BirEx JOURNAL
Budapest International Research in Exact Sciences
Medical, Biological, Argiculture, Engineering
Science and other related areas
e-ISSN : 2655-7827
p-ISSN : 2655-7835
http://www.bircu-journal.com/index.php/birex

Effects of Turmeric Ethanol Extract on Pancreatic Amylase Enzyme in Doxorubicin-Induced Wistar Mice

Wen Shiqi[1], Florenly[2], Johannes Bastira[3], Fioni[4]
[1,2,3,4]*Departement of Medicine, Faculty of Medicine, Universitas Prima Indonesia*
ly@unprimdn.ac.id

Abstract: *Type II diabetes mellitus indicates the presence of chronic hyperglycemia and results from progressive failure of pancreatic β cells in which the amount of insulin is insufficient to meet metabolic demands and is characterized by peripheral insulin resistance and impaired insulin secretion. Turmeric (Curcuma longa Linn) has been widely researched and used for antioxidants, antivirals, anti-inflammatories, as well as its potential as a functional food for pancreatic-related diseases. The study's goal was to test the effectiveness of turmeric ethanol extract against pancreatic amylase enzymes in doxorubicin-induced Wistar mice (lowered fasting KGD, 2 hoursPP, HbA1C, in doxorubicin-induced male mice). This type of research is an experimental study, in early March 2021, conducted at the Laboratory of Pharmaceutical Pharmacology of the University of North Sumatra. The animals used in the study were male rats weighing 150–200g. The results in the treatment group iii treatment group (ethanol extract Curcuma longa 600g) had blood sugar levels of 74.2014 ± 3.78 mg/ml had a neggant difference (P<0.05) with the negative control group and did not have a significant difference (P>0.05) with the positive control group. Ethanol extract Curcuma longa doses of 200 mg/kg bb, 400 mg/kg bb, and 600 mg/kg bb had significantly different blood glucose levels decreased activity (P<0.05) with negative control groups given only CMC-Na and doxorubicin. Curcuma longa extract doses of 200 mg/kg bb, 400 mg/kg bb, and 600 mg/kg bb had significantly different hbA1c reduction activity (P<0.05) with negative control groups given only CMC-Na and doxorubicin. Advice, for researchers, can further do antidiabetic testing by looking at insulin expression after doxorubicin-induced and tested measurement of antioxidant levels such as GSH and Catalase.*
Keywords: *curcuma longa; amylase; pancreas; doxorubicin*

I. Introduction

Type II diabetes mellitus, indicates the presence of chronic hyperglycemia and results from progressive failure of pancreatic β cells in which the amount of insulin is insufficient to meet metabolic demands (Delgado-León et al. 2018), characterized by peripheral insulin resistance and impaired insulin secretion (Heart et al. 2016). The risk of chronic complications that can occur is coronary heart disease and stroke, kidney failure, retinopathy, and diabetic gangrene (Permana and Hospital 2000). In clinical practice, risk models that identify patients at risk of developing dm disease (diabetes mellitus) due to the use of drug ingredients, such as doxorubicin (Heart et al. 2016). Vitamin E is one type of antioxidant that is effective in clearing free radicals in the body (El Hadi, Vettor, and Rossato 2018), inhibits the production of reactive oxygen species (ROS) molecules when fat undergoes oxidation and during the spread of free radical reactions (Rizvi et al. 2014).

Turmeric (Curcuma longa Linn) has been investigated and used for antioxidants, antivirals, anti-inflammatory, antifungal, protection against the liver, gastrointestinal effects, dissolving gallstones, anticarcinogenic, antimicrobial, cardiovascular (Razavi 2021), it's potential as a functional food for pancreatic-related diseases (Devaraj et al. 2014), protective

drugs against kidneys, anticancer, anticholesterol, and others (Itokawa et al. 2008). The study aimed to test the effectiveness of turmeric ethanol extract against pancreatic amylase enzymes in doxorubicin-induced Wistar mice (lowered fasting KGD, 2 hoursPP, HbA1C, in doxorubicin-induced male mice).

II. Review of Literature

Curcumin is the most important element among the natural curcuminoids found in the turmeric plant (Curcuma longa). Curcumin derivatives have been evaluated for bioactivity and structure-activity relationships (SAR=Structure-activity relationships). (Itokawa et al. 2008). The efficacy and interesting properties of chemical physics of curcumin compounds, making this plant used as a lead compound for the development of new drug compounds (Wanninger et al. 2015). Curcumin, its main active constituent, is very powerful and antioxidants such as vitamins C, E, and Beta-Carotene, making the use of turmeric the consumer's choice for cancer prevention, liver protection, kidney protection, anti-aging, anti-inflammatory activity, anti-spasmodic and analgetic function (Ahmad et al. 2010). The National Cancer Institute has clarified that turmeric plants are non-toxic, even at high doses, so they are recognized as safe ingredients (GRAS=Generally recognized as safe) (Itokawa et al. 2008).

