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Improvement of Pancreatic Langerhans Islets by Curcuminoid, S-Methyl Cysteine and Its Combination: An Immunohistochemistry Analysis

[1,2]E. Sovia, [1]E.Y. Sukandar, [1]J.I. Sigit and [1]L.D.N. Sasongko
[1]School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia
[2]Faculty of Medicine, Jenderal Achmad Yani University, Cimahi, Indonesia

Abstract: Turmeric and garlic have been known to have antibiabetes effect. Therefore, both can be used as an alternative therapy in the treatment of diabetes. However, their mechanism of action in lowering blood glucose is still unknown. In this study, we investigated the effect of curcuminoid, S-methyl cysteine and its combination on blood glucose level and improvement of pancreatic damage in alloxan induced diabetes mice. Curcuminoid, S-methyl cysteine and their combination were given for 21 days. Then, blood glucose levels were measured and mice were sacrificed and pancreas were isolated for immunohistochemical analysis. The results showed a significant difference of blood glucose levels between control group with treatment group. Adminstration of curcuminoid, S-methyl cysteine and its combination can lower blood glucose levels significantly. Immunohistochemical analysis of pancreas showed a darker color intensity in the group given by curcuminoid and S-methyl cysteine compared with the control group. While the group given by its combination showed minor change compared with the control group. In conclusion curcuminoid, S-methyl cysteine and its combination can lower blood glucose levels. Curcuminoid and S-methyl cysteine could repair damage of pancreas caused by the induction of alloxan while its combination showed only a minor improvement.

Key words: Diabetes, alloxan, blood glucose level, immunohistochemistry, pancreas

INTRODUCTION

The pancreas consists of two major tissue types, namely the acini which secrete digestive juices and the islets of Langerhans which secrete insulin and glucagon. Insulin synthesis and secretion is stimulated by glucose which stimulates the β cell to take up extracellular calcium. The cation appears to trigger a contractile mechanism, whereby the microtubules participate in the movement of insulin containing granule towards the cell membrane, where granule fuse and the granule contents are released into the extracellular space by exocytosis. Meanwhile, Glucagon is rapidly secreted when plasma glucose concentrations fall and secretion is inhibited when glucose concentrations rise (Greenstein and Wood, 2008).

The development of diabetes mellitus is closely related to the development of insulin resistance. However, insulin resistance alone can not fully explain the occurrence of hyperglycemia, because the failure of beta cell function plays an important role in the pathogenesis of diabetes mellitus. It is proved that the insulin secretion decreased progressively when blood glucose rises. In general, the loss of first phase insulin showed beta cell damage. Therefore, increasing insulin secretion does effective treatment of diabetes mellitus. Sulfonylurea (SU) is included among drugs that increase insulin secretion. In general, guideline such as IDF 1999, ADA 2004 and the European national guidelines recommend SU as the first choice of mono therapy and as combination therapy with other anti diabetic drugs. Second generation SU a safer but carry the same efficacy, these drugs have been used for several years (Stingl and Schernthaner, 2007).

The usage of plants, plant extracts or plant derived pure chemicals to treat disease become a therapeutic modality which has stood the test of time (Ansari and Inamdar, 2010). Turmeric and garlic have been known to have antidiabetic effects in preclinical studies (Hussain, 2002; Kuroda *et al*., 2005; Thomson *et al*., 2007; Eidi *et al*., 2006; Shariatzadeh *et al*., 2008, Haque *et al*., 2011). Clinical trial of activity of a combination of turmeric and garlic as antidiabetic against type-2 diabetes patients with dyslipidemia have also been conducted (Sukandar *et al*., 2010). Curcuminoid and S-methyl cysteine which are components of turmeric and garlic have also been known to have antidiabetic effects (Arun and Nalini, 2002; Sheela *et al*., 1995; Sheela and Augusti, 1992). However, the action mechanism of curcuminoid and S-methyl cysteine as antidiabetic still unclear.

