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Exploration of the Effectiveness of Turmeric Ethanol Extract against the Enzyme Amylase Pancreas in Doxorubicin-Induced Wistar Rats

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Abstract: **The most common diabetes mellitus is type two diabetes mellitus (T2D). Type II diabetes mellitus exhibits chronic hyperglycemia and is thought to result from the progressive failure of pancreatic β-cells that do not secrete enough insulin to meet metabolic needs. Curcumin content in turmeric has been studied and used as an antioxidant, antiviral, anti-inflammatory, antifungal, liver protection, gastrointestinal effects, dissolving gallstones, anticarcinogenic, antimicrobial, cardiovascular, and others. This study aims to test the pancreoprotective activity of turmeric ethanol extract (EEK) in experimental animals by measuring biochemical parameters of temporary blood sugar levels, HbA1c, and amylase enzyme. This type of research is practical and was conducted in March 2022. This research was conducted at the Pharmacy Laboratory of the University of North Sumatra, Medan. A total of 24 male white Wistar rats (Rattus norvegicus) were purchased from the Medan Bintang street animal market. The results of the phytochemical screening of turmeric ethanol extract showed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids—phytochemical screening results. The administration of turmeric ethanol extract showed a decrease in blood sugar levels which was inversely proportional to the increase in the dose of temulawak ethanol extract—the average HbA1c level of each treatment group. The lowest level in the standard group was 21.34 ± 0.66 ng/ml, and the highest level in the negative control group was 71.12 ± 3.44 ng/ml. Statistically, the negative control group had a significant difference (P<0.05) from the positive control group. This indicates that increasing the dose of turmeric ethanol extract can reduce HbA1c levels. The ethanol extract of turmeric reduces blood glucose levels, significantly different (P<0.05) from the negative control group, which was only given CMC-Na and doxorubicin. The turmeric extract at different doses had a very other HbA1c lowering activity (P<0.05) than the negative control group, which was only given CMC-Na and doxorubicin.**

Keywords: **turmeric, pancreas, doxorubicin.**

I. INTRODUCTION

Various epidemiological studies have shown an increasing trend in the incidence and prevalence of type II diabetes mellitus in multiple parts of the world, including Indonesia (Abdul et al., 2020). The most common diabetes mellitus is type two diabetes mellitus (T2D). Type II diabetes mellitus exhibits chronic hyperglycemia and is thought to result from the progressive failure of pancreatic β-cells that do not secrete enough insulin to meet metabolic demands (Hameed et al., 2015). Doxorubicin is used to control primary and metastatic tumors. Still, it often produces toxicity in normal tissues, and the associated side effects often outweigh its clinical benefits and worsen patients' quality of life (Marcu, 2022).

The various biological and pharmacological activities of turmeric's curcumin content have been investigated and used for antioxidant, antiviral, anti-inflammatory, antifungal, protection of the liver, gastrointestinal effects, dissolving gallstones, anticarcinogenic, antimicrobial, cardiovascular, tonic for the digestive system, eliminating worms in the gastrointestinal tract (Nabofa et al., 2018), enhancing the immune system, antifertility, menstrual disorders, anti-Diabetes Mellitus, hypolipidemic, protection against worms in the digestive tract, improve the immune system, antifertility, menstrual disorders, anti-Diabetes Mellitus, hypolipidemic, protection of the urinary tract and kidneys, anti-blood clotting, increase

appetite, cough, rheumatism, sinusitis, and anti-HIV (Fioni, 2021); (Muthia Milasari, 2019). Based on this background, the researcher was encouraged to test the pancreoprotective activity of turmeric ethanol extract (EEK) in experimental animals by measuring biochemical parameters of blood sugar levels, HbA1c, amylase enzymes, and conducting histopathological studies of the pancreas of experimental animals.

II. LITERATURE REVIEW

Curcumin is a yellow-colored compound found in turmeric rhizomes, commonly seen as curcuminoids, a mixture between curcumin, demetoxicurcumin, and bisdemethoxycurcumin. Curcumin compounds' attractive efficacy and physical-chemical properties make them a lead combination for developing new drug compounds. Curcuma Longa can be used as a protective drug against the kidneys, anticancer, antioxidant, treatment against osteoarthritis, a natural dye in food, anti-inflammatory, anticholesterol, and others. Pancreototoxicity is one of the side effects of the anthracycline antibiotic chemotherapy drug Doxorubicin, which causes an imbalance between free oxygen radicals and antioxidants (Teiten et al., 2014).

