ACTIVITY OF CATALASE ENZYME IN DIABETIC RAT (*RATTUS NORVEGICUS*) MODELS AFTER ADMINISTRATION OF ETHANOL EXTRACT OF *JENGKOL* (*ARCHIDENDRON PAUCIFLORUM*) FRUIT PEEL

Desak Made Malini[1], Retnaningtyas Siska Dianty[2], Madihah[1]

[1,2] *Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jl. Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang 45363, West Java, Indonesia.*
*Correspondent author: *desak.made@unpad.ac.id*

ABSTRACT

Jengkol fruit peel (Archidendron pauciflorum) from the Fabaceae family is one of the herbal plants used by the community in Karangwangi Village, Cianjur, West Java, as a remedy for diabetes mellitus (DM). DM is known to increase oxidative stress and cause high production of free radicals, thus requiring enzymatic antioxidants, such as catalase, to counteract free radicals. The aim of this study was to evaluate the activity of the catalase enzyme in diabetic rat (Rattus norvegicus) models after the administration of ethanol extract from jengkol (Archidendron pauciflorum) fruit peel (EEOJFP). The method used was experimental with a Completely Randomized Design (CRD) involving six treatments: diabetic rats; diabetic rats treated with glibenclamide at a dose of 10 mg/kg BW as a comparison; diabetic rats treated with EEOJPF at doses of 385, 770, and 1540 mg/kg BW; and non-diabetic rats, each with four replicates. The results showed that all treatments did not significantly affect catalase enzyme activity in blood cells. The concentration of catalase enzyme activity in the liver did not differ significantly from the group given glibenclamide, although this enzyme activity was lower compared to both the positive and negative controls. The conclusion of this study is that EEOJFP is not yet effective in significantly increasing catalase enzyme activity in blood cells and the liver of diabetic rat models, but its effect is comparable to that of glibenclamide.

Keywords: *Activity, Liver, Catalase, Jengkol Fruit Peel, Blood Cells.*

1. INTRODUCTION

The use of herbal plants in the field of health by the Indonesian people has been widely practiced and represents a local wisdom of its own. One of the plants extensively utilized in Indonesia for health purposes is the jengkol plant (*Archidendron pauciflorum*). Ethnobotanical studies conducted in Karangwangi Village, Cianjur, West Java, show that the infusion of jengkol fruit peel (*A. pauciflorum*) is used by the local community in Karangwangi as a remedy for diabetes mellitus (DM) [1]. DM is a metabolic disorder characterized by hyperglycemia, which is caused by damage to insulin secretion and/or insulin action [2]. Diabetes is also a prevalent condition among the Indonesian population.

In managing DM, patients are typically given medication to address the symptoms. Glibenclamide is one of many oral sulfonylurea drugs used to control blood sugar levels [3]. This medication stimulates insulin secretion and reduces glycogen storage by the liver to lower blood sugar levels [4]. The side effects of glibenclamide are generally mild, including gastrointestinal disturbances, central nervous system effects, and weight gain [5]. The side effects of this medication have led to increasing research into traditional diabetes remedies using herbal plants.

Extracts of *Bauhinia tomentosa* root at a dose of 500 mg/kg BW and *Cajanus cajan* leaf extract at a dose of 400 mg/kg BW can lower blood sugar levels in Wistar rats, both male and female, induced with alloxan [6] [7]. Extracts of *Afzelia africana* bark at a dose of 200 mg/kg BW can lower fasting blood glucose levels in male Wistar rats induced with streptozotocin [8]. The *jengkol* plant (*A. pauciflorum*) is known to have properties that can lower blood glucose levels, making it a viable option for development as an anti-diabetes remedy by the community of Karangwangi Village. The seeds, bark, fruit peel, and leaves of jengkol contain various flavonoid derivatives, procyanidins, fatty acids, terpenoids, vitamin E, and alkaloids [9], as well as saponins, glycosides, and steroids (triterpenoids) [10].

