

**PJN**

ISSN 1680-5194

PAKISTAN JOURNAL OF  
**NUTRITION**

**ANSI***net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: [editorpjn@gmail.com](mailto:editorpjn@gmail.com)

## Comparative Effect of Tempe and Soymilk on Fasting Blood Glucose, Insulin Level and Pancreatic Beta Cell Expression (Study on Streptozotocin-Induced Diabetic Rats)

Siti Harnina Bintari<sup>1</sup>, Natalia Desy Putriningtyas<sup>2</sup>, Kartika Nugraheni<sup>3</sup>, Nyoman Suci Widyastiti<sup>4</sup>, Edi Dharmana<sup>5</sup> and Andrew Johan<sup>6</sup>

<sup>1</sup>Department of Biology, Faculty of Mathematics and Sciences, Semarang State University, Semarang, Central Java, Indonesia

<sup>2</sup>Magister of Nutrition, <sup>4</sup>Department of Clinical Pathology,

<sup>5</sup>Department of Parasitology, <sup>6</sup>Department of Biochemistry, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia

<sup>3</sup>Department of Nutrition, Faculty of Nursing and Health Science, University of Muhammadiyah, Semarang, Central Java, Indonesia

**Abstract:** Hyperglycemia in diabetes mellitus due to pancreatic beta cell destruction can cause the raising of free radicals production. Soy isoflavone-containing diets have been reported to be beneficial in diabetes because they show potential antioxidant and antihyperglycemia activities. This study was conducted to analyze the difference between isoflavone aglycones in tempe and isoflavone glycosides in soymilk on beta cell function including insulin secretion, fasting blood glucose (FBG) and insulin expression of pancreatic beta cells. Thirty *sprague dawley* (SD) male rats were randomly divided into 3 following groups: (K1) diabetic control (P1) tempe flour 1.8 g (P2) soymilk powder 1.35 g. The treatment were given everyday for 28 days via oral gavage. FBG was measured using the GOD-PAP method, serum insulin was measured using ELISA, insulin expression analysis was done by immunohistochemical. Value of p less than 5% ( $p < 0.05$ ) was considered statistically significant. Tempe flour significantly decrease FBG level better than soy milk and control group ( $p < 0.01$ ). Although both groups showed an increase in serum insulin level after intervention, there was no significant different between them ( $p = 0.639$ ). There were also a significantly decrease in FBG level on soymilk group compared to control ( $p < 0.01$ ). The mean insulin expression on K1, P1 and P2 were  $2.67 \pm 2.34$ ,  $6.17 \pm 1.47$  and  $6.83 \pm 1.17$ , respectively. The insulin expression of both groups were not significantly different ( $p = 0.405$ ). It is concluded that tempe flour shows a better anti-diabetic activity than soymilk.

**Key words:** Tempe, soymilk, diabetes, fasting blood glucose, beta cell expression

### INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases marked by hyperglycemia due to the impairment of insulin secretion, insulin action, or both (American Diabetes Association, 2010; Indonesian Endocrinology Association, 2013; International Diabetes Federation, 2014).

Secondary hyperglycemia due to diabetes is closely related to the damage and impairment to the functions of several associated organs including eyes, kidney and liver, resulting in various complication that influence the patient's quality of life (International Diabetes Federation, 2014; American Diabetes Association, 2012).

Diabetes is a serious chronic disease in Indonesia. Based on data from the IDF in 2013, Indonesia was ranked seventh in the top 10 countries for number of

people with diabetes (20-79 years) and is expected to rise to sixth rank in 2035 (International Diabetes Federation, 2014). The result of Basic Health Research, from Indonesian Ministry of Health in 2007 showed that among the causes of death in Indonesia, diabetes ranks as the second highest cause of death, contributing to 14.7% nationwide (Research and Development Agency of Indonesian Ministry of Health, 2007).

Insulin is produced in specific amount to cater the metabolic requirements during healthy condition. Pancreatic beta cells can detect the change in blood sugar level and respond by releasing insulin. Decreased ability to sense and secrete insulin in diabetics would result in the impairment of carbohydrate, fat and protein metabolism, often reflected by dyslipidemia, which is prevalent among diabetics (Hsu *et al.*, 2003). In a person with diabetes, LDL particles

transform to become smaller and denser as well as to possess atherogenic features (Goldberg, 2001; American Diabetes Association, 2003). To this date, there has not been any pharmacologic agent that could restore the function of insulin to glucose response. Therefore, insulinotropic agent is highly necessary to help blood glucose control in diabetic patients (Gilbert and Liu, 2013). It has been reported that soy isoflavone increases glucose and lipid metabolism through the antidiabetic mechanism of PPAR (Peroxisome Proliferator-Activated Receptor), a receptor regulating gene transcription that plays a role in glucose and lipid metabolism homeostasis in the cell. Soy isoflavone may increase insulin secretion without changing the blood glucose level and may reduce the concentration of adiponectin in the plasma in type 1 DM patients (Kalaiselan *et al.*, 2010). Soy isoflavone extract also proven to improve glucose tolerance in diabetic rats (Shim *et al.*, 2007). Isoflavone genistein can protect the beta pancreatic cells from apoptosis as well as stimulation its regeneration and proliferation (Gilbert and Liu, 2013). During the process of fermentation, the beta-glucosidase enzyme hydrolyzes isoflavone glycoside to form isoflavone aglycone, a more active form of Bavia *et al.* (2012).