III. RESEARCH METHODS

This research type is experimental, carried out in November 2022. This research was conducted at the Pharmacy Laboratory of the University of North Sumatra Medan.

Tools, materials, and experimental animals

The tools used are surgical tools, microscopes, syringes 1 ml, syringe 3 ml, oral sonde, centrifuge, tube, animal balance sheet, analytical balance, beaker glass, mortar, stamper, spatulas, parchment paper, measuring flask, spectrophotometer, cuvette, micropipette, microtome, water bath, and object-glass, Glucometer + stick. The ingredients used in this study were EEBM, Doxorubicin, NaCl, 10% formalin, chloroform, CMC-Na, rats, virgin coconut oil, reagents, liquid paraffin, toluene, acetone, EEK (Turmeric ethanol extract). Twenty-four male Wistar rats (Rattus norvegicus) were bought from the Bintang Medan street animal market.

Phytochemical Screening of Turmeric Ethanol Extract

Phytochemical screening of extracts was carried out at the Biology Laboratory of the Faculty of Pharmacy, University of North Sumatra, by examining compounds of alkaloid groups, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids.

Solution Manufacturing

Solution manufacturing includes a 0.5% w/v CMC-Na suspension and a 200, 400, and 600 mg/kg bb dose EEK suspension.

1. Manufacture of 0.5% sodium methyl cellulose carboxy suspension

Weighed CMC-Na powder 0.5 gams, sprinkled in a lump filled with enough hot water, developed for 15 minutes, ground homogeneously, then put into a 100 ml measuring flask, and then enough to the marking line.

2. Manufacture of turmeric ethanol extract suspension

Turmeric ethanol extract was weighed 200, 400, and 600 mg, respectively, then put into a mortar and added 0.5% CMC-Na suspension was little by little while grinding until homogeneous, then put in a 10 ml measuring flask and sufficed to the marked line with CMC-Na suspension.

I was testing the pancreoprotective activity of turmeric ethanol extract (EEK) in vivo.

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

a. Group I (Normal Group): Na-CMC Suspension

b. Group II (Negatip Group): Doxorubicin-injected mice

c. Group III (Positive control): male wistar rats (Rattus norvegicus) induced doxorubicin + Vitamin E 1 % BB

d. Group IV (Treatment 1): male wistar rats (Rattus norvegicus) induced doxorubicin + 200 mg/kgbb EEK

e. Group V (Treatment 2): male wistar rats (Rattus norvegicus) induced doxorubisin + 400 mg/kgbb EEK

f. Group VI (Treatment 3): male wistar rats (Rattus norvegicus) induced doxorubicin + 600 mg/kgbb EEK

Induction of pancreatic damage using doxorubicin 5 mg/kg bb intraperitoneally on days 1, 7, 14, and 20, then EEK suspensions are administered daily at 200 mg/kg bb (body weight), 400 mg/kg bb and 600 mg/kg bb. Rats were pre-fasted for approximately 18 hours (not fed, but still given a drink). The rat was anesthetized with chloroform and then tethered to the surgical board on all four limbs. The chest cavity is dissected and blood in the heart as much as 2 ml is taken using a 3 ml syringe. The blood is then transferred into a blood tube, then centrifuged for 10 minutes at a speed of 3000-4000 rpm so that 2 layers are produced, namely serum / supernatant and its precipitate. The serum layer is taken, then accommodated in microtubes and stored in a refrigerator at a temperature of -4°C. Blood serum is used for examination of KGD fasting, KGD2 hours post-prandial, HbA1c. The pancreas organs of rats were taken, histopathological preparations were made (Gede et al., 2012).