The extract of *A. pauciflorum* seeds at a dose of 50 g/kg BW administered for 12 days can lower blood sugar levels in streptozotocin-induced diabetic model rats [11]. Besides lowering blood glucose levels, the flavonoids in *jengkol* can also reduce free radicals, thus being classified as free radical scavenging compounds [12]. DM can increase oxidative stress and lead to high production of free radicals while reducing the capability of antioxidant defenses [13]. Antioxidants as protectors against oxidative stress can be categorized into enzymatic and non-enzymatic types. Enzymatic antioxidants include catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (Gpx) [14]. Catalase is an enzyme in the hydroperoxidase group that catalyzes substrates like hydrogen peroxide (H2O2) and organic peroxides to prevent lipid peroxidation in cell membranes and acts as a free radical scavenger [14]. The levels of catalase enzyme in the pancreas of Wistar rats, both male and female, with STZ-induced diabetes at a dose of 50 mg/kg BW, show an increase after administration of *Moringa oleifera* leaf extract at a dose of 150 mg/kg BW [15]. This indicates that plant extracts containing flavonoid compounds have the potential to enhance catalase enzyme activity under oxidative stress conditions. Therefore, a study was conducted to investigate the effect of ethanol extract of *jengkol* fruit peel (EEOJFP) on the activity of catalase enzyme in Wistar rats with STZ-induced diabetes.

2. Materials and Methods

2.1 Preparation of Ethanol Extract of *Jengkol* Fruit Peel

The extraction process was carried out by maceration using 70% ethanol as solvent. The steps for making the extract included washing and drying the jengkol peel and then grinding it into raw powder using a blender. The raw powder obtained was placed in a container and mixed with 70% ethanol in a ratio of 1:10 (weight/volume). It was then left to stand for three cycles of 24 hours each, and the maceration solution was collected. The obtained maceration solution was concentrated using a rotary evaporator at a temperature of 40 °C [16].

2.2 Animals, treatments, and experimental approach.

Twenty-four female Wistar rats (*Rattus norvegicus*) 8-12 weeks with an average weight of 182.6 grams were used in this study. The rats were maintained in an animal house under controlled temperature (22-30℃), 12 hours light-dark cycle, and fed with standard pellet CP 511 (5 gr/100 gr BW/day) and water ad-libitum. A diabetic model of rats was induced intravenously by streptozotocin (STZ) of dose 65 mg/kg BW in a 0.1 M citrate buffer solution (pH 4.5). The STZ-induced rats with blood glucose ≥250 mg/dl in 72 hours were indicated as having diabetes. The rats were randomly divided into six treatment groups with four replicates each: (1) control group (0.5% of Carboxymethyl Cellulose (CMC); (2) Positive control (streptozotocin 65 mg/kg BW; (3) Pb group (10 mg/kg BW of Glibenclamide as commercial drug comparison); (4) P1 (385 mg/kg BW of EEOJFP); P2 (770 mg/kg BW of EEOJFP); and P3 (1540 mg/kg BW of EEOJFP). All treatments were given with gavage for 14 days consecutively. On the 15th day, blood samples were collected through the tail puncture and placed in Ethylene Diamine Tetra-acetic Acid (EDTA)-coated tubes. All the rats were then sacrificed by cervical dislocation and the liver was isolated. Blood and liver samples were used to measure catalase enzyme activity [17]; 18].

3. Results and Discussion

The results of the average catalase enzyme activity measurements in blood cells and liver organ using the Aebi and Cohen methods are shown in Table 1. The catalase enzyme activity in blood cells of the diabetic test animal group (PC) was higher compared to the non-diabetic test animal group (NC). This is due to an imbalance between the enzymatic antioxidant catalase and the production of Reactive Oxygen Species (ROS), particularly under diabetic conditions induced by streptozotocin (STZ). STZ can disrupt antioxidant enzymes, leading to an imbalance in ROS production, such as hydroxyl radicals (HO), superoxide anions (O2), and hydrogen peroxide

(H2O2) [19]. Meanwhile, cells need to be protected from increased oxidative states by antioxidant enzymes. Therefore, the increased catalase enzyme activity in the diabetic rat group is suspected to be due to increased ROS production.