Corresponding Author: Evi Sovia, School of Pharmacy, Bandung Institute of Technology, Jl. Ganesha 10, Bandung 40132, Indonesia Tel/Fax: +62-22-2504852

Immunohistochemistry (IHC) is a technical inspection carried out in several veterinary laboratories to establish the diagnosis and for research purposes. IHC is a reliable technique for diagnosis as well as for research of infectious diseases and cancer in animals. The basis of the IHC is very simple and involves three disciplines namely: immunology, histology and chemistry. The basic concept of the IHC is reacting antigen (Ag) on the tissue with specific antibodies (Ab). Binding of antigen with antibody is shown by histochemical color reaction that can be viewed with a microscope under normal or ultraviolet light. Although the concept is simple, the IHC method becomes more complex with the desire to improve the sensitivity and inspection specification. Previous studies used Immunohistochemical analysis to detect insulin (Kojima *et al*., 2004). In this study, Immunohistochemical analysis to detect insulin and glucagon were performed.

In the present study, the effect of curcuminoid, S-methyl cysteine and their combination on blood glucose levels in alloxan induced diabetes mice were evaluated. In addition, immunohistochemical analysis of the pancreas of mice was also done to determine its action mechanism.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Pharmacology, School of Pharmacy, ITB from April to June 2010. The Ethics Committee of Hasan Sadikin Hospital, Bandung, approved all procedures.

Fifty male mice of Swiss-Webster strain six to eight weeks old and weighed between 25-30 g were used in this study. Mice were divided into five groups, each consisting of ten mice. Before treatment, mice were adapted for one week in a cage with room temperature (±25°C) and given access to food and drink.

Alloxan (Sigma, St. Louis, MO, USA), dissolved in 0.9% sodium chloride, was injected intravenously in mice with a single dose of 50 mg kg^{-1} body weight. Normal mice were injected with 0.9% sodium chloride in parallel with the treated mice. Diabetes examined 72 h after injection of alloxan. Blood samples were obtained from tail vein and blood glucose levels were checked by using glucometer (Life Scan, One Touch Ultra, Melitas, CA). After 72 h, fasting blood glucose levels were checked, mice that have a fasting blood glucose levels <145 mg dL^{-1} excluded from the study and the remainder is divided into four groups: control group, group given a dose of 25 mg kg^{-1} body weight of curcuminoid, group given a dose of 25 mg kg^{-1} body weight of S-methyl cysteine and group given a combination of curcuminoid and S-methyl cysteine with each dose of 12.5 mg kg^{-1} body weight. Control and normal group were given a solution of carboxy methyl cellulose 0.5%.

Immunohistochemical analysis: The pancreas was cut from paraffin blocks with a microtome (5 μm) and placed on an object glass which was coated by poly-L-lysin 10%. Immunohistochemistry performed to localize insulin and glucagon in the pancreas. Pieces were rehydrated with graded ethanol (100, 95 and 80%) and washed with deionized water before soaked in 1% hydrogen peroxide to inhibit endogenous peroxidase activity for 5 min. Then washed and soaked in PBS (Phosphatte Buffer Saline) for 2 min, then soaked in 5% BSA (Bovine Serum Albumin) in PBS to reduce non specific bond. Object glass were soaked for 2 h with primary antibody (guinea pig polyclonal to insulin or glucagons, Abcam-ab7842 1:50 dilution) in goat serum (50 μL for each object glass). Wash and soak with PBS for 2 min. Biotinylated goat antiguinea pig Immunoglobulin-G (Santa-Cruz-sc2440, 1:100 dilution) diluted with the same buffer and left for 30 min over an object glass, then washed and soaked with PBS for 2 min. Pieces were immersed in HRP (Horseradish Peroxidase) streptavidin complex for 30 min, then washed and soaked with PBS for 2 min, immersed in the HRP-substrate for 7 min and washed with deion water. Pieces were stained with haematoxylin, dehydrated in graded ethanol (80, 95, 100%), then cleaned with xylol 1 and xylol 2 and covered with glass cover.