Based on these backgrounds, the authors will study the influence of soy administration in two preparation: fermented soy in the form of tempe and unfermented soy in the form of soymilk powder, to the fasting glucose level, plasma insulin level and insulin expression in beta cell in the pancreas.

## MATERIALS AND METHODS

**Study design:** The study is an experimental study with pre-post test randomized controlled group trial design. The study was conducted in the Pusat Antar Universitas (PAU) of Gadjah Mada University, Yogyakarta, Pathology Anatomy Laboratory of Kariadi Hospital, Diponegoro University Semarang, Pathology Anatomy Laboratory of Elisabeth Hospital Semarang and GAKI Laboratory of Faculty of Medicine, Diponegoro University Semarang.

**Tempe and soymilk powder preparation:** The soy used in this study was the local variety soy from Grobogan (small district near Semarang, Central Java). The production of tempe was assisted by one of the tempe maker of Krobokan area in Semarang, who had received the training of hygienic tempe production from the Industry and Cooperative Business Agency of Semarang city, while the production of soy milk and tempe flour was conducted in Salatiga. The study used fresh tempe that has been fermented for 18-24 h using the methods of tempe making according to Bintari (Bintari, 2013). Soy milk powder used in this study was produced using direct oil-free pan fry method (Indonesian P Ministry of Reaserch and Technology, 2013).

The standard of diabetes mellitus diet suggested the intake of 2-3 exchanger unit of vegetable protein a day

(Food exchange list and diet planning for diabetes mellitus, 2007). The study utilized the standard of 3 vegetable-based protein exchanger unit that is equivalent with 150 g of tempe and 75 g of soy (Indonesian Nutritionist Associaton, 2002). The amount was then converted to rat dosage (200 g) to acquire 2.7 g of tempe and 1.35 g of soy. Tempe processing into powder resulted quantitative shrinking to 1/3 of the original weight to produce a dosage of 1.8 g of tempe flour (Bintari, 2007). The isoflavone content in 150 g of tempe is approximately 154.05 and 160.5 mg in 75 g of soy (Nakajima *et al.*, 2005; Song *et al.*, 1998).

**Animals and diets:** The samples used in this study is male *Sprague dawley* rats aged 6-8 weeks with body weight of 170-200 g obtained from PAU UGM. The calculation of sample size was performed using Federer formula and minimum sample size of 9 rats were obtained. The rats were adapted for 7 days and fed with standardized pellet diet. In this study, 30 rats were used and grouped randomly into 3 groups, including:

- K : Diabetic control group with the administration of aquadest
- P1 : First intervention group with the administration of tempe flour at 1.8 g/day dissolved in 3 ml of water
- P2 : Second intervention group with the administration of soymilk powder at 1.35 g/day dissolved in 3 ml of water

**Induction of diabetic rats:** The induction of hyperglycemic rat model in Szkudelski with STZ dosage of 65 g/kg of body weight and NA 230 mg/kg of body weight. SD rats were then weighed then the chosen ones were adapted for 7 days, placed in individual cages with the temperature of 28-32°C, adequate lighting (12 dark/light cycle). SD rats were subjected to 6 hours fasting *ad libitum* and then their blood was drawn from the retro orbital plexus to undergo fasting blood glucose level (FPG). SD rats were induced by STZ intra peritoneally then their FPG level was examined on the fifth day post-STZ induction and rats with blood glucose level of  $\geq 200$  mg/dL were chosen.

**Measurement and analytical procedures:** The blood glucose level measurement was performed during the adaptation time, prior to and after the induction with STZ+NA and once a week during the study. The blood glucose level was measured with the GOD-PAP method using blood serum sample. The lipid profile examination was measured using the CHOD-PAP and GPO-PAP methods. The measurement of serum insulin level was performed before and after the intervention ends. Insulin level was measured using ELISA method in the GAKI Laboratory of Faculty of Medicine, Diponegor University Semarang using blood serum samples. The reagent DRG Insulin ELISA Kit with catalogue number EIA-2048 was used for insulin level examination.

**Pancreatic biopsy:** The working principle of this method is staining pancreatic beta-cell for histologic analysis using the reagents mouse monoclonal antibody insulin primary antibody (beta subunit Ab-5, Clone: INSO5/2D11-H5) Cat.#1378 from NeoMarker Lab Vision. Allred score calculation was performed manually using 400 time magnification in minimum field of 10 Langerhans island while counting the proportion score and intensity score. Allred score is the sum of proportion and intensity score ranged 0-8 (Allred, 1998). Readings were performed by two independent experts.

**Statistical analysis:** Data was analyzed using one way ANOVA and expressed in mean±SE. Statistical analyses were performed using SPSS Statistic 20. p-value ≤0.05 was considered significant.

## RESULTS

There was a significant difference in initial body weight and body weight gain in experimental group during the experiment ( $p < 0.01$ ). Body weight in control group decreased significantly ( $p < 0.01$ ) during the treatment (Table 1).

The fasting blood glucose level in tempe and soy milk groups underwent significant reduction each week ( $p < 0.01$ ). However, the reduction of blood glucose level in the tempe group was more significant than the reduction of blood glucose in the soy milk group ( $p < 0.01$ ). The control group that did not receive any intervention has an increase of blood glucose level each week ( $p < 0.01$ ) (Table 2).