The treatment with Glibenclamide at a dose of 10 mg/kg BW (Pb) and EEOJFP at doses of 385 (P1), 770 (P2), and 1540 (P3) mg/kg BW in diabetic rats showed lower catalase enzyme activity in blood cells compared to the diabetic rats (PC), but closer to the values in non-diabetic rats (NC). Statistical analysis using the Kruskal-Wallis test indicates that all treatments did not significantly affect catalase enzyme activity in blood cells compared to the diabetic rats (PC).

Table 1: Catalase Enzyme Activity in Blood Cells and Liver Organs of Diabetic Rat Models

Treatment group	Average of catalase enzyme activity	
	Blood Cells (IU/mg)	Liver (IU/mg)
Negative control (CMC Na 0,5 %)	1,00 ± 0,014	4,76 ± 0,33
Positive control (STZ 65 mg/kg BW)	1,01 ± 0,007	5,63 ± 0,462
Pb (Glibenklamid 10 mg/kg BW)	0,99 ± 0,018	2,70 ± 0,414[ab]
P1 (EEOJFP 385 mg/kg BW)	1,00 ± 0,008	2,01 ± 0,038 [ab]
P2 (EEOJFP 770 mg/kg BW)	1,00 ± 0,002	2,15 ± 0,425[ab]
P3 (EEOJFP 1540 mg/kg BW)	1,00 ± 0,011	2,02 ± 0,541[ab]

Note: Data are presented as ($\bar{X}$) ± SD and analyzed using the Kruskal-Wallis and Mann-Whitney tests with a 95% confidence level. [a)] Significantly different from CN; [b)] Significantly different from PC.

A decrease in catalase enzyme activity can occur in test animals treated with STZ [20]. This decrease in catalase enzyme activity is caused by an increase in free radicals or factors related to humoral immunity. There are the relationship between the dose of a drug or plant extract and the humoral immune response of lymphocytes in the blood. Therefore, changes in catalase enzyme activity, which plays a role in reducing free radicals, are influenced by humoral immune factors in blood cells following treatment with plant extracts [21];[22]. Catalase enzyme activity levels in the liver organ show that diabetic test animals (PC) experienced a decrease in catalase enzyme activity compared to non-diabetic test animals (NC). This finding is consistent with the research by Florence et al. [23], which states that catalase enzyme activity in the liver of diabetic test animals is lower compared to non-diabetic test animals. The decrease in catalase enzyme activity is attributed to enzyme inactivation or glycation [24]. The aldehyde binding reaction on proteins progresses as browning and oxidation reactions, leading to the accumulation of chemically modified proteins in tissues [25].

The observed opposite catalase enzyme activity in this study is suspected to be due to differences in the sex of the test animals used. Florence et al. [23] used male Wistar rats, while this study used female Wistar rats. There is a difference in sensitivity to STZ among rodents depending on their sex [26]. Sensitivity in female rats is reduced due to the protective effect of the hormone estradiol on β pancreatic cells from apoptosis caused by oxidative stress [27]. Therefore, it is suspected that the female diabetic rats (PC) did not experience excessive oxidative stress, allowing the catalase enzyme to remain active and increase its activity in the liver organ.

