

## **Effect of Antihypoglycemic *Sechium edule* Jacq. Swartz. Etanol Extract on Histopathologic Changes in Hyperglycemic *Mus musculus* L.**

**Jekson Martiar Siahaan**

Department of Physiology, Faculty of Medicine, Methodist Indonesia University, Medan

### **ABSTRACT**

**Background:** Streptozotocin as diabetogenic can damage the pancreatic  $\beta$  cells of animals tried through the oxidative stress process to increase blood sugar levels. Giving ethanolic extract of squash fruit has hypoglycemia effect because it contains flavonoids that act as antioxidants and antihyperglycemia. This study aimed is analyze the effect of ethanol extract of squash fruit to decrease blood sugar level and the change of pancreatic  $\beta$  cell diameter.

**Subjects and Method:** This study was an experimental study with post-randomized controlled group design, using male white mice (*Mus musculus* L.) DD Webster strains randomized into 4 groups: negative control group, positive control group, group with extract ethanol of 100 mg/kgBB, and a group of ethanol extract of 200 mg/kgBW of pumpkin.

**Results:** The results showed a significant reduction in blood sugar levels if compared with the control group. The presence of changes in  $\beta$  pancreas cell diameter on ethanol extract of 100 mg/kgBB and 200 mg/kgBB.

**Conclusion:** The conclusion of this study is the extract of ethanol fruit of 200mg/kgBB squash significantly reduce blood sugar level of mice, the change of  $\beta$  pancreas cell diameter on ethanol extract of 100mg/kgBB and 200mg/kgBB.

**Keywords:** streptozotocin, antihypoglycemia, flavonoid

### **Correspondence:**

Jekson Martiar Siahaan. Physiology Teaching Staff of Medicine Faculty, Methodist University of Indonesia Medan, Jl. Setia Budi Pasar 2, Medan 20132, Indonesia.

### **BACKGROUND**

Streptozotocin (STZ) has a chemical name 2-Deoxy-2-[[[(methylnitrosoamino)carbonyl]amino]D-glucopyranose, obtained from *Streptomyces achromogenes* and structurally derived from nitrosourea (Akbarzadeh et al., 2007; Nugroho, 2006; Srinivasan and Ramarao, 2007). STZ has antineoplastic, antibiotic and diabeto-genetic effects (Raza and John, 2012; Akbarzadeh, et al., 2007; Srinivasan and Ramarao, 2007). Its use as a diabetogenic was first performed by Rakietyen in dogs and mice in 1963. STZ evokes free radicals that play a role in destroying pancreatic  $\beta$  cells. The STZ mechanism is mediated primarily by NO formation and reactive oxygen generation. Superoxide anion reactive oxygen for-

mation and increased activity of xanthine oxidase caused by STZ in mitochondria. STZ inhibits the krebs cycle and decreases mitochondrial oxygen consumption. The limited production of mitochondrial ATP subsequently results in a drastically reducing nucleotide of pancreatic  $\beta$  cells. Increased ATP defo-phyzation will spur substrate increases for the enzyme xanthine oxidase (pancreatic  $\beta$  cells have high activity on this enzyme), and further increase uric acid production. Xanthine oxidase will catalyze the formation of an active superoxide anion formation. Based on the superoxide anion formation process, hydrogen peroxide and superoxide radicals are formed. NO and reactive oxygen are the

main causes of pancreatic  $\beta$  cell damage (Srinivasan and Ramarao, 2007).

Persistent hyperglycemia in people with Diabetes Mellitus (DM) will lead to increased oxidative stress due to an imbalance between free radicals and natural antioxidants formed by the body. Increased oxidative stress can occur in Type 1 DM and Type 2 DM. Type 1 DM and oxidative stress will damage pancreatic  $\beta$  cells while Type 2 DM will cause disruption of insulin production, release, and insulin function (Sheikhpour et al., 2013).