The fasting insulin level in the control group was increased, although the increase was not statistically significant ( $p = 0.118$ ) (Table 3). The intervention group also demonstrated significantly increased fasting insulin level after tempe administration ( $p = 0.007$ ) and soy milk ( $p = 0.003$ ). However, there was no significant difference between the increase of fasting insulin level in the tempe group compared to the soy milk group ( $p = 0.0607$ ). Based on Allred Score, The highest mean ( $6.83 \pm 1.17$ ) of results was obtained in the group administered with soymilk (Table 4).

The expression of insulin from pancreatic beta cells between the tempe flour group and the soy milk group showed no significant difference ( $p = 0.405$ ). The results showed that both the administration of soy tempe flour with the dose of 1.8 g/200 g body weight and the administration of soy milk with the dose of 1.35 g/200 g body weight for 28 days provided the same influence in fixing the damage to pancreatic beta cell due to STZ induction (Table 5).

The results of ANOVA analysis in the three groups for pancreatic beta-cell insulin expression showed p value of  $< 0.01$  that could be concluded that there is a difference of beta cell insulin expression of pancreatic beta cells between the three study groups (Table 6).

The significant group in this study is the tempe flour intervention group with the control group ( $p = 0.006$ ) and

the soy milk intervention group with control group ( $p = 0.001$ ) (Table 7).

Table 1: Initial and final body weight of the control and experimental groups

	Control	Tempe	Soymilk
Initial weight (g)	179.50±17.43	172.50±6.11	173.50±2.89
Final weight (g)	167.50±17.27	193.83±6.34	193.17±19.82

## DISCUSSION

Soy is one of the beans species originated from East Asia. For Asian population, it is the dietary protein source to supplement the animal protein sources (Allred, 1998). Epidemiologic studies showed that soy consumption or specific complement of soy is beneficial for health due to its potential effects in preventing diseases associated with lifestyle like cardiovascular diseases, several types of cancer, osteoporosis and even menopausal symptoms in middle-aged women. Several soy components, like protein, isoflavone, oligosaccharides and sterol have been proven as FOSHU (Food for Specified Health Uses) by the Japanese Health Department. FOSHU is functional food with a license for health claims (Sugano, 2005). As with the Japanese, FDA has also allow several soy components with proven positive health effects to be mentioned in product wrappings (Yang *et al.*, 2011).

Tempe is the product of soy fermentation with the help of the microorganism *Rhizopus oligosporus*. Tempe has been consumed by major of Indonesian population for more than 500 years and acknowledged as a national food (Yang *et al.*, 2011; Kwon *et al.*, 2010). Soy fermentation increases the activity of antioxidants. The type and concentration of isoflavone produced during the fermentation process depends on the inoculum being utilized. The most commonly used inoculum include *Rhizopus sp*, *M. luteus* and *Bacillus epidermis* (Nout and Kiers, 2005).

During the fermentation, the enzyme beta-glucosidase hydrolyses isoflavone glycoside into isoflavone aglycone, a more active form of the substance (Bavia *et al.*, 2012). Soy isoflavone is a group of active biologic substances with estrogen-like chemical structure. Several epidemiological researches have associated high isoflavone consumption with decreased risk of having diabetes and diabetes-related complications, including cardiovascular diseases. The evidence demonstrated that soy isoflavone has antihyperlipidemic effect through antioxidant mechanism, a function similar to that of estrogen and involved in the genes regulating lipogenesis and lipolysis (Lu *et al.*, 2008).

Blood glucose level is also one of the indicators of hyperglycemia. STZ-induced rats demonstrated increased blood glucose level. STZ has a selective toxicity towards pancreatic cells and does not damage other endocrine cells or exocrine parenchyma. SD rats would have their blood analyzed on day 5 after STZ induction to measure the fasting blood glucose and if the results showed the value of  $\geq 200$  mg/dL, the SD rat

Table 2: Fasting serum glucose and insulin level of the 3 groups after treatment for 4 weeks

Groups	DM	Glucose (mg/dL)			
		1st week	2nd week	3rd week	4th week
Control	218.68±7.11	224.96±2.40	22.66±2.71	229.10±2.46	230.60±2.43
Tempe flour	215.52±3.19	205.58±3.18	165.20±3.32	117.31±2.61	88.73±2.35
Soymilk	216.40±8.73	207.16±8.65	182.09±8.92	153.52±7.95	135.06±7.59

Table 3: Fasting serum insulin before and after treatment

	Control	Tempe flour	Soymilk
Insulin pretest (ng/mL)	0.19±0.28	0.13±0.28	0.12±0.83
Insulin posttest (ng/mL)	0.22±0.18	0.24±0.22	0.23±0.67

Table 4: Table of analysis of the expression of insulin in beta-pancreatic cells in the groups

Groups	N	Allred score (PS+IS)			p*
		Minimum	Maximum	Mean	
Control	6	0.00	6.00	2.67±2.34	0.421*
Tempe flour	6	4.00	8.00	6.17±1.47	0.804*
Soymilk	6	5.00	8.00	6.83±1.17	0.421*

Table 5: Results of insulin expression difference test of beta-pancreatic cells in the intervention group

Groups	N	Allred score (PS+IS)			p
		Min.	Max.	Mean	
Tempe flour	0.19±0.28	6	4.00	8.00	6.17±1.47
Soymilk	0.22±0.18	6	5.00	8.00	6.83±1.17

\*Independent test in two groups. Min.: Minimum. Max.: Maximum

Table 6: Test results of the difference of pancreatic beta cells insulin expression in all groups

Groups	Allred score (PS+IS)			p
	Control	Tempe flour	Soymilk	
TS Allred score	2.67±2.34	6.17±1.47	6.83±1.17	0.000*

\*Significant one way ANOVA test

Table 7: Post hoc test result for beta-pancreatic ceell insulin expression between the groups

Groups	p
Control-tempe flour	0.006*
Control-Soymilk	0.001*
Tempe flour-Soymilk	0.562

\*LSD test, significant

would be determined as hyperglycemic (Szkudelski, 2012; Rachmawati, 2012). Fasting blood glucose examination in this study is performed to monitor the development of SD rats during the intervention.