Diabetic rats treated with glibenclamide at 10 mg/kg BW (Pb) had lower liver catalase enzyme activity, but this was significantly different from both the positive control (PC) and negative control (NC). However, the catalase enzyme activity in diabetic rats treated with glibenclamide did not significantly differ from that in rats treated with EEOJPF. This finding is consistent with research by Bukan et al. [28] which showed that catalase enzyme activity increased in the liver of STZ-induced diabetic animals after being treated with glibenclamide at a dose of 5 mg/kg BW. It is known that glibenclamide can normalize blood glucose levels in diabetic patients by enhancing insulin's ability to reduce glycogen production in the liver and increasing peripheral glucose utilization [29]. Hyperglycemia can cause an imbalance in oxidative status in the body, leading to a decrease in antioxidant enzyme activity, including catalase. The normalization of blood glucose levels following glibenclamide treatment is suspected to help restore catalase enzyme activity, but the EEOJPF appears to be more effective than glibenclamide.

In test animals treated with EEOJFP, it was found that diabetic rats treated with EEOJFP at a dose of 770 mg/kg BW (P2) had lower average catalase enzyme activity values, but this was significantly different from both the positive control (PC) and negative control (NC). However, statistical analysis using the Kruskal-Wallis test indicated that all treatments affected liver catalase enzyme activity. This finding contrasts with the research by Sherien et al. (2015) [30], which reported an increase in catalase enzyme activity in diabetic test animals treated

with *Caesalpinia ferrea* leaf extract at a dose of 500 mg/kg body weight. Although both extracts come from the same family, it cannot be concluded that the same dose will increase catalase enzyme activity in diabetic test animals. Both types of plants from the Fabaceae family have almost identical phytochemical contents. Phytochemical screening showed that C. ferrea leaf extract contains glycosides, tannins, and phenolic compounds such as flavonoids [30], The EEOJFP contains chemical compounds such as alkaloids, flavonoids, saponins, and phenols [31]. Flavonoid compounds, in addition to lowering blood glucose levels, can also function to scavenge or reduce free radicals, thus being referred to as free radical scavengers [12].

The mechanism of catalase enzyme activity in blood cells and liver organs does not differ. the mechanism of catalase activity as an antioxidant involves catalyzing the breakdown of H_2O_2 into H_2O and O_2, which does not affect the catalase enzyme levels [32]. However, catalase enzyme activity is highest in the liver [33]. Therefore, it is suspected that catalase enzyme activity in the liver is higher compared to that in blood cells. The effect of flavonoids on catalase enzyme activity remains contradictory. The increase in catalase enzyme activity due to flavonoids may be due to the activation of flavonoid compounds in the redox signaling pathway Nrf2, which regulates phase II detoxification and antioxidant enzymes [34]. On the other hand, the catalase enzyme activity can be inhibited by flavonoids [35]. Inhibition of catalase enzyme activity is due to the formation of hydrogen bonds between catalase and flavonoids, resulting in changes to the secondary or tertiary structure of catalase. Therefore, it is assumed that the flavonoid content in the ethanol extract of jengkol fruit peel may have the potential to decrease, rather than increase, catalase enzyme activity. Active flavonoid compounds need to be identified to confirm their direct effect on catalase enzyme activity in diabetic rat models.

4. CONCLUSIONS

The conclusion of this study is that EEOJFP is not yet effective in significantly increasing catalase enzyme activity in blood cells and the liver of diabetic rat models, but its effect is comparable to that of glibenclamide.

5. REFERENCES

[1] Kamilawati, F. 2015. Inventarisasi Tumbuhan yang Digunakan Masyarakat sebagai Obat Diabetes di Desa Karangwangi, Kabupaten Cianjur, Jawa Barat. *Laporan Kerja Praktek*. Bandung: Universitas Padjadjaran.

[2] American Diabetes Association. 2013. Standards of Medical Care in Diabetes. *Diabetes Care*. 36(1) : 11-66.

[3] Nathan, D. M., J.B. Buse, *and* M.B. Davidson. 2009. ADA/EASD concensus statement and algorithm for the treatment of Type 2 diabetes. *Diabet Care*. 1 : 193-203.

[4] Rendell, M. 2004. The role of sulphonylureas in the management of type 2 diabetes mellitus. *Drug*. 64 (12) : 1339-58.