Oxidative stress in pancreatic  $\beta$  cells destroys proteins, enzymes, lipid membranes, DNA and reduces immune and antioxidant responses, increases levels of lipid peroxidase and *proinflammatory* cytokines (Moussa, 2008). Chronic hyperglycemia can damage tissues including pancreatic islet cells. Various biochemical mechanisms due to glucose toxicity causing oxidative stress can be through 6 mechanisms namely methylglyoxal and glycerin nonenzymatic proteins, polyol sorbitol pathways (aldose reductase), activation of hexosamine metabolism, activation of protein c kinase, and oxidative phosphorylation and glucose autotoxidation (Setiawan and Eko, 2005; Robertson, 2004; Sheikhpour, 2013; Shradha, 2010; Atalay, 2002).

Flavonoids including phenolic compounds, secondary metabolites produced by green plants except algae, can be found in cereals, vegetables and fruits. Flavonoids commonly found are flavones and flavones with C- and O-glycosides, C- and O-glycoside isoflavones, C- and O-glycoside flavanones, C- and O-glycosides, and dihydrochloric, proanthocyanidin and anthocyanin, auron O glycosides, and dihydroflavoneol O-glycosides while the main flavonoids are flavans, flavanones, flavones, flavonols, flavanols, flavanonols,

cetechins, anthocyanidins and isoflavones (Brahmachari, 2011; Rohyami, 2008; Redha, 2010).

Flavonoids are antidiabetic compounds by blocking glucose uptake in the intestines, improving glucose tolerance, disturbing carbohydrate metabolism through inhibition of enzyme  $\alpha$  amylase and enzyme  $\alpha$  glucosidase, stimulating glucose uptake by peripheral tissue. In addition, flavonoids also stimulate insulin production (*insulin secretagogues*) and act like insulin stimulating glycogen synthesis (*insulin mimetics*) (Brahmachari, 2011; Piparo, 2008; Getha et al., 2010). Flavonoids can repair damaged pancreatic tissue due to DNA alkylation by STZ so that insulin secretion increases and blood glucose levels go down (Suryani et al., 2013).

The oxidative stress caused by streptozotocin is a major cause of damage from pancreatic  $\beta$  cells (Srinivasan and Ramarao, 2007). The pancreatic histopathologic changes may be a decrease in the number and diameter of pancreatic  $\beta$  cells (Suarsana et al., 2010; Erwin et al., 2012; Ridwan et al., 2012). A decrease in the number of pancreatic  $\beta$  cells in hyperglycemic animals began to appear on day 7 and continued to decline until day 28. Increasing the number of pancreatic  $\beta$  cells is caused by the body's own healing mechanisms through the improvement of  $\beta$  cells and new cell divisions (mitosis) that occur gradually (Erwin et al., 2012). A decrease in the number of pancreatic  $\beta$  cells results in pancreatic  $\beta$ -cell diameter, normal  $\beta$  pancreatic  $\beta$ -cell diameter 100-400  $\mu\text{m}$  (Ridwan et al., 2012).

---

## SUBJECTS AND METHOD

This study was a design experimental study of *post test randomized controlled group design*. The sample was male white mouse

(*Mus musculus* L.) DD Webster strain was obtained by *simple random sampling* method. The samples were randomized into 4 groups: negative control group, positive control group (STZ 60 mg/ kgBB), group receiving STZ 60 mg/ kgBW and ethanol extract of 100 mg/ kgBB, and STZ 60 mg/ kgBB and extract ethanol fruit chayote 200 mg/ kgBB. Mice (*Mus musculus* L.) DD Webster DM strain if on the fourth day after STZ administration, blood sugar level

≥250 mg/ dl. When blood sugar levels had risen then the ethanol extract of the squash was given until the 28th day.

## RESULTS

The experimental animals used in this study were healthy trial animals with normal blood glucose (KGD). We performed KGD measurements using GlucoDr glucometer to make sure the animals tried normal.