STZ induction in this study is performed to create a model of type 1 diabetes mellitus mediated by the activity of immune system and cause delayed onset diabetes through beta-cell damages and immunologic injury. STZ administration damages the pancreatic beta cells, while NA administration is aimed to protect insulin secretion in balancing the action of STZ as a diabetogenic agent (Szkudelski, 2012).

STZ will be transported by beta cells through GLUT-2 and cause DNA damage and consequently increase the activity of poly ADP ribose polymerase (PARP-1) to repair DNA. The overactivity of PARP-1 results in intracellular depletion of NAD<sup>+</sup> and ATP and hypersecretion of insulin would result in necrosis. NA administration plays a role in the inhibition of PARP-1 and as the precursor NAD<sup>+</sup> and ATP exposed by STZ (Szkudelski, 2012).

STZ can cause the deaths of pancreatic beta cells through DNA alkylation, causing the DNA to break because of the activation of poly ADP-ribocylation, triggering the cellular NAD<sup>+</sup> depletion and reducing ATP content. This condition causes the inhibition of synthesis and secretion of insulin, then increasing the blood glucose level (Szkudelski, 2001).

High blood glucose level will increase oxidative stress through both enzymatic and non-enzymatic process. Changes in protein function, for example NAPH oxidase to disrupt and damage the cellular function and produce *reactive oxygen intermediates* that could oxidize LDL, occurred during the enzymatic process while non-enzymatic process will change gene expression (growth factor and cytokine) and disrupt antioxidant defenses (increasing oxidative stress) that eventually cause beta cell damage (Sheetz and King, 2002).

There are four isoflavone forms in soy, including (1) aglycone shapes (free form) including genistein, daidzein and glisitein, (2) glycosides including genistin, daidzin and glisetin, (3) acetyl glycosides including 6''-O-asetilgenistin, 6''-O-asetildaizdin and 6''-O-asetilglisetin, (4) malonilglycosides including 6''-O-malonilgenistin, 6''-O-malonildaizdin, 6''-O-malonilglisetin. Current studies have shown that isoflavone possesses antidiabetic activity through the glucose uptake inhibition in the intestinal brush border, the activity of alpha-glucosidase, tirosin kinase inhibitor and PPAR-alpha and PPAR-gamma agonist (Cheng *et al.*, 2004).

The influence of soy isoflavone to the reduction of blood sugar level is thought to be caused by the stimulus of genistein to the beta pancreatic cells to increase the production of insulin to make it capable of increasing glucose uptake by cells (Esteves *et al.*, 2011). The influence is proven by the administration of genistein to experimental animals and *in vitro* studies using adipocytes and insulinoma cells, showing that the bioactive components formed from isoflavoneoid during fermentation process may activate a series of signals to stimulate insulin release (Kwon *et al.*, 2011; Jonas *et al.*, 1995).

Lee and Lee (2001) stated that the hypoglycemic effect of isoflavone to the blood glucose control in cases of diabetes is through the mechanism of alpha-glucosidase inhibitor in the intestinal brush border (Lee and Lee, 2001).

Lee's study (2006) to DM mice with the diet supplementation of genistein at 600 mg/kg and protein isolates at 200 mg/kg showed significant results to the reduction of blood glucose level and increased insulin level (Lee, 2006). Alpha-glucosidase is the main enzyme in the process of carbohydrate digestion. alpha-