[5] Soegondo, S. 2004. Prinsip Pengobatan Diabetes, Insulin dan Obat Hipoglikemik Oral. Dalam: Penatalaksanaan Diabetes Mellitus Terpadu. *Skripsi*. Fakultas Kedokteran, Universitas Indonesia.

[6] Mashirkar V.J., V.N. Deshmukh, J.K. Jadhav, *and* D.K. Sarkar. 2008. Antidiabetic activity of the ethanolic extract of *Pseudarthria viscida* root againts alloxan induced diabetes in albino rats. *Res J Pharmaceut Tech*. 1 :541-546.

[7] Adaobi, C.E., A.A. Peter, C.O. Charles, *and* B.O. Chinwe. 2010. Experimental evidence for the antidiabetic activity of *Cajanus cajan* leaves in rats. *J Basic Clin Pharm*. 1:81-84.

[8] Oyedemi S.O., E.A. Adewusi, O.A. Aiyegoro, *and* D.A. Akinpelu. 2011. Antidiabetetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic wistar rats. *Asian Pac J Trop Biomed*. 1 : 353-358.

[9] Bunawan, H., L. Dusik., S.N. Bunawan., *and* N.M. Amin. 2013. Bonaty, traditional uses, phytochemistry and pharmacology of *Archidendron jiringa* : A review. *Global J of Pharmacol* 7(4) :474-478.

[10] Wahyuni, N.Y., N. Mayasari, dan Abun. 2012. Pengaruh penggunaan ekstrak kulit jengkol (Pithecellobium jiringa (Jack) Prain) dalam ransum terhadap nilai hematologi ayam Broiler. *e-Jurnal Mahasiswa* 1(1). jurnal.unpad.ac.id. [9 juli 2017].

[11] Shukri, R., S. Mohamed, N.M. Mustapha *and* A.A. Hamid. 2011. Evaluating the toxic and beneficial effects of jering beans (*Archidendron jiringa*) in normal and diabetic rats. *J Sci Food Agri*. 91(14) : 2697-2706.

[12] Giorgio, P., 2000. Flavonoid as Antioxidant. *J Nat Prod*. 63 : 1035- 1045.

[13] Descorbeth, M. *and* M.B. Anand-Srivastava. 2010. Role of oxidative stress in high-glucose and diabetes-induced increase expression of Gq/11α proteins and associated signaling in vascular smooth muscle cell. *Free Radical Bio Med*. 49(9) :1395-405.

[15] Gupta, R., M. Mathur, V.K. Bajaj, P. Katariya, S. Yadav, *and* R. Kamal. Evaluation of diabetic and antioxidant [14] Kohen, R. *and* Nyska, A. 2002. Oxidation of Biological System: Oxidative Stress Phenomena, Antioxidant, Redox Reaction and Methods for Their Quantification. *Toxicol Pathol*. 30 :620-650activity *Moringa oleifera* in experimental diabetes. *J Diabetes*. 4(2) :164-71.

[16] Khan, B.A., N. Akhtar, A. Rasul, T. Mahmood, H.S. Khan, S.U. Zaman, M. Iqbal and G. Murtaza. 2012. Investigation of Effects of Extraction Solvent/Technique on The Antioxidant Activity of *Cassia fistula* L. *J.Med. Plants Res*. 6(3): 500-503.

[17] Cohen, G., D. Dorothy, *and* M. Judith. 1970. Measurement of Catalase Activity in Tissue Extracts. *Analytic Biochem*. 34: 30-38.

[18] Aebi, H. 1984. Catalase in Vitro. *Methods in Enzymol*. 105(1).

[19] Qujeq, D. *and* T. Rezvani. 2007. Catalase (antioxidant enzyme) activity in streptozotocin-induced diabetic rats. *Int J Diabetes & Metabolism*. 15 :22-24.