Analysis of blood sugar levels ad random

1. Measured with a glucometer with a stick
2. Ad random and post-prandial blood sugar reference values: adults (up to 140 mg/dl; complete blood up to 120 mg/dl).
3. Fasting blood sugar reference value: adults (70 – 110 mg/dl)

HbA1c analysis

1. HbA1c can be measured by several methods, such as affinity chromatography, electrophoresis, immunoassay, or boronate affinity method.
2. The specimen used for HbA1c measurement is capillary or venous blood with anticogulants (EDTA, citrate, or heparin).
3. Referral value: normal person (4.0 – 6.0 %), well-controlled DM (less than 7 %), controlled DM (7.0 – 8.0 %), uncontrolled DM (more than 8.0 %).

Data Analysis

The data were analyzed using the Shapiro-wilk method to see the normality of the data. If the data is normally distributed ($P > 0.05$), proceed using the One Way ANOVA method to determine the average difference between the groups. If there is a difference, ($P < 0.05$) followed by the Post Hoc Tukey HSD test to see the real difference between treatments. But if the distributed data is abnormal then the Kruskal-Wallis test is used.

IV. RESULTS AND DISCUSSION

The phytochemical screening results of turmeric ethanol extract obtained showed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, steroids / triterpenoids. The results of phytochemical screening of turmeric ethanol extract can be seen in Table 1.

Table 1. Turmeric ethanol extract screening results

No.	Screening	Result
1.	Flavonoids	+
2.	Alkaloids	+
3.	Saponins	+
4.	Tanins	+
5.	Glycosides	+
6.	Steroids/Triterpenoids	+

Description: (+) : there is

(-) : none

Results of blood sugar levels after administration of doxorubicin

Each group including the normal group, the positive control group, the negative control group, the I treatment group, the II treatment group, and the III treatment group were given doxorubicin with a dose of 8 mg / kgBB which was then given on days 7, 14, and 21, and subsequent KGD checks were carried out on days 5, 10, 15, and 20. The results of the data can be seen in the table below:

Table 2. KGD Measurement Data on days 5, 10, 15, and 20

No.	Treatment group	Kadar gula darah (mg/dl)				
		0	5	10	15	20
1.	Normal Group (Not DOX induced)	71,28 ± 0,41	82,38 ± 1,99	80,58 ± 0,24	74,68 ± 1,85	72,21 ± 1,33
2.	Negative Group (DOX + CMC)	72,10 ± 1,12	244,41 ± 4,14	272,82 ± 3,51	277,05 ± 0,93	276,57 ± 11,56
3.	Positive Group (DOX + Vitamin E)	74,5 ±0,04	182,3 ± 4,44	177,21 ± 1,11	169,82 ± 0,94	85,19 ± 11,57
4.	Treatment group I (DOX + 200 mg/kgBB)	75,44 ± 0,87	236,22 ± 4,609	222,97 ± 2,22	204,11 ± 1,763	132,16 ± 8,12
5.	Treatment group II (DOX + 400 mg/kgBB)	72,16 ± 0,07	192,03 ± 4,22	182,522 ± 2,22	165,70 ± 4,31	99,55 ± 4,40
6.	Treatment group III (DOX + 600 mg/kgBB)	71,42 ± 0,59	184,42 ± 4,89	176,66 ± 3,66	144,807 ± 3,24	73,113 ± 3,22

Based on Table 2. showed the administration of turmeric ethanol extract in doxorubicin-induced rats. The normal group had the lowest blood sugar levels at the end of the 20th day of the study, namely 72.21 ± 1.33 mg/ml had significant differences ($P<0.05$) against the negative control group, treatment group I, treatment group II, and did not have significant differences ($P>0.05$) with the positive control group and treatment group III. The negative control group had the highest blood sugar levels of 276.57 ± 11.56 mg/ml and had significant differences ($P<0.05$) from the normal group, positive control group, treatment group I, treatment group II, and treatment group III. In the extract treatment group, the III treatment group had blood sugar levels of 73,113 ± 3.22 mg/ml had significant differences ($P<0.05$) from the negative control group and did not have a significant difference ($P>0.05$) with the positive control group. And in the group of turmeric ethanol extract administration showed a decrease in blood sugar levels inversely proportional to an increase in the dose of ethanol curcuma extract.

In previous studies (Izzati, 2010), research has been conducted on the antioxidant effect of the isolation of phenolic compounds from turmeric rhizomes against 1,1-diphenyl-2-picrylhidrazil (DPPH), it is known that turmeric rhizomes have antioxidant activity.