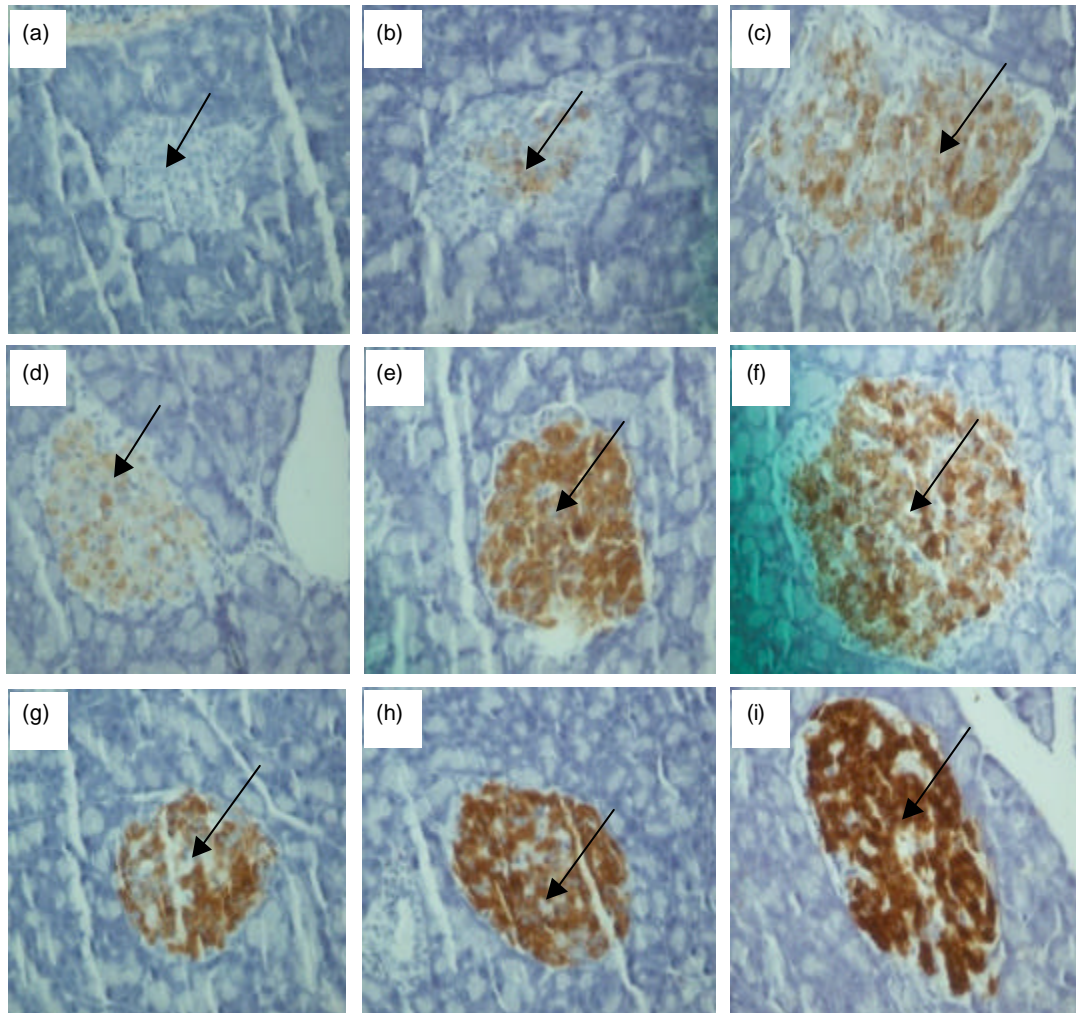


Fig. 1: Histopathologic figure of Allred score with Ab staining. Insulin, 400x magnification. (a) INS negative in the K1 group, Allred score 0 (-+0), (b) INS positive for K1, Allred score 3 (2+1), (c) INS positive in K1, Allred score 6 (2+4), (d) INS positive, beta-cell cytoplasm with brown staining in P1 group, Allred score 4 (2+2), (e) INS positif, beta-cell cytoplasm with brown staining in P1 group, Allred score 6 (3+3), (f) INS positif, beta-cell cytoplasm with brown staining in P1 group, Allred score 7 (4+3), (g) INS positif, beta-cell cytoplasm stained with brown staining in P2 group, Allred score 5 (3+2), (h) INS positif, beta-cell cytoplasm stained with brown staining in P2 group, Allred score 7 (3+4) and (i) INS positif, beta-cell cytoplasm with brown staining in P group, Allred score 8 (3+5)

glucosidase plays a role as the catalisator of hydrolysis for 1,4-alpha-glycosides bonding existing in carbohydrate and releases the alpha-glucose chain and causing increased blood glucose level. Alpha-glucosidase inhibitor is antagonistic to the activity of alpha-glucosidase, thus it may delay the absorption of carbohydrate and suppress the increase of blood glucose level (Wu *et al.*, 2012). The results of McCue *et al.* study (2005) showed that soy fermented by *Rhizopus oligosporus* yeast has a higher anti-alpha-glucosidase compared to other soy products fermented by *Lentinus edodes* yeast (McCue *et al.*, 2005).

The absorption of carbohydrate and glucose is the main target in blood glucose control. alpha-amylase and alpha-glucosidase are the main enzymes responsible for carbohydrate metabolism to form glucose. Glucose is absorbed from intestinal enterocytes by certain transporters. The inhibition of digestive enzymes may reduce glucose release and absorption in the small intestines, which would result in the decrease of postprandial hyperglycemia (Hanhineva *et al.*, 2010).

The mechanism of blood glucose level reduction by soy isoflavone, predominantly genistein and daidzein also

occurs through the peroxisome-proliferator activated receptor (PPAR). The activation of PPAR-gamma by genistein and daidzein causes an increase in gene expression that functions to code GLUT-4 and induce the upregulation of GLUT-4 needed as glucose transporter to enter the cells and utilized as energy source (Kavanagh *et al.*, 2008; Cho, 2006). The difference between fasting blood glucose reduction between the tempe flour group and soy milk group is caused by the form of isoflavone compound in both of these groups. Isoflavone aglycone in tempe flour has a higher bioavailability compared to isoflavone glycosides in soy milk.

Isoflavone is absorbed by the body in the form of aglycone. The changes in isoflavone glycosides to aglycone in tempe occurs during the fermentation process with the help of beta-glucosidase enzyme originated from *Rhizopus oligosporus*. Isoflavone aglycone can be detected in the blood 30 min after ingestion and reach its peak level 1 hour post-ingestion (Kwon *et al.*, 2011; Day *et al.*, 2000; Cassidy *et al.*, 2006; Larkin *et al.*, 2008).