**Table 1. KGD before induction with STZ 60 mg/ kgBW**

P1 (Negative Control)		P2 (Positive Control)		P3 (Ethanol Extract of the Squash 100 mg/kgBB)		P4 (Ethanol Extract of the Squash 200 mg/kgBB)	
U1	142	U1	162	U1	184	U1	121
U2	138	U2	148	U2	152	U2	197
U3	116	U3	166	U3	152	U3	116
U4	154	U4	175	U4	188	U4	176
U5	140	U5	198	U5	136	U5	198
U6	130	U6	184	U6	162	U6	184
U7	126	U7	91	U7	118	U7	143

Description: U1 (Repeat 1), U2 (Repeat 2) U3 (Repeat 3), U4 (Repeat 4), U5 (Repeat 5) U6 (Repeat 6), U7 (Repeat 7).

Based on the measurement of KGD white male mice before induced STZ (Table 1), white male mice did not have DM. KGD male white mouse called DM in this study was ≥250 mg/ dl. Studyer used STZ at a

dose of 60 mg/ kgBW, administered intraperitoneally (Park and Han, 2012) on day 4 of the KGD measurements shown in Table 2.

**Table 2. KGD after induction with STZ 60 mg/ kgBW**

Treatment	P1 Negative Control (mg/dl)	P2 Positive Control (mg/dl)	P3 Ethanol Extract of the Squash 100 mg/kgBB (mg/dl)	P4 Ethanol Extract of the Squash 200 mg/kgBB (mg/dl)
U1	122	218	254	372
U2	138	250	232	435
U3	116	303	267	398
U4	154	314	264	200
U5	140	309	375	323
U6	120	276	299	438
U7	126	377	360	310

Description: U1 (Repeat 1), U2 (Repeat 2) U3 (Repeat 3), U4 (Repeat 4), U5 (Repeat 5) U6 (Repeat 6), U7 (Repeat 7).

Based on Table 2, it appeared that P1 group had KGD <250 mg/ dl while P2, P3 and P4 groups had KGD ≥250 mg/ dl. Since the data were normally distributed and the data

variance was the same, Anova test (Table 3) was performed.

Table 3 showed that the KGD mean in the group P1 = 129.67 ± 14.50; group P2 = 292.43 ± 51.01; group P3 = 314.0 ± 49.97,

and group P4 = 340.17 ± 83.40. The mean of KGD in group P2, P3, and P4 ≥250 indicated that this group had DM. Anova

test results obtained p <0.001, it meant there were differences statistically significant KGD mean in each group.

**Table 3. Anova KGD test results after being induced with STZ 60 mg/ kgBW**

Group	Blood Sugar I					p
	Mean	Median	SD	95% CI		
				Low	Up	
P1	129.67	124.00	14.50	114.45	144.88	<0.001
P2	292.43	303.00	51.01	245.25	339.61	
P3	314.00	315.50	49.97	261.56	366.44	
P4	340.17	347.50	83.40	252.64	427.69	

Description: P1 is Negative control (mg/ dl), P2 is Positive control (mg/ dl), P3 is Ethanol extract Squash Fruit 100 mg (mg/ dl), P4 is Ethanol Extract of SquashFruit 200 mg (mg/ dl)

An increase in KGD of the experimental animals ≥250 mg/ dl was used as the basis of ethanol extract of the squash fruit in the treatment group. The dose of extract of ethanol extract of the squash fruit in the treatment group P3 was given 100 mg/

kgBB and in the treatment group P4 was given 200 mg kg per day. KGD measurements were done every 7 days ie day 7, day 14, day 21 and 28<sup>th</sup> day. The results of blood glucose measurements were shown in Table 5, 6, 7, and 8.