[20] Shanmugasundaram, R., D.V. Kalpana, S.P. Tresina, R.M. Veerabahu. 2011. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Senna auriculata* (L.) Roxb. leaves in alloxan induced diabetic rats. *J PharmTech Res*. 3(2) :747-756.

[21] Anderson, D., T.W. Yu, B.J. Phillips, *and* P. Schmezer. 1994. The effect of various antioxidant and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. *Mutat Res*. 307 : 261-271.

[22] Slaga, T.J. 1995. Inhibition of the induction of cancer by antioxidants. *Advances in Experimental Medical Biology*. 369 : 167-174.

[23] Florence, N.T., M. Z. Benoit., K. Jonas, T. Alexandra, D. D. P. Désiré, K. Pierre, *and* D. Théophile. 2013. Antidiabetic and antioxidant effect of *Annona muricata* (Annonaceae) aqueous extract on streptozotocin-induced diabetic rats. *J of Pharmacol*. 151 : 784-790.

[24] Yan, H., *and* J.J. Harding. 1997. Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. *Biochem J*. 328 :599-605.

[25] Haffner, S.M. 1999. The importance of hyperglycemia in the non fasting state to development cardiovascular state. *Endocrine Review*. 19(5) :583-92.

[26] Deeds, M.C., J.M. Anderson, A.S. Armstrong, D.A. Gastineaeu, H.J. Hiddinga, and A. Jahangir. 2011. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. *Lab Anim*. 45(3) : 131-40.

[27] Le May, C., K. Chu, M. Hu, C.S. Ortega, E.R. Simpson, *and* K.S. Korach. 2006. Estrogens protect pancreatic beta-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. *Prod of the Nat Academy of Sci*. 103 (24) : 9232–7.

[28] Bukan, N., B. Sancak, A. Bilgihan, F. Kosova, G. Bugdayci, *and* Altan, N. 2004. The effects of the sulfonylurea glyburide on glutathione peroxidase, superoxide dismutase and catalase activities in the heart tissue of streptozotocin-induced diabetic rat. *Met and Find in Exp and Clin Pharmacol*. 26 (7) : 519−522.

[29] Nazaroglu, N.K., A. Sepici-Dincel, *and* N. Altan. 2009. The effects of sulfonylurea glyburide on superoxide dismutase, catalase, and glutathione peroxidase activities in the brain tissue of streptozotocin-induced diabetic rat. *J of Diabetes and Its Complication*. 23 : 209-213.

[30] Sherien, K.H., M.E. Nermin, M.M. Amria, *and* M.H. Maha. 2015. Hypoglycemic and antioxidant activities of *Cesalpinia ferrea* Martius leaf extract in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed*. 5(6) :462-471.

[31] Sihombing, J.R., Abdi Dharma, Z. Chaidir, Almahdy, E. Fachrial, *and* E. Munaf. 2015. Phytochemical screening and antioxidant activities of 31 fruit peel extract from Sumatra, Indonesia. *J of Chem and Pharmaceutic Res*. 7(11) : 190-196.

[32] Kumar, V., A.K. Abbas, N. Fausto, J.C. Aster, *and* J.C. Hauth. 2008. Robbins and Cotran Pathologic Basic of Disease. Eight edition. *Cell Adapt, Cell Inj, and Cell Death*. 1 :16-18.

[33] Eisner, T. *and* D.J. Aneshansley. 1999. Spray aiming in the bombardier beetle: photographic evidence. *Proc Natl Acad Sci*. 96 (17) : 9705–9709.

[34] Elavarasan, J., P. Velusarny., T. Ganesan., S.K. Ramakhrisnan., D. Rajasekaran, *and* K. Periandavan. 2012. Flavonoids. *J of Phar and Pharmacol*. 64 :1472-1482.

[35] Krych, J *and* Gebicka, L. 2013. Catalase is inhibited by flavonoids. *Inter J of Bio Macromol*. 58 :148-153.