Isoflavone glycosides in soy milk can be changed into aglycone form with the help of beta-glucosidase enzyme and *lactase phlorizin hydrolase* (LPH) enzyme contained in the digestive system, especially in small intestines. The conversion of isoflavone glycosides into aglycone require 2-4 h post-ingestion. Isoflavone glycosides reaches its peak level 4-8 h post-ingestion. The study by Piskula *et al.* (1999) suggested that genistein and daidzein is absorbed more easily with the plasma concentration 4 times higher than the glycosides form. This proves that isoflavone aglycone in tempe flour is more effective in reducing fasting blood glucose level compared to isoflavone glycosides in soy milk (Hanhineva *et al.*, 2010; Day *et al.*, 2000; Cassidy *et al.*, 2006; Larkin *et al.*, 2008; Piskula *et al.*, 1999).

The decrease in the sensitivity and secretion of insulin resulted in the blood glucose level disorder. To date, there has not been any pharmacologic agent that could restore the function of insulin response to the glucose response, thus the insulinotropic agent is urgently needed to help control blood glucose level in diabetic patients (Gilbert and Liu, 2013). Genistein and daidzein increase insulin secretion in the administration of certain dosage. Liu *et al.* (2006) suggested high-soy-isoflavone diet (0.334 g/kg diet) may increase the production of insulin (Lu *et al.*, 2008). Soy isoflavone protects the beta pancreatic cells from glucose toxicity and improve the function of beta pancreatic cells. Preventing beta pancreatic cells from further damage may increase insulin secretion. The improvement of serum insulin level in diabetic rats administered with soy products may also be used by other insulinotropic substances contained in soy, stimulating insulin

secretion from the beta cells. Lee (2006) stated that genistein supplementation and soy protein in diabetic rats may improve insulin production (McCue *et al.*, 2005). Soy isoflavone protects pancreatic beta cells from blood glucose level. Hypoglycemic effects from genistein and soy protein are derived from serum insulin level and improvement of periphery glucose metabolism.

Liu *et al.* (2006) and Fu *et al.* (2010) suggested that genistein modulates insulin secretion through the cAMP/PKA activation mechanism. Genistein induces the proliferation of INS1 and pancreatic beta cell islets through the signal of cAMP/PKA-dependent ERK1/2 activation pathway. *Cyclic adenosine monophosphate* (cAMP) is the central molecule in several cellular system and plays important role in insulin secretion. The accumulation of intracellular cAMP increases glucose-mediated insulin secretion through the activation of protein kinase A (PKA). Extracellular-regulated protein kinases (ERK1/2) plays a role in cellular responses stimulated by environment, like proliferation, differentiation and apoptosis. The increase of intracellular cAMP activates ERK1/2, causing the beta cells to proliferate and protect itself from apoptosis that eventually increase insulin secretion (Liu *et al.*, 2006; Fu *et al.*, 2010).

The recovery of beta cell function is expected to derive from the improvement of blood glucose control, because hyperglycemia may interrupt with insulin response. In diabetes mellitus type 1 cases, the improvement of insulin secretion is presumed to be preceded by the improvement of blood glucose control. The improvement of pancreatic beta cell function in type 1 diabetes mellitus cases may be initiated by improved blood glucose control that impacts the increase of insulin secretion and vice versa (Steele *et al.*, 2004).

Insulin secretion by pancreatic beta cells involves several reaction that is a potential target to the role of isoflavone in diabetes mellitus. In the condition of high blood glucose level, pancreatic beta cells respond to the increase of insulin requirements through several mechanism, including increasing insulin secretion. The release of insulin from pancreatic beta cells is one of the chain reaction initiated by glucose uptake by GLUT-2 (Hanhineva *et al.*, 2010).

Isoflavone as one of the flavonoids has a high antioxidant activity. Gilbert and Liu (2013) suggested that the role of isoflavone genistein to the function of pancreatic beta cells is caused by the ability of genistein to stimulate regeneration of proliferation of beta cells. In diabetes, ROS is produced predominantly through the non- enzymatic glycation reaction (AGEs) that can occur to several tissues and cause complications in diabetes (Gilbert and Liu, 2013). Blood glucose level reduction due to antioxidant causes the decrease of glucose toxicity and prevent the decrease of beta cell mass and insulin secretion (Kaneto *et al.*, 1999).

The result of King and Bursill (1998) and Fu and Liu (2006)'s study suggested that genistein exposure for a relatively long period through daily good consumption may increase the insulin secretion function from pancreatic beta cells. The result of this study is in support of those studies, where the administration of tempe flour and soy milk as one of the source of genistein may help improving the function of pancreatic beta cells in diabetes (King and Bursill, 1998; Fu and Liu, 2009).

**Conclusion:** Overall, both tempe and soymilk can help lower blood sugar level, increase insulin secretion and improve pancreatic beta-cells. Tempe, which known as Indonesian traditional fermented soybean, has a better antidiabetic effect than soymilk because isoflavone aglycones found in tempe are biologically more active than isoflavone glycosides in soymilk. However, more studies are needed to better understand the exact effect of fermented soybean on diabetes.

#### ACKNOWLEDGEMENT

This study partially supported by The *Unggulan* Scholarship from Indonesian Ministry of Education and Culture.