**Table 4. Anova KGD test after giving ethanol extract of squash fruit in day 7<sup>th</sup>**

Group	Blood Sugar II				p
	Mean	SD	95% CI		
			Lower Limit	Upper Limit	
P1	129.67	14.50	114.45	144.88	<0.001
P2	274.71	53.42	225.31	324.12	
P3	207.67	34.82	171.13	244.21	
P4	209.50	23.98	184.33	234.67	

Description: P1 Negative control (mg/ dl), P2 Positive control (mg/ dl), P3 ethanol extract of the squash 100 mg (mg/ dl), P4 ethanol extract of the squash 200 mg (mg/ dl)

Based on the Anova test resulted in Table 4, it appeared that the KGD mean group P1 = 129.67 ± 14.50; group P2 = 274.71 ± 53.42; group P3 = 207.67 ± 34.82, whereas in the P4 group = 209.50 ± 23.98, there was a decrease of KGD in the treatment group while the negative control did not decrease at all and the positive control decreased. Based on Anova test results, it was obtained p <0.001 which meant there was a statistically significant difference in KGD mean in each group.

Anova test results in Table 5 showed that KGD mean happened in the 14<sup>th</sup> day in

group P1 = 129.67 ± 14.50; group P2 = 274.71 ± 53.42; group P3 = 170.83 ± 25.31, and group P4 = 172.83 ± 21.91. There was a decrease of KGD mean in treatment group while in the control group there was no decrease of KGD mean. The P3 and P4 groups given the extract decreased KGD, while P1 and P2 did not decrease the KGD. Anova test results, obtained p <0.001 which meant there was a difference of average KGD in each group and statistically significant.

**Table 5. Anova KGD test after giving ethanol extract of squash fruit in 14<sup>th</sup> day**

Group	Blood Sugar III					P
	Mean	Median	SD	95% CI		
				Lower Limit	Upper Limit	
P1	129.67	124.00	14.50	114.45	144.88	<0.001
P2	274.71	268.00	53.42	225.31	324.12	
P3	170.83	160.50	25.31	144.27	197.39	
P4	172.83	177.00	21.91	149.84	195.83	

Description: P1 Negative control (mg/ dl), P2 Positive control (mg/ dl), P3 ethanolic extract of squash fruit 100 mg (mg/ dl), P4 ethanol extract of squash fruit 200 mg (mg/ dl)

**Table 6. Anova KGD test after giving ethanol extract of squash fruit in 21<sup>st</sup> day**

Group	Blood Sugar IV					P
	Mean	Median	SD	95% CI		
				Lower Limit	Upper Limit	
P1	129.67	124.00	14.50	114.45	144.88	<0.001
P2	176.71	184.00	18.87	159.26	194.17	
P3	127.50	138.00	29.86	96.16	158.84	
P4	125.17	128.50	18.10	106.17	144.17	

Description: P1 Negative control (mg/ dl), P2 Positive control (mg/ dl), P3 ethanolic extract of squash fruit 100 mg (mg/ dl), P4 ethanol extract of squash fruit 200 mg (mg/ dl)

Based on the Anova test results in table 6, it showed the mean of KGD in group P1 =  $129.67 \pm 14.50$ , group P2 = average  $176.71 \pm 18.87$ , group P3 = mean  $127.50 \pm 29.86$ , while KGD group P4 =  $125.17 \pm 18.10$  average. KGD increased in the mean decrease in KGD occurred in groups P2, P3,

and P4, whereas in group P1 there was no decrease in KGD. In the Anova test, the value of P = 0.000 meant that there was significant difference ( $\alpha$  5%,  $p < 0,05$ ), it could be concluded that there was difference of KGD mean in each group.

**Table 7. Anova KGD test after giving ethanol extract of squash fruit in 28<sup>th</sup> day**

Group	Blood Sugar V					P
	Mean	Median	SD	95% CI		
				Lower Limit	Upper Limit	
P1	127.67	128.50	12.925	114.10	141.23	0.001
P2	184.29	186.00	21.242	164.64	203.93	
P3	145.33	145.50	11.130	133.65	157.01	
P4	133.50	130.00	38.188	93.42	173.58	

