 mg/kg body weight)	71,4	± 0,58	74,204	± 3,78	2,784

HbA1c measurements were made using the Rat HbA1c Kit by ELISA method, which was read by absorbance with a microplate reader at a wavelength of 450 nm. This method is based on the principle of measuring antigens or antibodies both relatively and quantitatively. HbA1c levels were obtained by measuring absorbance in the presence of the addition of standard solutions of 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml, 1.562 ng/ml. The absorbance value of each concentration can be seen in Table 4.3 as follows.

Table 3: Absorbance of HbA1c

Standard HbA1c concentration	Absorbance (450nm)
1,526	0,122
3,125	0,206
6,25	0,336
12,5	0,478
25	0,745
50	1,223
100	2,248

Table 4: HbA1c concentration in rat blood

Treatment group	Treatment group	Rata-rata konsentrasi HbA1c ± SD (ng/ml)
Normal Group (Not induced by DOX)	Normal group (CMC)	20,22 ± 0,74
Negative Group (DOX + CMC)	Negative control group (DOX+CMC)	75,12 ± 0,23
Positive Group (DOX + Vitamin E)	Positive control group (DOX+VitE)	25,51 ± 0,45
Treatment group I (DOX + 100 mg/kg body weight)	Treatment group I (DOX + 100 mg/ kg body weight)	47,22 ± 2,78
Treatment group II (DOX + 150 mg/kg body weight)	Treatment group II (DOX + 150 mg/ kg body weight)	31,54 ± 1,45
Treatment group III (DOX + 200 mg/kg body weight)	Treatment group III (DOX + 200 mg/ kg body weight)	22,34 ± 1,12

Table 4. shows the average HbA1c levels of each treatment group. The table shows the lowest level in the standard group, 20.22 ± 0.74 ng/ml, and the highest level in the negative control group, 75.12 ± 0.23 ng/ml. Statistically, the negative control group has a significant difference (P <0.05) from the positive control group, treatment group I, treatment group II, and treatment group III. The positive control group did not have a significant difference (P <0.05) with the regular group and treatment group III and had a difference (P>0.05) with the negative control group, treatment group I, and treatment group II. This study shows that increasing the dose of turmeric ethanol extract reduces HbA1c levels.

Table 5: Amylase enzyme levels in rat blood

No.	Kelompok perlakuan	Enzim amilase ± SD (mg/ml)
1	Normal group (CMC)	102,11 ± 0,43
2	Negative control group (DOX+CMC)	267,12 ± 0,28
3	Positive control group (DOX+Vit E)	134,14 ± 0,33
4	Treatment group I (DOX + 100 mg/ kg body weight)	253,81 ± 4,23
5	Treatment group II (DOX + 150 mg/ kg body weight)	167,77 ± 4,11
6	Treatment group III (DOX + 200 mg/ kg body weight)	125,12 ± 3,89

The data is in Table 5. shows that there is a decrease in amylase enzyme activity in the extract; this indicates a reduction of enzyme levels with an increase in the dose of turmeric ethanol extract. And in the negative control group that was only given doxorubicin showed that there was an increase in amylase enzyme activity. One of the enzymes included in hydrolase is amylase. Based on statistics, there was a significant difference (P < 0.05) between the negative control group, which was only given doxorubicin, the positive control group, regular treatment group I, treatment group II, and treatment group III. The class of amylase enzymes includes α-amylase, β-amylase, glucoamylase, and pullulanase. α-amylase has the specificity of randomly cutting α-1,4-glycoside bonds on starch and will not cut branches that have α-1,6 glycoside bonds. The result of α-amylase digestion is short linear maltodextrins: glucose, maltose, maltotriose, maltotetraose, maltopentose, maltohexose, and α-dextrin. Doxorubicin was previously shown to inhibit insulin secretion by the islets of Langerhans *in vitro* at doses below those used in chemotherapy therapy, suggesting the possibility of it being a possible target for chemotherapy-induced diabetes [6, 7]. Although the mechanism of doxorubicin toxicity has been characterized in various tumor cell types, the agency responsible for doxorubicin toxicity in pancreatic islets or β-cells has never been determined [8].

Conclusions

Curcuma longa ethanol extract doses of 100 mg / kgBB, 150 mg / kgBB, and 200 mg / kgBB have the activity of reducing blood glucose levels with significantly different (P <0.05) with the negative control group, which is only given CMC-Na and doxorubicin. Curcuma longa extract doses of 100 mg/kgBB, 150 mg/kgBB, and 200 mg/kgBB have the activity of reducing HbA1c levels with significantly different (P < 0.05) with the negative control group, which is only given CMC-Na and doxorubicin.

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