Statistical analysis: The data obtained is shown in Mean±SD. Statistical analysis using SPSS 14.0. Student t-test was used to compare blood glucose level before and after treatment. The significant level was set at 5%.

RESULTS AND DISCUSSION

Diabetes Mellitus (DM) type-2 is a heterogeneous disorder characterized by insulin resistance and relative insulin deficiency or dysfunction of β cells. Insulin resistance is characterized by an increase in lipolysis and free fatty acid production, increased hepatic glucose production and decrease glucose uptake of skeletal muscle. Free fatty acids indirectly cause hyperglycemia by stimulating hepatic glucose production. β cell dysfunction occurs progressively and contribute to the worsening of blood sugar control (Oki and Isley, 2002).

According to The United Kingdom Prospective Diabetes Study (UKPDS) the main cause of progression of type-2 diabetes mellitus is due to β cell failure. Therefore, the most effective way to control blood glucose level is by preventing the failure of β cell function. In this present study, we investigated the effect of curcuminoid, S-methyl cysteine and its combination on α and β cells repair (Madsbad, 2007).

Result in this study showed that after 21 days of treatment the group receiving curcuminoid, S-methyl cysteine and its combination have a lower blood glucose

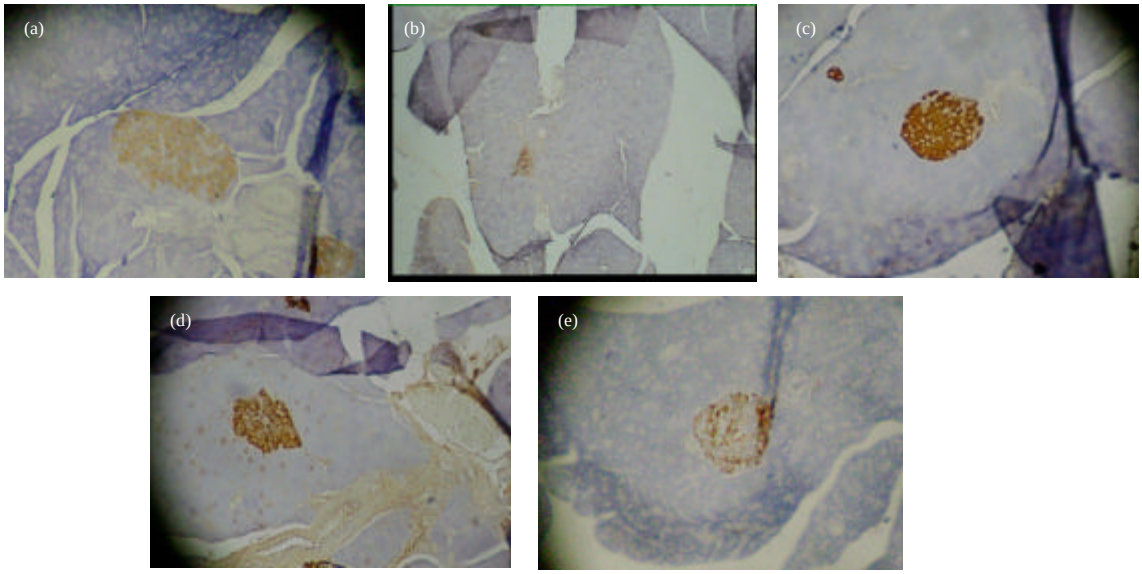

Fig. 1: Result of insulin immunohistochemical analysis of mice pancreas for all treatment groups. (a) Normal group, (b) alloxan induced diabetic mice group, (c) group that received curcuminoid dose of 25 mg kg^{-1} of body weight, (d) group that received S-methyl cysteine dose of 25 mg kg^{-1} of body weight and (e) group that received a combination of curcuminoid and S-methyl cysteine each dose of 12.5 mg kg^{-1} of body weight

