THE EXTRACT SOYBEAN OF LOCAL VARIETIES AS REDUCE ADVANCED GLYCATION END PRODUCTS AND HEMOGLOBIN A1C IN TYPE 2 DIABETES MICE

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INTRODUCTION

The data from a global study, in 2011, showed that the number of diabetes mellitus (DM) patients had reached 366 million and is expected to increase to 552 million by 2030. DM prevalence in Indonesia is rising from year to year, and many people are still in productive age. Diabetic complications are the systemic vascular disease (accelerated atherosclerosis), cardiovascular disease, the microvascular disease in the eye as a cause of blindness and retinal degeneration (diabetic retinopathy) [1], cataracts, and renal impairment as a cause of renal failure and diabetic neuropathy [2]. The prevalence of Type 2 diabetes (T2D) patients is quite large, usually due to the relative failure of β-cells and insulin resistance, especially in people with underweight or obese. Usually once diabetes is detected, this syndrome has developed and there have been one or two complications. Complications in diabetes seem to correlate with blood glucose concentration so that excessive glucose is suspected to be a major cause of tissue damage [3]. This phenomenon is caused by the condition of hyperglycemia in vivo role in the process of free radical formation [4]. Hyperglycemia causes auto-oxidation of glucose, protein glycation, and activation of polypeptide metabolism pathways which further accelerate the formation of reactive oxygen compounds [5,6].

Soybean is a type of plant with polyphenol compounds that are reported to have various biological activities, which are beneficial to health. Certain components of soybean, especially isoflavones, saponins, Vitamin C, and tocopherols, are associated with the protective effect of oxidative stress. Food intake of soybean seeds is known to reduce risk factors for chronic diseases. Certain components of soybean, especially isoflavones, saponins, Vitamin C, and tocopherols, are associated with the protective effect of oxidative stress [13].

Christyaningsih proved that soy extract can lower pollutants levels and lipid peroxidation in the blood [14]. Yulia using other varieties of soybean suggests varying biological activity in decreasing lipid peroxidation activity, potentially as an antioxidant [15]. Further, research conducted by Yulia proving of several varieties of soybean seeds that exist, Argomulyo varieties provide the highest antioxidant effect [16]. The high content of isoflavones in soybeans has also proven to be significantly beneficial for postmenopausal women in terms of increasing levels of collagen, elasticity, and skin epithelial thickness [17]. In addition to isoflavones, soybean seeds of Detam-2 variety proved to contain other active antioxidant compounds hexadecanoic acid methyl ester and hexadecanoic acid ethyl ester. Isoflavones in soybeans are also shown to inhibit bone resorption and stimulate bone formation in menopausal women [18,19]. This study aims to analyze the effect
of soybean extract formulation (Glycine max [L.] Merr.) on AGEs and HbA1c. The use of this experimental animal was declared ethical by the Research Ethics Committee of Health Polytechnics, Surabaya.

MATERIALS AND METHODS
This study has an experimental design of study and randomized pretest-posttest control group design. The experimental animals used were mice Balb/C, male, 10-week-old, weighing 20–35 g, healthy physically with clear-eyed features, glossy fur, and active motion, obtained from the Biochemistry Laboratory, Airlangga University. The use of this animal has been authorized by the Research Ethics Committee of Politeknik Kesehatan Kemenkes Surabaya. The animals were acclimatized for about 1 week, given high carbohydrate and lipid feed for 2 weeks and drinking water ad libitum.

Preparation of sample
T2D animal models: 28 male mice were fasting for ±4 h and then the mice measured blood glucose levels and body weight. The next 2 h (after the tail injury dries), the mice were injected with streptozotocin solution at 55 mg/kg BW intraperitoneally, then the mice were fed and drank 10% dextrose solution ad libitum then left in the cage for 2 days. On the 3rd day, after the mice were fasting ±4 h, measured blood glucose levels. The results of blood glucose measurements showed that the mice had been conditioned to T2D [20]. The mice were randomly assigned to seven treatment groups with four replications, i.e. four control groups and three groups of soybean extract treatment. During the treatment, the mice were fed and drank ad libitum and on day 12th, measurements of blood glucose, HbA1c, and AGE levels were measured.