Vitamin E is a fat-soluble micronutrient consisting of 8 chemical compounds (Tocochromanols): 4 tocopherols (α, β, γ, δ) and 4 tocotrienols (α, β, γ, δ), all of which are powerful membrane-soluble antioxidants, and exhibit biological activity of d-α-tocopherol (Rizvi et al. 2014). Vitamin E effective treatment of Bronchopulmonary dysplasia (BPD) (Cosby A. Stone. et al 2018), can be used as a prevention of side effects of radiation, antiviral, anti-tumor, anti-cancer, lower cholesterol, anti-oxidant function (Cosby A. Stone. et al 2018).

The pancreas is a large retroperitoneal organ located just behind the posterior wall of the lower sac, on the supracolic compound floor of the abdominal cavity. (Mahadevan 2019). The pancreatic enzymes each specialize in digesting specific compounds found in chyme, among others, amylase, trypsin, lipase. The function of the pancreas, among others, as homeostasis of blood glucose, the system of regulating the function of the pancreas (Pandol 2015).

Glycemic control is monitored by measuring glucose using the patient's plasma and laboratory analysis of glycated hemoglobin (HbA1c), the most accurate way of determining high blood sugar levels over the past two to three months (Utomo, Wungouw, and Marunduh 2015). In addition to measurements of venous plasma glucose, hbA1c concentrations in the blood can also be used to diagnose diabetes mellitus (Heart et al. 2016). Doxorubicin (DOX) is one of the most effective and important antineoplastic agents in clinical use, but the use of this drug affects the heart and kidneys, there is evidence that Dox toxicity also extends to other organs such as the pancreas and brain. To date there has been no efficient therapeutic strategy available to ward off Doxorubicin-induced pancreatic tissue injury (Oktem et al. 2011);(Ewer and Ewer 2010).

III. Research Methods

This type of research is an experimental study, in early March 2021, conducted at the Laboratory of Pharmaceutical Pharmacology of the University of North Sumatera.

3.1 Tool

The tools used are surgical tools, microscopes, 1 ml syringe, 3 ml syringe, oral sonde, sentifuge, tube, animal balance, analytical balance, beaker glass, mortar, stamfer, spatula, parchment paper, measuring pumpkin, spectrophotometer, kuvet, micro pipette, microtom, water handler, and object glass, Glucometer + stick.

3.2 Material

The ingredients used in the study are EEBM, Doksorubicin, NaCl, 10% formalin, chloroform, CMC-Na, Tikus, Virgin coconut oil, reagents, liquid paraffin, toluents, and acetone, EEK (turmeric ethanol extract).

3.3 Experimental Animals

The animals used in the study were male rats weighing 150–200g. Before the study began, the test animals were acclimatized for one week with room temperature conditions (22-25°C), under a light/dark 12-hour cycle, given pellets and drinking water tap ad libitum.

3.4 Turmeric Ethanol Extract (Curcuma longa)

Turmeric rhizomes are braced and dried in the dryer cabinet for 3 days. The manufacture of turmeric ethanol extract is done in maceration with a 96% ethanol solvent. As much as 500 grams of turmeric rhizome simplistic powder are put in a glass container, added 96% ethanol as much as 3.75 L, cover, leave for 5 days protected from light while often stirred, stalk, squeeze, wash the dregs with a liquid to taste until obtained 4 L. Transfer it into a closed vessel, leave it in a cool place, protected from light for 2 days. Grounded or filtered. The results obtained are checked with Rotary Evaporator until most of the solvent evaporates and continues the evaporation process on the water handler until a viscous extract (turmeric ethanol extract) is obtained. (Depkes RI, 1979).

3.5 Phytochemical Screening of Turmeric Ethanol Extract

Screening of phytochemical extracts was conducted in the Biology Laboratory of the Faculty of Pharmacy, University of North Sumatra by examination of alkaloid compounds, flavonoids, glycosides, saponins, tannins, and steroids / triterpenoids. Examination of alkaloids, flavonoids. Flavonoids are positive if there is a red or yellow or orange color in the amyl alcohol layer. Examination of glycosides, saponins, tannins, steroids/triterpenoids (Buckley 1966).

3.6 Solution Making

Solution making includes the manufacture of CMC-Na suspension 0.5% b/v and turmeric ethanol extract suspension doses of 200, 400, and 600 mg/kgbb.