Flavonoids found in turmeric ethanol extract have antidiabetic activity. In this study, free radicals produced by doxorubicin, namely semiquinone metabolite compounds, have adverse activities including damaging the pancreas so which can cause a decrease in insulin production. Turmeric rhizomes contain chemical compounds curcumin, zedoarin, gum, resins, starch, saponins, flavonoids, polyphenols, and essential oils such as cineol, camphene, zingiberene, borneol, and camphor. Turmeric rhizome contains 1-2.5% evaporating oil with the main components of the sesquiterpene, namely curcumin. The evaporated oil contains more than 20 components such as kurzerenon (zedoarin) which is the largest component, kurzerena, pyroquinekuzerenone, curcumin, curcumenone, epicurkumenol, curcumol (curcumenol), isokurkumenol, procurcumenol, dehydrokurdone, furanodienon, isofuranodienon, furanodiene, zederon, and kurdion. Essential oils found in turmeric native to India also contain 1,8-cineol (15.9%) and germakron (9.0%) (Fioni, 2021).

Table 3. Percent decrease in KGD (%) on days 0 and 20

No.	Treatment group	Blood sugar levels (mg)		(%)
		0	20	Increased glucose levels
1.	Normal Group (Not DOX induced)	71,28 ± 0,41	72,27 ± 1,23	6,2
2.	Negative Group (DOX + CMC)	72,10 ± 1,12	280,51 ± 12,80	71,42
3.	Positive Group (DOX + Vitamin E)	74,5 ±0,04	85,19 ± 11,57	13,88
4.	Treatment group I (DOX + 200 mg/kgBB)	75,44 ± 0,87	132,16 ± 8,12	42,11
5.	Treatment group II (DOX + 400 mg/kgBB)	72,16 ± 0,07	99,55 ± 4,40	25,78
6.	Treatment group III (DOX + 600 mg/kgBB)	71,42 ± 0,58	74,204 ± 3,78	3,82

HbA1c Level Results

HbA1c measurements were carried out using the Rat HbA1c Kit by the ELISA method, which was read 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 measurement of absorbance with the addition of a standard solution 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 absorbant values of each concentration can be seen in Table 4. as follows.

Table 4. HbA1c absorbance

HbA1c standart 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 5. HbA1c concentration in rat blood

Treatment group	Average HbA1c concentration ± elementary school (mg/ml)
Normal group (CMC)	21,34± 0,66
Negative control group (DOX+CMC)	71,12 ± 3,44
Positive control group (DOX+VitE)	26,34 ± 1,72
Treatment group I (DOX + 200 mg/kgBB)	48,13 ± 0,66
Treatment group II (DOX + 400 mg/kgBB)	34,33 ± 1,53
Treatment group III (DOX + 600 mg/kgBB)	25,01 ± 1,23

Table 5. showed average levels of HbA1c in each treatment group. The table shows the lowest levels, namely the normal group, namely 21.34 ± 0.66 mg/ml, and the highest level in the negative control group, which is 71.12 ± 3.44 ng/ml. Statistically, the negative control group had significant differences ($P<0.05$) from the positive control group, treatment group I, treatment group II, and treatment group III. A positive control group did not have significant differences ($P<0.05$) with the normal group and treatment group III and had differences ($P>0.05$) with the negative control group, treatment group I, and treatment group II. In this study, it was shown that increasing the dose of turmeric ethanol extract lowered the levels of HbA1c. Hemoglobin A1c or HbA1c is a minor component of hemoglobin that binds to glucose. HbA1c is referred to as glycosylation or glycosylated hemoglobin or glycohemoglobin. Hemoglobin is an oxygen-carrying pigment that gives the red color to red blood cells and is also the dominant protein in red blood cells.

Turmeric (Curcuma longa) is one of the spices that is widely used as a food ingredient and also in traditional medicine. The active content of turmeric, namely curcumin, has been widely studied and proven to have biological activity as an anti-inflammatory, anticancer, antioxidant, antidislipidemia, and antidiabetic. Curcumin can be obtained from turmeric extracted with ethanol solvent (Cobra, 2019). Turmeric ethanol extract has antioxidant activity and it is possible to lower glucose levels in the blood. Antioxidant compounds act as inhibitors used to prevent autoxidation, so the best way to reduce oxidative stress is to reduce free radicals or optimize the body's defenses by multiplying antioxidants (Wahyuningtyas, Permana, and Wiadnyani, 2017).