Extraction
Soybeans were obtained from the Research Institute of various beans and tubers, Malang, East Java, with varieties of Wilis, Gema, and Argomulyo. The soybean seeds obtained from Baltikabi, Malang, are certified and harvested at the same plant age. The soybean seeds are ground with a blender, and the powder that has been obtained is then sieved with a mesh size of 20 to form a finer and more uniform powder. Soybean seed powder that has been sieved then weighed to determine the weight. Eliminated fat content with hexane (pa) using kinetic maceration tool [21]. The extraction results were concentrated using a rotary evaporator. The concentration was obtained with water bath electric until the extract was obtained.

Soy extract dissolved in Na- CMC with equal doses of 1 g of soybeans (Anjasmoro/ Detam II/ Argomulyo)/kg BW/oral/day/mice, given in the morning with NGT feeding tube [22]. At the end of the study, the mice were sacrificed by cervical dislocation; their blood was drawn and surgically removed for liver and pancreatic tissue.

The analysis of AGEs, Oxiselect™ AGE competitive ELISA kit, cat. no STA-817
This test uses the technique of immunoassay enzyme competitive inhibition (ELISA). A monoclonal antibody specific to AGEs labeled on a microplate. Competitive inhibition reactions between biotin labeled AGEs and those not labeled AGEs (found on standards or samples) with antibodies coated specifically for AGEs. After incubation, the unbound conjugate will be separated during washing. Next, add the conjugated Avidin-horseradish peroxidase (HRP) to each well on the microplate and then incubate. The number of conjugated HRP bonds is inversely proportional to the concentration of AGEs in the sample. After addition of the substrate solution, the measured color intensity is inversely proportional to the concentration of AGEs in the sample, which is read in the ELISA Reader tool.

The analysis of HbA1c kit Sigma-Aldrich cat. code: BCR405RM
Hb glycosylate or HbA1c can be measured by several methods such as affinity chromatography, electrophoresis, immunoassay, or boronate affinity method. Specimens used for the measurement of HbA1c: Capillary or venous blood with anticoagulants (EDTA, citrate, or heparin).

The location of the research at Biochemistry Laboratory of Airlangga University and the analysis examination was held at Research Institute of Media Husada Surabaya Corp., Laboratory of Pharmacy Faculty, Airlangga University, Surabaya.

RESULTS
A total of 28 mice were divided into seven groups with the following details:
1. Negative control: Normal mice with induction of soy extract
2. Pre-test: T2D mice
3. Placebo control: T2D mice with solvent induction (CMC Na)
4. Positive control: T2D mice with metformin
5. Test 1: T2D mice with Wilis variety of soybean extract induction
6. Test 2: T2D mice with Gema variety of soybean extract induction
7. Test 3: T2D mice with Argomulyo variety of soybean extract induction

After the treatment and observation of the animal, try in accordance with the design made then obtained the data in Fig 1.

The average of blood glucose in mice before the diabetic model was 127.1±29 mg/dl, after being conditioned into diabetic model had blood glucose level of 235.1±113 mg/dl. It shows the animal model making the process of the diabetic model is said to be successful, more visible with Fig 1.

Fig. 1: Glucose in blood (mg/dl) levels in Type 2 diabetes animal models
The extraction of various soybean (G. max [L] Merr.) was given to AGEs in mice as T2D animal models.

Table 1 shows that the T2D group (pretest) has the highest AGEs level, where there are many intermediate processes of dicarbonyl gum using a reactive carbonyl group attached to amino acids. The application of soy extract in T2D mice decreased AGEs product even though the AGEs were above the mice group with metformin.

**Table 1: AGEs level (μg/ml) for each treatment group**

| Groups               | AGEs (μg/ml) | ANOVA test P value |
|----------------------|-------------|--------------------|
| Negative control     | 1.400±0.6245| 0.066              |
| Pre-test             | 3.600±3.078 |                    |
| Placebo              | 1.725±0.188 |                    |
| Positive control     | 0.575±0.050 |                    |
| T2D+Wils extract     | 1.025±0.631 |                    |
| T2D+Gema extract     | 1.000±0.4243|                    |
| T2D+Argomulyo extract| 1.033±0.6658|                    |

ANOVA test results on AGEs in mice of all groups did not have significant differences ($P = 0.06$).