#### REFERENCES

- American Diabetes Association, 2010. Standard of medical care in diabetes. *Diabetes Care*, 1: 11-61.
- American Diabetes Association, 2012. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 1: 64-71.
- American Diabetes Association, 2003. Management of dyslipidemia in adults with diabetes. *Diabetes Care*, 26: 83-86.
- Allred, D.C., 1998. Scoring immunostained slides. *Modern Pathol.*, 11: 155-168.
- Bavia, A.C.F., C.E. Da Sila, M.P. Ferreira, L.R. Santos, J.M.G. Mandarino and M.C. Carrao-Panizzi, 2012. Chemical composition of tempeh from soybean cultivars specially developed for human consumption. *Science Technol. Aliment*, 32: 613-620.
- Bintari, S.H., 2013. Pasteurization for hygienic tempe. *GSTF J. Bio.*, 2: 39-44.
- Bintari, S.H., 2007. Isoflavone effect in proliferation and apoptosis breast cancer cell on mice (*Mus musculus*) strain C<sub>3</sub>H with AgNORs, p53, Cas-3 and Bcl-2 parameters [dissertation]. [Semarang]: Diponegoro University. (In Bahasa Indonesia).
- Cheng, S.Y., N.S. Shaw, K.S. Tsai and C.Y. Chen, 2004. The hypoglycemic effects of soy isoflavones on postmenopausal women. *J Women Health*, 13: 1080-1086.
- Cho, K.W., 2014. Molecular mechanisms of anti-diabetic effects of soy isoflavones [dissertation on the internet]. [Indiana (USA)]: Purdue University; 2006 [cited 2014 June 6]. Available from: <http://docs.lib.purdue.edu/dissertations/AAI3251598/>.
- Cassidy, A., J.E. Brown, A. Hawdon, M.S. Faughnan, L.J. King and J. Millward *et al.*, 2006. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *J. Nutr.*, 136: 45-51.
- Day, A.J., F.J. Canada, J.C. Diaz, P.A. Kroon, R. Mclauchlan and C.B. Faulds *et al.*, 2000. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Lett.*, 468: 166-170.
- Esteves, E.A., J. Bressan, N.M. Costa, H.S. Martino, S.S. Donkin and J.A. Story, 2011. Modified soybean affects cholesterol metabolism in rats similarly to a commercial cultivar. *J. Med. Food*, 14: 1363-1369.
- Food exchange list and diet planning for diabetes mellitus, 2007. In: S. Waspadji, K. Sukardji, M. Octarina, editors. *Diet guidance for diabetes mellitus* 1st ed. Jakarta: Balai Penerbit FKUI, 2007. (In Bahasa Indonesia).
- Fu, Z., W. Zhang, W. Zhen, H. Lum, J. Nadler and J. Bassaganya-Riera *et al.*, 2010. Genistein induces pancreatic beta-cell proliferation through activation of multiple signaling pathways and prevents insulin-deficient diabetes in mice. *Endocrinol.*, 151: 3026-3037.
- Fu, Z. and D. Liu, 2009. Long-term exposure to genistein improves insulin secretory function of pancreatic beta-cells. *Eur. J. Pharmacol.*, 616: 321-327.
- Goldberg, I.J., 2001. Diabetic dyslipidemia: causes and consequences. *J. Clin. Endocrine Metab.*, 86: 965-971.
- Gilbert, E.R. and D. Liu, 2013. Anti-diabetic functions of soy isoflavone genistein: mechanism underlying its effects on pancreatic beta-cell function. *Food Funct.*, 4: 200-212.
- Hsu, C.S., W.C. Chiu and S.L. Yeh, 2003. Effects of soy isoflavone supplementation on plasma glucose, lipids and antioxidant enzyme activities in streptozotocin- induced diabetic rats. *Nutr. Res.*, 23: 65-75.
- Hanhineva, K., R. Torronen, I. Bondia-Pons, J. Pekkinen, M. Kolehmainen and H. Mykkanen *et al.*, 2010. Impact of dietary polyphenols on carbohydrate metabolism. *Int. J. Mol. Sci.*, 11: 1365-1402.
- Indonesian Endocrinology Association, 2013. Consensus of management and prevention of diabetes mellitus type 2 in Indonesia [Internet]; [cited 2013 June 6]. Available from: <http://perkeni.org/download/Konsensus%20DM%202011.zip> (In Bahasa Indonesia).
- International Diabetes Federation, 2014. *IDF diabetes atlas* 6th edition [Internet]; [cited 2014 Nov 9]. Available from: [www.idf.org/diabetesatlas](http://www.idf.org/diabetesatlas).
- Indonesian P Ministry of Reaserch and Technology, 2013. Soybean powder for drink [Internet]; [cited 2013 Apr 7]. Available from: [http://www.warintek.ristek.go.id/pangan\\_kesehatan/pangan/piwp/bubuk\\_kedelai.pdf](http://www.warintek.ristek.go.id/pangan_kesehatan/pangan/piwp/bubuk_kedelai.pdf) (In Bahasa Indonesia).