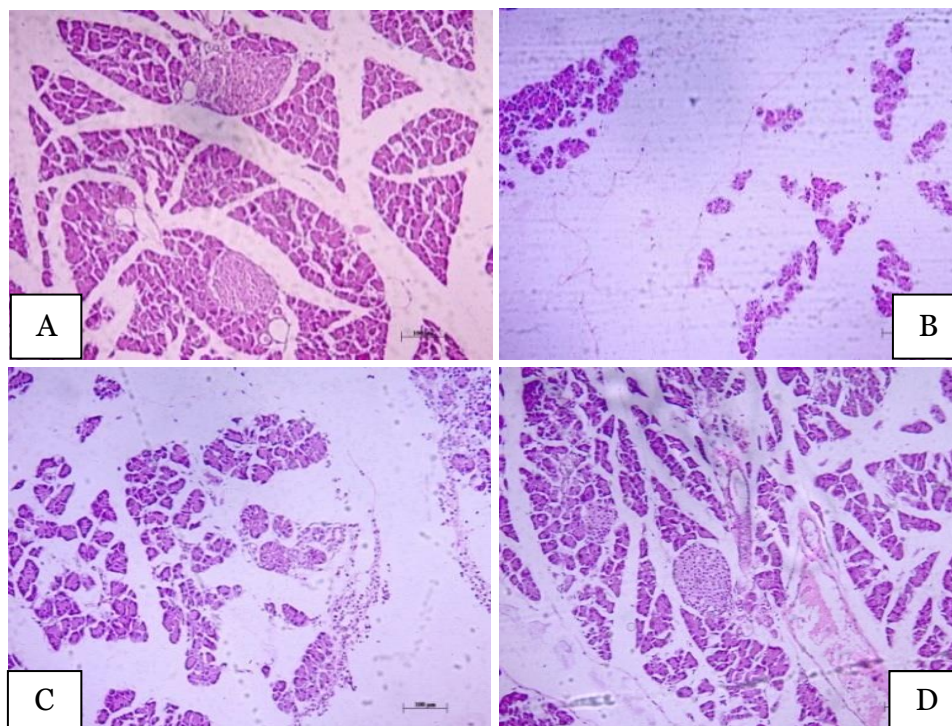
Description: P1 Negative control (mg/ dl), P2 Positive control (mg/ dl), P3 ethanolic extract of squash fruit 100 mg (mg/ dl), P4 ethanol extract of squash fruit 200 mg (mg/ dl)

Based on Table 7 above, it could be seen that the mean of KGD on day 28 in group P1 =  $129.67 \pm 12.92$ , group P2 average =  $184.29 \pm 21.24$ , group P3 average =  $145.33 \pm 11.13$ , group P4 =  $133.50 \pm 38.19$  was lower when compared to mean on P2, and

P3. The KGD rate rose in P2, P3, and P4 but still within normal limits while in group P1 there was no change of KGD. Anova test results obtained  $p = 0.001$  which meant there was a difference of average KGD in each group and statistically significant.

The result showed that there was improvement of pancreatic organ of male white mouse (*Mus musculus* L), DD

Webster strain in group P3 and P4, while in P2 the pancreas diameter decreased, as seen in Picture 1.



**Picture 1. Histopathology of pancreatic  $\beta$ -cell diameter**

**Description:** A. P1 (negative control) had a pancreatic  $\beta$  cell diameter of 238.7  $\mu\text{m}$  indicating that normal pancreatic  $\beta$  cell diameter (100-400  $\mu\text{m}$ ). B. Group P2 (positive control) showed diminished pancreatic  $\beta$  cells indicating pancreatic  $\beta$  cells damaged by the induction of Streptozotocin with a small size of 68.79  $\mu\text{m}$ . C. The P3 group showed that the pancreatic diameter of p-cell size was improved, measuring 93.11  $\mu\text{m}$ . D. P4 group showed that normal pancreatic  $\beta$  cell size, with pancreatic  $\beta$ -cell diameter size was 182.69  $\mu\text{m}$ .

Picture 1. Showed that the pancreas diameter backed to normal in the P4 treatment group while in the P3 treatment group did not return to normal but an increase in diameter when compared with positive control P2.