Table 1: Effect of curcuminoid, S-methyl cystein and its combination on blood glucose levels

	Blood glucose level (mg dL^{-1})	
Groups	Before treatment	After treatment
Curcuminoid	172.5±22.53	122.6±13.46*
S-methyl cystein	173.7±22.94	140.6±26.89*
Combination of curcuminoid-s methyl cystein	213.4±34.59	161.4±11.85*
Normal	124.8±33.39	139.7±30.41
Control	183.3±74.37	172.8±7.18

Values are expressed as Mean±SD (n = 10).*p<0.05: Significant difference of blood glucose level after treatment in each group compared to before treatment

levels compared to control group (Table 1). After treated with curcuminoid, s methyl cysteine and its combination for 21 days, blood glucose level decreased significantly by 122.6±13.46, 140.6±26.89 and 161.4±11.85 mg dL^{-1}, respectively. Results of this study support previous research that showed antidiabetic effects of curcuminoid and S-methyl cysteine (Joe *et al*., 2004; Strimpakos and Sharma, 2008; Nishiyama *et al*., 2005; Chattopadhyay *et al*., 2004; Hussain, 2002; Sheela *et al*., 1995; Sheela and Augusti, 1992).

Diabetes were inducted by using alloxan. Alloxan and its reduction, namely dialurat acid, initiated a redox cycle with superoxide radical formation. These radicals dysmutated into hydrogen peroxide. After that, a highly reactive hydroxyl radicals is formed by Fenton reaction. The work of Reactive Oxygen Species (ROS) with a massive increase in calcium sitosolik concentration cause a rapid destruction of beta cells (Szkudelski, 2001; Lenzen, 2008). From this experiment, it can be observe there were damaged of β cells that caused by alloxan (Fig. 1b). In addition, Fig. 2b showed that alloxan also damage the α cell so that glucogon levels also decreased.

At the end of the study, the pancreas of mice was isolated for IHC analysis. IHC analysis of insulin of normal group showed in Fig. 1a. IHC analysis of insulin on pancreatic mice that were given curcuminoid (Fig. 1c) and S-methyl cysteine (Fig. 1d) showed darker color intensity and larger size compared to control group (Fig. 1b). This indicated that curcuminoid and S-methyl cysteine were able to repair β cells damage caused by alloxan. However, the size of pancreas of curcuminoid and S-methyl cysteine group was smaller compared to normal group, although the color intensity were darker. IHC analysis of glucagon in the mice pancreas that were given curcuminoid (Fig. 2c) and S-methyl cysteine (Fig. 2d) also showed darker color intensity compared to control group (Fig. 2b). This indicated that curcuminoid and S-methyl cysteine also repaired-damaged α cells. However, the size of pancreas of curcuminoid and S-methyl cysteine group were smaller compared to normal group (Fig. 2a).

This study demonstrated that curcuminoid and S-methyl cysteine not only to have a protective effect but also to repair damage to the pancreas. Results of this study supported previous study that found that curcuminoid inhibited the formation of ROS associated with damage and dysfunction of the Langerhans islets