1. Carboxy methyl cellulose sodium suspension manufacturing 0.5%.
   Weighed CMC-Na powder 0.5 gam, sprinkled in lumpang containing enough hot water, developed for 15 minutes, then eroded homogeneously then put into a measuring pumpkin 100 ml, then shaved up to the sign line.
2. Manufacture of turmeric ethanol extract suspension.
   Turmeric ethanol extract is weighed at 200, 400, and 600 mg, respectively, then put into lumpang and added a 0.5% CMC-Na suspension little by little while being eroded until homogeneous, then put into a 10 ml measuring pumpkin and shaved up to the sign line with CMC-Na suspension.

3.7 Testing the Pancreoproprotective Activity of Turmeric Ethanol Extract (EEK) in Vivo

This test was conducted using male wistar rats as subjects. The in vivo test on the experiment used 24 (twenty-four) healthy mice weighing about 170 g ± 10%, then divided into 4 (four) groups and each group consisting of 5 (five) mice:

a. Group I (Normal Group): Suspension of Na-CMC
b. Group II (Negative Group): Rats injected with doxorubicin
c. Group III (Positive control): male wistar (Rattus norvegicus) mice induced doxorubicin + Vitamin E 1% BB
d. Group IV (Treatment 1): male wistar (Rattus norvegicus) rats induced doxorubicin + 200 mg/kgbb EEK
e. Group V (Treatment 2): male wistar (Rattus norvegicus) rats induced doxorubicin + 400 mg/kgbb EEK
f. Group VI (Treatment 3): male wistar rat (Rattus norvegicus) male induced doxorubicin + 600 mg/kgbb EEK

The number of mice used in each group is calculated using the Federer Formula (n-1)(t-1) ≥ 15, (n)=number of repetitions, (t)=number of groups, with the result n ≥ 4. So each group consisted of 4 mice or 4 repetitions. Induction of pancreatic damage using doxorubicin 5 mg / kg bb intraperitonial on days 1, 7, 14, and 20 then EEK suspension is given daily at doses of 200 mg / kg bb, 400 mg / kg bb, and 600 mg / kg bb. Rats were satisfied first for approximately 18 hours (not fed, but still given a drink). Mice sedated with chloroform were then tethered to surgical boards 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 split. Blood is then transferred into the blood tube, then concentrated for 10 minutes at a speed of 3000-4000 rpm so that 2 layers are produced, namely serum/supernatant and sediment. The serum layer is taken, then accommodated in microtubes, and stored in a refrigerator temperature of -4 °C. Blood serum is used for KGDpuasa examination, KGD2 postprandial hours, HbA1c. The pancreas of the mouse is taken each treatment, made histopathological preparations.

3.8 Analysis of Blood Sugar Levels ad Random

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

3.9 Analysis of HbA1c

- HbA1c can be measured by several methods, such as affinity chromatography, electrophoresis, immunoassay, or boronate affinity methods.
- Specimens used for HbA1c measurement are capillary or venous blood with anticogulants (EDTA, citrate, or heparin).
- 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%).

3.10 Analysis of Blood Alpha Amylase Enzymes

Principle: The substrate (4,6-ethylidene-p-nitrophenyl-a-D-maltoheptaoside) will be decomposed by the enzyme alpha amylase, where the result is that oligosaccharides are hydrolyzed by a-glucosides producing glucose and p-nitrophenol. The increase in p-nitrophenol was proportional to the activity of a-amylase in the sample. Tools used Chemical Alayzer Cobas 6000 (C510+E610), scanner, centrifuse, test tube, test tube rack (L. Wiradewi & S. Dharma 2017).

3.11 Preparation of Pancreatic Tissue

Preparation of pancreatic tissue in accordance with the procedure outlined by (Rabiah, Berata, and Suri 2015). The organs are fixated with a 10% solution of formalin for 3-4 hours, then with acetone 3 times (each for 2 hours). After that, cleaning (cleaning) using toluene as much as 3 times (each 1-2 hours). The process of embedding (soaking) samples in liquid paraffin at a temperature of 60-70 ° C as much as 3 times (each for 2 hours), then the process of printing paraffin blocks. The cutting stage of paraffin blocks is done using microtome so that sheets are obtained with a thickness of 5 μm. The sheet is placed in a water handler whose temperature is 30 ° C then placed in the object-glass and heated in the oven for 2-3 minutes. The resulting sheet is observed under a light microscope with a magnification of 10x40, observed the number of necrosis and normal cells.