## DISCUSSION

Male white mice can be induced into DM with STZ, this mechanism is mediated primarily by the formation of ROS-free radicals, RNs that cause destruction of pancreatic  $\beta$  cells (Srinivasan and Ramarao, 2007). There were significant differences in blood sugar levels between groups, where significant decreases occurred in the group given ethanol extract of squash fruit 100

mg/ kgBB and 200 mg/ kgBW. The group receiving the 200 mg dose experienced the lowest decrease of KGD, this result was similar to Maity et al., 2013. The decrease of KGD in the treatment group was due to the giving of the ethanol extract of the flasks containing flavanoid (Siciliano et al., 2004; Marliana et al. , 2005). Flavonoids are antidiabetes by interfering with glucose uptake in the intestine, improving glucose

tolerance, disturbing carbohydrate metabolism through inhibition of enzyme  $\alpha$  amylase and enzyme  $\alpha$  glucosidase, stimulating glucose uptake in peripheral tissues besides flavonoids also stimulating *insulin secretagogues* and act like stimulating insulin synthesis of glycogen (*insulin mimetics*) (Brahmachari, 2011; Piparo, 2008; Getha, et al., 2010).

The decrease in KGD in P2 group may occur due to self-healing mechanisms by the body through repair of pancreatic  $\beta$  cells and new cell division (mitosis) that occur gradually. A decrease in the number of pancreatic  $\beta$  cells in hyperglycemic animals began to appear on day 7 and continued to decline until the 28th day (Erwin et al., 2012). The decrease of KGD in P3 and P4 groups had begun to be seen after administration of ethanol extract of squash fruit on the 7th day and returned normal on day 28. This situation indicates that in P3 and P4 the decrease of KGD due to flavonoid effect contained in ethanol extract of peanut pump while in group P2 decrease of KGD start happening on 21st day caused by repair mechanism of pancreatic  $\beta$  cells indicated by improvement of KGD mouse .

The results showed that the pancreatic  $\beta$  cell diameter was different in treatment group P3 and P4, due to flavonoids in ethanol extract of Pumpkin Siam (*S. edule* Jacq Swartz) and actlike an antioxidant by repairing damage of pancreatic tissue due to DNA alkylation by STZ so that secretion increased insulin and decreased blood glucose levels (Suryani et al., 2013).

Based on the results and discussion of the study, it can be concluded that the ethanol extract of the squash fruit 200 mg/kgBB can significantly decrease the blood sugar level of mice, there is difference of pancreatic  $\beta$  cell diameter group given

ethanol extract of squash fruit 200 mg/kgBB compared to ethanol extract group of squash fruit 100 mg/ kgBB and control group.

---

#### REFERENCE

---

- Akbarzadeh AD, Norouzian MR, Mehrabi Sh, Jamshidi, Farhangi, Verdi AA, Mofidian SMA, Rad BL (2007). Induction Of DM By Streptozotocin In Rats. *Indian Journal of Clinical Biochemistry*. 22 (2): 60-64.
- Atalay M, Laaksonen DE (2002). DM, oxidative stress and physical exercise. *Journal of Sports Science and Medicine*. 1: 1-14.
- Brahmachari, Goutam (2011). Bioflavonoids with promising anti-diabetic potentials: A critical survey. *Study signpost*, 6619370:187-212.
- Erwin, Etriwati, Rusli (2012). Mencit (*Mus musculus*) galur balb-c yang diinduk-sikan streptozotosin berulang sebagai hewan model DM. *Jurnal Kedokteran Hewan* 6(1).
- Gethaa, Sahgal, Ramanathan S (2010). Brine Shrimp Lethality and Acute Oral Toxicity Studies on *Swetwnia Mahagoni* (Lin) Jacq. Seed Methanolic Extract. *Pharmacognosy Study*. 2(4): 215-220.
- Moussa SA (2008). Oxidative stress in diabetes mellitus. *Rom J Biophys* 18(3): 225-36.
- Nugroho AE (2006). Animal models of diabetes mellitus: Pathology and mechanism of some diabetogenics. *Biodiversitas, J Biol Divers* 7(4):378-82.
- Park, Han MH, Ji Sook (2012). Hypoglycemic Effect of *Padina arbore-scens* Extract in Streptozotocin-induced Diabetic Mice, *Prev Nutr Food Sci*. 17: 239 - 244.