Table 2 shows HbA1c in the T2D group with the induction of Gema and Argomulyo varieties of soybean extract having values below the normal mice group.

**Table 2: The HbA1c (mmol/mol) levels in each group of mice**

| Groups               | HbA1c (mmol/mol) | ANOVA test P value |
|----------------------|------------------|--------------------|
| Negative control     | 27.30±1.155      | 0.003              |
| Pre-test             | 54.00±20.461     |                    |
| Placebo              | 34.25±5.439      |                    |
| Positive control     | 25.25±4.573      |                    |
| T2D+Wils extract     | 28.50±5.066      |                    |
| T2D+Gema extract     | 26.50±3.323      |                    |
| T2D+Argomulyo extract| 24.00±2.646      |                    |

ANOVA test results for HbA1c levels in blood showed no significant difference ($P = 0.003$). The result of LSD test between the groups was found that the group of T2D mice (pre-test) which had significantly different HbA1c levels with the soybean and metformin group but did not have a significant difference with the control placebo group (T2D+CMC solvent).

**DISCUSSION**

The initial stage of the Maillard reaction, also known as glycation, is glucose reacts with protein amino groups (NH₂) form a Schiff base. This reaction occurs quickly and reversibly, depending on the substrate concentration. Schiff base then converts into ketamine called a more stable amorous product (e.g. HbA1c). Free reactive carbonyl groups from this amadori product are responsible for some of the biological consequences of glycation. Products Amadori can be degraded to various carbonyl compounds more highly reactive such as 3-deoxyglucosone to react again with the amino groups are free to form intermediate products glycation which the intermediate products of these contribute to the formation of AGEs include dicarbonyl intermediates such as 3-deoxyglucosone, glyoxal, and methylglyoxal (formed by glucose auto-oxidation and glycolipid products). Early glycation products and intermediate undergo a complex series of chemical arrangements further, to produce a compound of AGEs stable and irreversible, with a tendency to trigger ROS and interact with specific cell surface structures.

The fraction of total AGEs with relevant effects not only for the structure and function of proteins but also as mediators of biological responses has been characterized in tissues. These compounds include as follows:

1. Imidazoline formed by the 3-deoxyglucosone reaction and the arginine residue in the protein
2. N-ε-carboxymethyl-lysine, a CM analog that is formed by the reaction of methylglyoxal with lysine
3. Glyoxal-lysine dimer
4. Methyl-glyoxal-lysine dimer, an imidazolium cross-link formed by glyoxal or methylglyoxal reactions with lysine residues in proteins [23].

AGEs make cellular damage through pathways of AGE receptors and through intracellular reactive oxygen species (ROS) as well as the reciprocal AGEs process with ROS that will produce either one. ROS activates signaling pathways in the form of mitogen-activated protein kinase, protein kinase C, Janus kinase/signal transducer, and activator of transcription, which has effects on proinflammatory and profibrotic cytokines [7]. AGEs were highest in the T2D group (pre-test), where many intermediate dicarbonyl glycation processes occurred using reactive carbonyl groups bonded with amino acids. The application of soy extract in T2D mice decreased AGEs product even though the AGEs were above the mice group with metformin.

**CONCLUSIONS**

The soybean extract (G. max [L] Merr) effect on the lowest rate of AGEs in mice as T2D animal models is Gema variety, although there is no statistically significant difference with other varieties. The soybean extract (G. max [L] Merr) effect on the lowest rate of HbA1c in mice as T2D model animals is Argomulyo variety although there is no statistically significant difference with other varietal groups.

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**AUTHOR’S CONTRIBUTIONS**

Juliana Christyaningsih has provided the design, intellectual content, innovations, and protocol for conducting the experiment along with mentorship, and sincerely authored the article. Taufiqurrahman has majority...
CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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