- Indonesian Nutritionist Association, 2002. Department of Nutrition Cipto Mangunkusumo Hospital. Food exchange list, Jakarta: Gramedia Pustaka Utama. (In Bahasa Indonesia).
- Jonas, J.C., T.D. Plant, P. Gilon, P. Detimary, M. Nenguin and J.C. Henguin, 1995. Multiple effects and stimulation of insulin secretion by the tyrosine kinase inhibitor genistein in normal mouse islets. *Br. J. Pharmacol.*, 114: 872-880.
- Kalaiselan, V., M. Kalaivani, A. Vijayakumar, K. Sureshkumar and K. Venkateskumar, 2010. Current knowledge and future direction of research on soy isoflavones as a therapeutic agents. *Pharmacogn Rev.*, 8: 111-117.
- Kwon, D.Y., J.W. Daily 3rd, H.K. Kim and S. Park, 2010. Antidiabetic effects of fermented soybean products on type 2 diabetes. *Nutr. Res.*, 30: 1-13.
- Kwon, D.Y., S.M. Hong, I.S. Ahn, M.J. Kim, H.J. Yang and S. Park, 2011. Isoflavonoids and peptides from meju, long-term fermented soybeans increase insulin sensitivity and exert insulinotropic effects *in vitro*. *Nutr.*, 27: 244-252.
- Kavanagh, K., K.L. Jones, L. Zhang, D.M. Flynn, M.K. Shadoan and J.D. Wagner, 2008. High isoflavone soy diet increases insulin secretion without decreasing insulin sensitivity in premenopausal nonhuman primates. *Nutr. Res.*, 28: 368-376.
- Kaneto, H., Y. Kajimoto, J. Miyagawa, T. Matsuoka, Y. Fujitani and Y. Umayahara *et al.*, 1999. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes*, 48: 2398-2406.
- King, R.A. and D.B. Bursill, 1998. Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. *Am. J. Clin. Nutr.*, 67: 867-72.
- Larkin, T., W.E. Price and L. Astheimer, 2008. The key importance of soy isoflavone bioavailability to understanding health benefits. *Crit. Rev. Food Sci. Nutr.*, 48: 538-552.
- Lu, M.P., R. Wang, X. Song, R. Chibbar, X. Wang and L. Wu *et al.*, 2008. Dietary soy isoflavones increase insulin secretion and prevent the development of diabetic cataracts in streptozotocin-induced diabetic rats. *Nutr. Res.*, 28: 464-471.
- Lee, D.S. and S.H. Lee, 2001. Genistein, a soy isoflavone, is a potent alpha-glucosidase inhibitor. *FEBS Lett.*, 501: 84-86.
- Lee, J.S., 2006. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats. *Life Sci.*, 79: 1578-1584.
- Liu, D., W. Zhen, Z. Yang, J.D. Carter, H. Si and K.A. Reynolds, 2006. Genistein acutely stimulates insulin secretion in pancreatic beta-cells through a cAMP-dependent protein kinase pathway. *Diabetes*, 55: 1043-1050.
- McCue, P., Y.I. Kwon and K. Shetty, 2005. Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean. *Asia Pac. J. Clin. Nutr.*, 14: 145-152.
- Nakajima, N., N. Nozaki, K. Ishihara, A. Ishikawa and H. Tsuji, 2005. Analysis of isoflavone content in tempeh, a fermented soybean and preparation of a new isoflavone-enriched tempeh. *J. Biosci. Bioeng.*, 100: 685-687.
- Nout, M.J. and J.L. Kiers, 2005. Tempe fermentation, innovation and functionality: update into the third millenium. *J. Appl. Microbiol.*, 98: 789-805.
- Piskula, M.K., J. Yamakoshi and Y. Iwai, 1999. Daidzein and genistein but not their glucosides are absorbed from the rat stomach. *FEBS Lett.*, 447: 287-291.
- Research and Development Agency of Indonesian Ministry of Health, 2007. Basic Health Research (RISKESDAS) National Report 2007 [Internet]; [cited 2013 June 6]. Available from: <https://www.k4health.org/sites/default/files/laporanNasional%20Riskesdas%202007.pdf> (In Bahasa Indonesia).
- Rachmawati, B., 2012. Suplementasi folate, kadar homocysteine, nitric oxide dan petanda retinopati diabetik studi pada tikus sprague dawley diabetes. *M. Med. Indon.*, 46: 100-107. (In Bahasa Indonesia).
- Shim, J.Y., K.O. Kim, B.H. Seo and H.S. Lee, 2007. Soybean isoflavone extract improves glucose tolerance and raises the survival rate in streptozotocin-induced diabetic rats. *Nutr. Res. Pract.*, 1: 266-272.
- Song, T., K. Barua, G. Buseman and P.A. Murphy, 1998. Soy isoflavone analysis: quality control and a new internal standard. *Am. J. Clin. Nutr.*, 68: 1474-1479.
- Sugano, M., 2005. Nutritional implications of soy. In: Sugano M, editor. *Soy in health and disease prevention*. Boca Raton: CRC Press.
- Szkudelski, T., 2012. Streptozotocin-nicotinamide-induced diabetes in the rat characteristics of the experimental model. *Exp. Biol. Med.*, 237: 481-490.
- Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol. Res.*, 50: 537-546.
- Sheetz, M.J. and G.L. King, 2002. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA*, 288: 2579-2588.
- Steele, C., W.A. Hagopian, S. Gitelman, U. Masharani, M. Cavaghan and K.I. Rother *et al.*, 2004. Insulin secretion in type 1 diabetes. *Diabetes*, 53: 426-433.
- Wu, C., J. Shen, P. He, Y. Chen, L. Li and L. Zhang *et al.*, 2012. The alpha-glucosidase inhibiting isoflavones isolated from *Belamcanda chinensis* leaf extract. *Rec. Nat. Prod.*, 6: 110-120.
- Yang, B., Y. Chen, T. Xu, Y. Yu, T. Huang and X. Hu *et al.*, 2011. Systematic review and meta-analysis of soy products consumption in patients with type 2 diabetes mellitus. *Asia Pac. J. Clin. Nutr.*, 20: 593-602.