- Raza, Haider, John A (2012). Streptozotocin-Induced Cytotoxicity, Oxidative Stress and Mitochondrial Dysfunction in Human Hepatoma HepG2 Cells. *Int. J. Mol. Sci.* 13, 5751-5767; doi:10.3390/ijms1305-5751.
- Redha A (2010). Flavonoid: Struktur, Sifat Antioksidatif Dan Peranannya dalam Sistem Biologis. *Jurnal Belian.* 9(2).
- Ridwan A, Astrian RT, Barlian A (2012). Pengukuran Efek Antidiabetes Polifenol (Polyphenon 60) Berdasarkan Kadar Glukosa Darah dan Histologi Pankreas Mencit (*Mus musculus* L.) S.W. Jantan yang Dikondisikan DM. *Jurnal Matematika & Sains.* 17(2).
- Robertson RP, Harmon JS (2007). Pancreatic islet  $\beta$ -cell and oxidative stress: The importance of glutathione peroxidase. *FEBS Lett* 581(19): 3743–8.
- Rohyami Y (2008). Penentuan Kandungan Flavonoid dari Ekstrak Metanol Daging Buah Mahkota Dewa (*Phaleria macrocarpa* Scheff Boerl). *Logika.* 5(1).
- Maity S, Firdous SM, Debnath R (2013). Evaluation of antidiabetic activity of ethanolic extract of *Sechium edule* fruits in alloxan-induced diabetic rats. *World J Pharm Pharm Sci* 2(5): 3612–21.
- Marliana SD, Suryanti V (2005). Skrining fitokimia dan analisis kromatografi lapis tipis komponen kimia buah labu siam (*Sechium edule* Jacq.Swartz.) dalam Ekstrak Etanol. *Biofarmasi* 3(1):26–31.
- Setiawan B, Suhartono E. (2005). Stres Oksidatif dan Peran Antioksidan pada DM. *Majalah Kedokteran Indonesia.* 55(2). Available from [http://mki.idionline.org/index.php?uPage=mki.mki\\_dl&smod=mki&sp=public&key=MTItMTQ](http://mki.idionline.org/index.php?uPage=mki.mki_dl&smod=mki&sp=public&key=MTItMTQ).
- Suryani N, Tinny EH, Aulanni'am (2013). Pengaruh Ekstrak Metanol Biji Mahoni terhadap Peningkatan Kadar Insulin, Penurunan Ekspresi TNF- $\alpha$  dan Perbaikan Jaringan Pankreas Tikus DM. *Jurnal Kedokteran Brawijaya.* 27(3)
- Piparo Elo, Scheib H, Frei N, Williamson G, Grigorov M, Chou CJ (2008). Flavonoids for controlling starch digestion: structural requirements for inhibiting human  $\alpha$ -amylase 3555–61.
- Shradha B, Sisodia SS (2010). DM, Dyslipidemia, Antioxidant And Status Of Oxidative Stress. *International Journal of Study in Ayurveda & Pharmacy,* 1(1).
- Sheikhpour R (2013). Diabetes and oxidative stress : The mechanism and action. *Iran J diabetes Obes* 5(1).
- Siciliano T, De Tommasi N, Morelli I, Braca A (2004). Study of flavonoids of *Sechium edule* (Jacq) Swartz (Cucurbitaceae) different edible organs by liquid chromatography photodiode array mass spectrometry. *Journal of Agricultural and Food Chemistry:* 6510–6515.
- Srinivasan K, Ramarao P (2007). Animal models in type 2 diabetes study: an overview. *Indian J Med Res* 125(3): 451–72.
- SuarsanaIN, Priosoeryanto BP, Bintang M, Wresdiyati T (2010). Profil Glukosa Darah dan Ultrastruktur Sel B Pankreas Tikus yang Diinduksi Senyawa Aloksan. *JITV,* 15(2): 118-123.