3.12 Coding Blood Samples

Trials of blood samples of male wistar rats (Rattus norvegicus) taken from each group, taken 3 wistar rats (Rattus norvegicus) males randomly and given the following numbers:

1. Group I (Normal group): Na-CMC suspension, code 1-2-3
2. Group II (Negative control): male wistar (Rattus norvegicus) mice induced doxorubicin + Vit E 1% BB. Coded 5-6-7.
3. Group III (Positip control): Doxoruisin+ Vitamin E 1%BB induced mice coded 9-10-11.
4. Group IV (Treatment 1): male wistar (Rattus norvegicus) mice induced doxorubicin + 200 mg/kgbb EEK. Coded 13-14-15.
5. Group V (Treatment 2): male wistar (Rattus norvegicus) rats induced doxorubicin + 400 mg/kgbb. Coded 17-18-19.
6. Group VI (Treatment 3): male wistar (Rattus norvegicus) male rats induced doxorubicin + 600 mg/kgbb. Coded 21-22-23.

3.13 Data Analysis

The data was analyzed using the Shapiro-Wilk method to see the normality of the data. If the distributed data is normal ($P > 0.05$), then proceed with the One Way ANOVA method to determine the average difference between groups. If there is a difference ($P < 0.05$), followed by the Tukey HSD Post Hoc test to see the real difference between treatments. But if the distributed data is not normal then the Kruskal-Wallis test is used.

IV. Discussion

4.1 Phytochemical Screening of Turmeric Ethanol Extract

Table 1. Turmeric Ethanol Extract Screening Results

No.	Screening	Result
1.	Flavonoid	+
2.	Alkaloid	+
3.	Saponin	+
4.	Tanin	+
5.	Glikosida	+
6.	Steroid/triterpenoid	+

Information: (+): exist
(-): none

4.2 Results of Strengthening Blood Sugar Levels after Administration of Doxorubicin

Table 2. KGD 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 DOX induced)	71,28 ± 0,41	82,38 ± 1,99	80,58 ± 0,24	74,68 ± 1,85	73,27 ± 1,23
2.	Kelompok Negatif (DOX + CMC)	72,10 ± 1,12	244,41 ± 4,14	272,82 ± 3,51	277,05 ± 0,93	280,51 ± 12,80
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	74,204 ± 3,78

Table 2. showed the administration of turmeric ethanol extract in doxorubicin-induced mice. The normal group had the lowest blood sugar levels at the end of the 20th-day study of 73.27 ± 1.23 mg/ml had significant differences ($P<0.05$) in the negative control group, treatment group I, treatment group II, and had no significant differences ($P>0.05$) with positive control groups and treatment group III. The negative control group had the highest blood sugar levels of 280.51 ± 12.80 mg/ml had significant differences ($P<0.05$) with the normal group, positive control group, treatment group I, treatment group II, and treatment group III. In the extract treatment group, treatment group III had blood sugar levels of 74.2014 ± 3.78 mg/ml had a neggine difference ($P<0.05$) with 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 showed a decrease in blood sugar levels inversely proportional to an increase in the dose of turmeric ethanol extract.

Based on the literature search there is a lot of research on anticancer and antioxidant activity in turmeric rhizomes. In previous research (Saefudin, Syarif, and Chairul 2014), Research has conducted antioxidant effects of isolation of phenolic compounds from white turmeric rhizomes (Curcuma zedoaria Rosc.) against 1,1-diphenyl-2-picrylhydrazil (DPPH), it is known that white turmeric rhizomes (Curcuma zedoaria Rosc.) have antioxidant activity. Flavonoids found in turmeric ethanol extract have antidiabetic activity. In this study, free radicals produced by doxorubicin, a metabolite of semiquinone compounds, have adverse activities including damaging the pancreas so that it can cause a decrease in insulin production. (Bisht, Wagner, and Bulmer 2010); (Gašić et al. 2020); (Andrew 2010). This compound is a derivative of 2-phenyl chromite or 2-phenyl benzopiron. The function of flavonoids can cure inflammation because these compounds have anti-bacterial, anti-viral, antiseptic, antihistamine, reducing, antihypertensive, stimulating estrogen, antifungal and insecticide effects and antidiabetics (Den Hartogh, Gabriel, and Tsiani 2020).

A plant can have antioxidant activity if it contains compounds that can ward off free radicals such as phenols, flavonoids, and carotenoids. White turmeric rhizomes (Curcuma zedoaria Rosc.) contain chemical compounds curcumin, zedoarin, gum, resin, starch, saponins, flavonoids, polyphenols, and essential oils such as cineol, camphene, zingiberene, borneol, and camphor. Turmeric rhizome contains 1-2.5% evaporated oil with the main components of sesquiterpene, curcumin. The evaporated oil contains more than 20 components such as kurzerenon (zedoarin) which is the largest component, curcumin, pyrocurkuzerenon, curcumin,

curcumin, curcumin, epicurkumenol, curcumin, curcumin (curcumin), curcumin (curcuminol), isokurkumenol, prokurkumenol, dehydrokurdon, furanodienon, isfuranodienon, furanodiena, zederon, and kurdion. The essential oil found in turmeric native to India also contains 1.8-sineol (15.9%) and germaron (9.0%) (Safwan, Yuliani, and Pramono 2014).