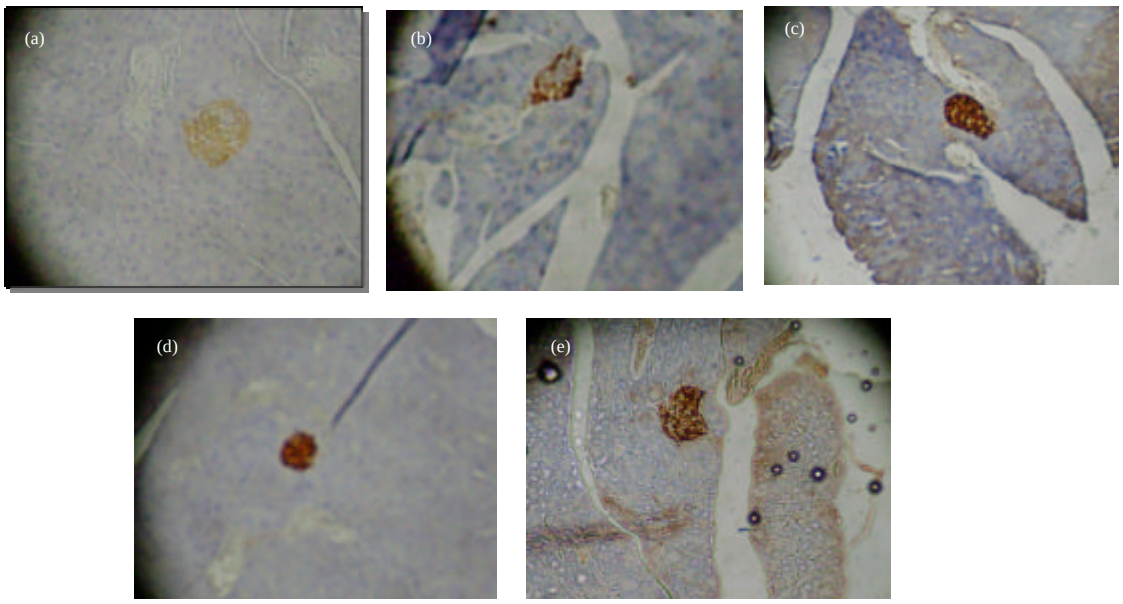

Fig. 2: Result of glucagon immunohistochemical analysis of mice pancreas for all treatment groups. (a) Normal group, (b) alloxan induced diabetic mice group, (c) group that received curcuminoid dose of 25 mg kg^{-1} of body weight, (d) group that received S-methyl cysteine dose of 25 mg kg^{-1} of body weight and (e) group that received a combination of curcuminoid and S-methyl cysteine each dose of 12.5 mg kg^{-1} of body weight

(Kanitkar and Bhonde, 2008). Curcuminoid were also reported to stimulate β-cell function that may contribute to the hypoglycemia effects caused by it (Best *et al*., 2007). Other studies have shown that curcuminoid protected Langerhans islets against oxidative stress induced by streptozotosin (Meghana *et al*., 2007). This indicated that curcuminoid acted as an antioxidant that can reduce oxidative stress induced by alloxan. Decrease in oxidative stress could decrease insulin resistance and inhibited beta cell damage caused by free radicals and glucose toxicity. Curcuminoid carries antioxidant activity which plays an important role in antidiabetes effects (Araujo and Leon, 2001; Joe *et al*., 2004; Strimpakos and Sharma, 2008). Action mechanism of S-methyl cysteine as antidiabetic still unclear but *in vivo* and *in vitro* studies showed that garlic acted as trigger for insulin secretion and also have antioxidant activity (Banerjee and Maulik, 2002; Al-Numair, 2009). Curcuminoid also showed to improve α cells. This effect can minimize the occurrence of hypoglycemia that often occurs in administration of oral antidiabetic drug.

Although the combination of curcuminoid and S-methyl cysteine significantly lowered blood glucose levels but the IHC analysis of insulin in the combination group showed little improvement of the pancreas (Fig. 1e). On the contrary, IHC analysis of glucagon showed greater improvement (Fig. 2e). This is probably caused by a lower doses on the combination that may require a longer time needed to repair the pancreas. This study supported previous studies that showed that reduced blood glucose levels only occurred 8 weeks after administration of a combination of turmeric and garlic extract (Sukandar *et al*., 2010). Another possibility is that the administration of combination has a different action mechanism with administration of its single.

CONCLUSION

Curcuminoid, S-methyl cysteine and their combination carry antidiabetic effects. Curcuminoids and S-methyl cysteine repair the pancreas damage but its combination showed only minor improvement.

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