Table 6. Amylase enzyme levels in rat blood

No.	Treatment group	Amylase enzyme ± SD (mg/ml)
1	Normal group (CMC)	105,62 ± 6,15
2	Negative control group (DOX+CMC)	280,71 ± 5,21
3	Positive control group (DOX+VitE)	171,81 ± 3,89
4	Treatment group I (DOX + 200 mg/kgBB)	266,55 ± 4,23
5	Treatment group II (DOX + 400 mg/kgBB)	158,91 ± 1,23
6	Treatment group III (DOX + 600 mg/kgBB)	119,01 ± 3,69

The data in the table shows that there is a decrease in the activity of the amylase enzyme in the extract, this indicates that there is a decrease in enzyme levels with an increase in the dose of turmeric ethanol extract. And in the negative control group that only doxorubicin was given showed that there was an increased activity of the enzyme amylase. One of the enzymes included in hydrolase is amylase. Based on statistics, significant differences ($P<0.05$) were obtained between the negative control group that was only given doxorubisin to the positive, normal, treatment group I, treatment group II, and treatment group III. Inhibition of the work of digestive enzymes will have an impact on decreasing the absorption of food substances in the body. Low absorption of food substances will result in diabetes mellitus disease has become the number three killer disease in Indonesia. One way to overcome diabetes mellitus is by inhibiting the work of enzymes that hydrolyze carbohydrates so as to reduce glucose absorption.

One of the enzymes that plays an important role in the breakdown of oligosaccharides and disaccharides into monosaccharides so that they are ready to be absorbed is the enzyme α-amylase. Inhibition of the enzyme α-amylase can delay and prolong the digestibility time of carbohydrates, causing a decrease in the rate of glucose absorption and preventing an increase in postpandrial glucose plasma levels (Cerutti et al., 2021). Doxorubicin was previously shown to inhibit insulin secretion by Langerhans islets in vitro at doses below those used in chemotherapy therapy, suggesting the possibility of it being a possible target for chemotherapy-induced diabetes (Heart et al., 2016). Although the toxicity mechanism of doxorubicin has been marked in different types of tumor cells, the mechanism responsible for the toxicity of doxorubicin on islands or cells β-pancreatic cells has never been determined. Doxorubicin can undergo a NADPH-dependent redox cycle with cytochrome P450 reductase and mouse liver microsomes and cardiac sarcosomes, demonstrating the role of superoxides and their reactive oxygen intermediate derivatives such as H2O2 in mediating the toxicity of doxorubicin in those tissues. Doxorubicin was previously shown to inhibit insulin secretion by rat isles in vitro at doses below those used in chemotherapy therapy, suggesting the possibility of it being a possible target for chemotherapy-induced diabetes. Although the toxicity mechanism of doxorubicin has been marked in different types of tumor cells the mechanism responsible for the toxicity of doxorubicin in islets or cells β cells of the pancreas has never been determined (Goyal et al., 2016).

Turmeric Extract (Curcuma longa L.) (Family Zingiberaceae) (TE) has a major component, curcumin, which is responsible for its biological action.5 Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a hydrophobic molecule that easily passes through the plasma membrane into the cytosol of human cells. This phenolic substance inhibits the initiation of tumors caused by a wide variety ofcarcinogens and has also been shown to inhibit the growth of many human cancer cell lines in vitro and has analgesic, anti-inflammatory, and antibacterial activity. The use of turmeric powder against bile disorders, anorexia, cough, diabetic wounds, liver disorders, rheumatism, andsinusitis has been reported by (Agne et al., 2010); (Silalahi, 2018).

V. CONCLUSION

Based on the results of research, observations and discussions, it can be concluded that the ethanol extract of curcuma longa has a significantly different activity of decreasing blood glucose spike ($P<0.05$) with a negative control group that is only given CMC-Na and doxorubicin. Curcuma longa extract at different doses, had a significantly different HbA1c-level reduction activity ($P<0.05$) with a negative control group that was only given CMC-Na and doxorubisin.

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