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

No.	Treatment group	Blood sugar levels (mg)		(%) Increased glucose levels
		0	20	
1.	Normal Group (Not DOX induced)	71,28 ± 0,41	72,27 ± 1,23	5,8
2.	Negative Group (DOX + CMC)	72,10 ± 1,12	280,51 ± 12,80	74,52
3.	Positive Group (DOX + Vitamin E)	74,5 ±0,04	85,19 ± 11,57	13,98
4.	Treatment group I (DOX + 200 mg/kgBB)	75,44 ± 0,87	132,16 ± 8,12	42,23
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

4.3 HbA1c Results

The HbA1c measurement was performed using the Rat HbA1c Kit with elisa method, which is 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 are obtained by absorbance measurement 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 absorbent value of each concentration can be seen in Table 4. as follows.

Table 4. Absorbance HbA1c

Standard concentration HbA1c	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

HbA1c levels are calculated by substituting the sample absorbant value (y) at a wavelength of 450 nm into the logarithmic regression line equation y = ax +b, obtained from the standard hbA1c curve so that the hba1c concentration value (x) results of HbA1c concentrations are then statistical analysis using One Way Analysis of Variant (ANOVA) obtained a significant difference in measurement results ($p<0.05$) between treatment groups. Results of the HbA1c test on mouse blood plasma can be seen in Table 5.

Table 5. Concentration of HbA1c in Rat Blood

Treatment group	Average concentration of HbA1c ± SD (ng/ml)
Normal group (CMC)	21,22 ± 0,68
Negative control group (DOX+CMC)	76,44 ± 3,12
Positive control group (DOX+VitE)	26,71 ± 1,31
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. They showed an average hbA1c level of each treatment group. The table shows the lowest levels, namely the normal group of 21.22 ± 0.68 ng/ml and the highest level in the negative control group which is 76.44 ± 3.12 ng/ml.

Statistically negative control groups had significant differences ($P<0.05$) with the positive control group, treatment group I, treatment group II, and treatment group III. The 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 negative control groups, treatment group I and treatment group II. This study showed that an increased dose of turmeric ethanol extract lowered HbA1c levels. Hemoglobin A1c or HbA1c is a minor component of hemoglobin that binds to glucose.

Turmeric (Curcuma longa) is one of the spices that are widely used as food ingredients and also traditional medicine. The active content of turmeric curcumin has been widely researched and proven to have biological activity as an anti-inflammatory, anticancer, antioxidant, antidislipidemia and antidiabetic. Curcumin can be obtained from turmeric extracted with ethanol solvents (Rezki, Anggoro, and MZ 2015). 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 autooxidation, so the best way to reduce oxidative stress is to reduce free radicals or optimize the body's defenses by multiplying antioxidants. In addition, antioxidants also protect tissues from oxidative damage.

4.4 Results in Amylase Enzyme Levels in Mice

Tabel 6. Amylase Enzyme Levels in Rat Blood

No.	Treatment group	Amylase enzyme ± SD (mg/ml)
1	Normal group (CMC)	107,22 ± 6,55
2	Negative control group (DOX+CMC)	281,77 ± 5,28
3	Positive control group (DOX+Vit E)	174,89 ± 3,89
4	Treatment group I (DOX + 100 mg/kgBB)	266,55 ± 4,23
5	Treatment group II (DOX + 300 mg/kgBB)	158,98 ± 1,11
6	Treatment group III (DOX + 500 mg/kgBB)	117,01 ± 3,89

The data on the table showed that there was a decrease in amylase enzyme activity in the extract, indicating that there was a decrease in enzyme levels with an increase in the dose of turmeric ethanol extract. And in the negative control group given only doxorubicin showed that there was increased activity of amylase enzymes. One of the enzymes included in hydrolase is amylase. Based on statistics, a significant difference ($P<0.05$) was found between the negative control group that was only given doxorubicin to the positive, normal, treatment group I, treatment group II, and treatment group III. Included in the amylase enzyme group are α-amylase, β-amylase, glucoamylase, and pullulanase. α -amylase has a specificity of cutting

the α-1,4-glycoside bond in starches randomly and will not cut branches that have α-1.6 glycoside bonds. The result of α-amylase digestion is short linear maltodextrin, which can be glucose, maltose, maltotriose, maltotetraose, maltopentose, maltohexose, and α-dextrin (Ariandi 2016).

Inhibition of the work of digestive enzymes will have an impact on the decreased absorption of food substances in the body. Low absorption of food substances will cause diabetes mellitus has become the number three killer disease in Indonesia (Rafika, 2016). One way to overcome diabetes mellitus is to inhibit the work of enzymes that hydrolyze carbohydrates to reduce glucose absorption. One of the enzymes that play an important role in the breakdown of oligosaccharides and disaccharides into monosaccharides so ready for absorbance is the enzyme α-amylase. Inhibition of the enzyme α-amylase can delay and prolong carbohydrate digestibility, causing decreased glucose absorption rate and preventing increased postprandial plasma glucose levels (Wardani 2018). Some types of natural ingredients are known to have activity as enzymes α-amylase inhibitors, namely in fruit skin water extracts and okra seeds (Abelmoscus esculentues (L.) Moench), oat ethanol extract, rice, and wheat (Nuryani 2013), methanol extract Cinnamomum zeylanicum, Artocarpus altilitis, Piper bethel, and Artocarpus heterophyllus (Joseph and Nair 2013), and one of them is turmeric ethanol extract (Pujiyanto and Raharja 2019).

Doxorubicin was previously shown to inhibit insulin secretion by Langerhans island in vitro at doses below that used in chemotherapy therapy, suggesting it likely became a possible target for chemotherapy-induced diabetes (Banjarnahor and Wangko 2013). Although the mechanism of doxorubicin toxicity has been characterized in many types of tumor cells (widely reviewed in Gewirtz, 1999 in Tacar et al., 2013), the mechanism responsible for doxorubicin toxicity in island or β-pancreatic cells was never determined. Doxorubicin may undergo a NADPH-dependent redox cycle with cytochrome P450 reductase and mouse liver microsomes and cardiac sarcosomes, demonstrating the role of superoxide and its reactive oxygen intermediate derivatives such as H2O2 in mediating doxorubicin toxicity in those tissues (Tacar, Sriamornsak, and Dass 2013).

The redox cycle of many compounds including quinone menadione is supported by insulin-secreting the pancreas of mice β-cell INS-1 832/13 and isolated murine islands from Langerhans, as our lab has shown before. The second significant mechanism of doxorubicin-mediated toxicity is through DNA damage caused by inhibition of DNA activity and topoisomerase, which results in apoptosis. The relative contribution of this mechanism to toxicity in β cells may differ (Heart et al. 2016). Amylase is an exocrine enzyme produced by pancreatic arrhynthage cells, and low serum amylase levels can be associated with endocrine diseases, such as metabolic syndrome and diabetes. We hypothesized that low serum amylase levels could be associated with impaired cell function β small islands in type 2 diabetes. Therefore, we investigated the relationship between serum amylase levels and island cell function β in patients with early type 2 diabetes.

The mechanism of doxorubicin damaging pancreatic cells including exposure to chemotherapy agents has been linked to an increased risk of type 2 diabetes (T2D), a disease characterized by peripheral insulin resistance and impaired glucose-stimulated insulin secretion (GSIS) from cells β the pancreas. Using cell lines β mice INS-1 832/13 and the pancreatic islands of isolated mice, we investigated the effects of the chemotherapy drug doxorubicin (Adriamycin) on pancreatic β cell survival and function. Exposure of INS-1 cells 832/13 to doxorubicin leads to decreased GSIS, cellular viability, increased cellular toxicity, immediately after 6 hours post-exposure. Doxorubicin interferes with the electron transport of plasma membranes (PMET), a pathway that depends on the reduction of NADPH / NADP equivalent, but fails to redox cycles in INS-1 cells 832/13. Although NADPH/NADP+ content was not affected, NADH/NAD+ content decreased in 4 hours post-exposure to doxorubicin, and was followed by a decrease in ATP content (Heart et al. 2016).

Turmeric extract (Curcuma longa L.) (Family Zingiberaceae) (TE) has a major component, curcumin, which is responsible for its biological actions. 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 of characterizes and has also been shown to inhibit the growth of many lines of human cancer cells 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, and sinusitis have been reported by (Anggraeny and Pramitaningastuti 2016).

V. Conclusion

Ethanol extract Curcuma longa doses of 200 mg/kgBB, 400 mg/kgBB, and 600 mg/kgBB had significantly different blood glucose levels decreased activity ($P<0.05$) with negative control groups given only CMC-Na and doxorubicin. Curcuma longa extract doses of 200 mg/kgBB, 400 mg/kgBB, and 600 mg/kgBB had significantly different hbA1c reduction activity ($P<0.05$) with negative control groups given only CMC-Na and doxorubicin. Advice, for researchers, can then be done antidiabetic testing by looking at insulin expression after doxorubicin-induced and tested measurement of antioxidant levels such as GSH, Catalase, and SOD.

References

Ahmad, Wasim, Azhar Hasan, Abdullah Ansari, and Tahera Tarannum. 2010. "Curcuma Longa, Linn – A Review." Hippocratic Journal of Unani Medicine 5(4):179–90.

Andrew, C. 2010. "Cyto- and DNA-Protective Dietary Compounds Author Griffith Research Online."

Anggraeny, Ebta Narasukma, and Anastasia Setyopuspito Pramitaningastuti. 2016. "Studi Uji Daya Antiinflamasi Dan Antipiretik Ekstrak Etanol Daun Lengkeng (Dimocarpus Longan Lour) Pada Tikus Putih Jantan (Rattus Norvegicus) Galur Wistar." Jurnal Ilmiah Farmasi 12(2):1–14.

Ariandi. 2016. "Pengenalan Enzim Amilase (Alpha-Amylase) dan Reaksi Enzimatisnya Menghidrolisis Amilosa Pati Menjadi Glukosa." Jurnal Dinamika 7(April):74–82.

Banjarnahor, Eka, and Sunny Wangko. 2013. "Sel Beta Pankreas Sintesis Dan Sekresi Insulin." Jurnal Biomedik (Jbm) 4(3).

Bisht, Kavita, Karl Heinz Wagner, and Andrew C. Bulmer. 2010. "Curcumin, Resveratrol and Flavonoids as Anti-Inflammatory, Cyto- and DNA-Protective Dietary Compounds." Toxicology 278(1):88–100.

Buckley, Joseph P. 1966. "Pharmaceutical Sciences (Np)." Science 151(3712):874–75.

Cosby A. Stone. et al. 2018. "Update on Vitamin E and Its Potential Role in Preventing or Treating Bronchopulmonary Dysplasia Cosby." HHS Public Access 113(4):366–78.

Delgado-León, Tania Guadalupe, José Manuel Sálas-Pacheco, Fernando Vazquez-Alaniz, Ángel Antonio Vértiz-Hernández, Olga Dania López-Guzmán, Eduardo Lozano-Guzmán, Aurora Martínez-Romero, Norma Úrtiz-Estrada, and Maribel Cervantes-Flores. 2018. "Apoptosis in Pancreatic β-Cells Is Induced by Arsenic and Atorvastatin in Wistar Rats with Diabetes Mellitus Type 2." Journal of Trace Elements in Medicine and Biology 46:144–49.

Devaraj, Sutha, Sabariah Ismail, Surash Ramanathan, and Mun Fei Yam. 2014. "Investigation of Antioxidant and Hepatoprotective Activity of Standardized Curcuma Xanthorrhiza Rhizome in Carbon Tetrachloride-Induced Hepatic Damaged Rats." Scientific World Journal 2014.

Ewer, Michael S., and Steven M. Ewer. 2010. “Cardiotoxicity of Anticancer Treatments: What the Cardiologist Needs to Know.” Nature Reviews Cardiology 7(10):564–75.
Gašić, Uroš, Ivanka Ćirić, Tomislav Pejčić, Dejan Radenković, Vladimir Djordjević, Siniša Radulović, and Živoslav Tešić. 2020. “Polyphenols as Possible Agents for Pancreatic Diseases.” Antioxidants 9(6):1–22.
El Hadi, Hamza, Roberto Vettor, and Marco Rossato. 2018. “Vitamin E as a Treatment for Nonalcoholic Fatty Liver Disease: Reality or Myth?” Antioxidants 7(1).
Den Hartogh, Danja J., Alessandra Gabriel, and Evangelia Tsiani. 2020. “Antidiabetic Properties of Curcumin I: Evidence from in Vitro Studies.” Nutrients 12(1).
Heart, Emma A., Shpetim Karandrea, Xiaomei Liang, Maren E. Balke, Patrick A. Beringer, Elyse M. Bobczynski, Delaine Zayas Bazán Burgos, Tiffany Richardson, and Joshua P. Gray. 2016. “Mechanisms of Doxorubicin Toxicity in Pancreatic β-Cells.” Toxicological Sciences 152(2):395–405.
Itokawa, Hideji, Qian Shi, Toshiyuki Akiyama, Susan L. Morris-Natschke, and Kuo Hsiung Lee. 2008. “Recent Advances in the Investigation of Curcuminoids.” Chinese Medicine 3:1–13.
Joseph, Baby, and Vrundha M. Nair. 2013. “Đặc Điểm Thực Vật.Pdf.” 3(2):273–92.
L. Wiradewi & S. Dharma. 2017. “Diktat Praktikum Kimia Klinik III Pemeriksaan Glukosa Urine Dan Glukosa Darah Kualitatif Dan Kuantitatif.” Bagian Patologi Klinik Fakultas Kedokteran Universitas Udayana 1–49.
Mahadevan, Vishy. 2019. “Anatomy of the Pancreas and Spleen.” Surgery (United Kingdom) 37(6):297–301.
Nuryani. 2013. “Potensi Subtitusi Beras Putih Dengan Beras Merah Sebagai Makanan Pokok Untuk Perlindungan Diabetes Melitus.” Media Gizi Masyarakat Indonesia 3(3):157–68.
Oktem, Gulperi, Sule Ayla, Ismail Seckin, Gamze Tanriverdi, Mujgan Cengiz, Mediha Eser, and B. C. Soner. 2011. “Doxorubicin Induced Nephrotoxicity: Protective Effect of Nicotinamide.” International Journal of Cell Biology 2011.
Pandol, Stephen J. 2015. “Normal Pancreatic Function.” Pancreapedia 13.
Permana, Hikmat, and Hasan Sadikin Hospital. 2000. “Komplikasi Kronik Dan Penyakit Penyerta Pada Diabetesi.”
Pujiyanto, Sri, and Budi Raharja. 2019. “Aktivitas Inhibitor Î±-Amilase Ekstrak Etanol Tanaman Brotowali (Tinospora Crispa L.).” Bioma : Berkala Ilmiah Biologi 21(2):126–31.
Rabiah, Erwanti, I. Berata, and Sam Suri. 2015. “Gambaran Histopatologi Ginjal Tikus Putih Yang Diberi Deksametason Dan Vitamin E.” Indonesia Medicus Veterinus 4(3):257–66.
Razavi, et al. 2021. “A Review of Therapeutic Potentials of Turmeric (Curcuma Longa) and Its Active Constituent, Curcumin, on Inflammatory Disorders, Pain, and Their Related Patents.” Phytotherapy Research (July):1–25.
Rezki, Rajian Sobri, Dwimas Anggoro, and Siswarni MZ. 2015. “Ekstraksi Multi Tahap Kurkumin Dari Kunyit (Curcumadomestica Valet) Menggunakan Pelarut Etanol.” Jurnal Teknik Kimia USU 29:29–34.
Rizvi, Saliha, Syed T. Raza, Faizal Ahmed, Absar Ahmad, Shania Abbas, and Farzana Mahdi. 2014. “The Role of Vitamin E in Human Health and Some Diseases.” Sultan Qaboos University Medical Journal 14(2):157–65.
Saefudin, Fauzia Syarif, and Chairul. 2014. “Antioxidant Potential and Proliferative Activity of Curcuma Zedoaria Rosc. Extract Oh Hela Cells.” Widyariset 17(3):381.
Safwan, Safwan, Sapto Yuliani, and Suwidjiyo Pramono. 2014. “Uji Aktivitas Minyak Atsiri Rimpang Kunyit (Curcuma Longa Linn) pada Tikus Sprague Dawley Model Demensia (Kajian Penghambat Aktivitas Asetilkolinesterase).” Kartika Jurnal Ilmiah Farmasi 2(2):20–26.
Tacar, Oktay, Pornsak Sriamornsak, and Crispin R. Dass. 2013. “Doxorubicin: An Update on

Anticancer Molecular Action, Toxicity and Novel Drug Delivery Systems." Journal of Pharmacy and Pharmacology 65(2):157–70.
Utomo, Mohammad R. S., Herlina Wungouw, and Sylvia Marunduh. 2015. "Kadar Hba1C Pada Pasien Diabetes Melitus Tipe 2 Di Puskesmas Bahu Kecamatan Malalayang Kota Manado." Jurnal E-Biomedik 3(1):3–11.
Wanninger, Simon, Volker Lorenz, Abdus Subhan, and Frank T. Edelmann. 2015. "Metal Complexes of Curcumin - Synthetic Strategies, Structures and Medicinal Applications." Chemical Society Reviews 44(15):4986–5002.
Wardani, Nela Agustin Kusuma. 2018. "Enzim α-Amilase Inhibitor Pada Ekstrak Air Kacang Merah (Phaseolus Vulgaris L.) Untuk Penanggulangan Diabetes Melitus." Jurnal Ilmu Pangan Dan Hasil Pertanian 1(2):50–59.