The Effect of *Mamordica charantia* L. Powder on The Status of Antioxidant Superoxide Dismutase in Liver and Kidney of Diabetic Rats

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ABSTRACT

The status of antioxidant superoxide dismutase (SOD) was reported decreased in the liver tissues of diabetic experimental Macaca fascicularis. *The aim of the study was to observe the effect of* pare (Mamordica charantia) *on the status of SOD in the liver and kidney of diabetic experimental rats. The SOD was localized using immunohistochemical technique. A total of 20 male Wistar rats were used for this study. They were divided into four groups: (1) negative control group, (2) positive control group (diabetes mellitus group/DM); (3) and (4) groups were DM groups that treated with 5 and 10% pare powder. The DM condition was achieved by alloxan (110 mg/KgBB) induction. Treatment of pare was done for 28 days. The results showed that* pare *increased the status of antioxidant SOD in the liver and kidney of diabetic experimental rats. The 10%* pare *powder gave better results than that of 5%. The results suggested that* pare *powder can be used to increase the status of antioxidant in the oxidative stress condition, such as diabetes mellitus.*

Key words : superoxide dismutase, *Mamordica charantia*, diabetes mellitus, liver, kidney, immunohistochemistry

INTRODUCTION

Diabetes mellitus (DM) is a carbohydrate metabolic disorder that was signed with high blood glucose level, more than 140 mg/dL. World Health Organization (WHO) survey showed that Indonesia has high number of DM patients, on fourth rank in the world after India, China, and America. In 2010 the number of DM patients in the world will be 239 millions and it will be 306 millions in 2020 (Mandrup-Poulsen 1998). DM condition increased 2-3 times risk of heart and kidney diseases and death, 10 times risk of gangrene, and 20 times of amputation, as well as hypertension (Schersten and Bitzen 1983).

Wresdiyati *et al.* (2003) reported that DM conditions decreased the level of intracellular antioxidant copper,zinc-superoxide dismutase (Cu,Zn-SOD) in liver tissues of diabetic experimental Macaques (*Macaca fascicularis*). These alterations may account for the diabetic condition inducing production of reactive oxygen species-free radical.

Increased levels of the reactive oxygen species, free radical, create a situation known as oxidative stress (Langseth 1995). This highly reactive oxygen can readily react with various biological macromolecules such as DNA, proteins, lipids, and caused protein destruction. The lesions in turn lead to various diseases and degenerative processes such as aging and carcinogenesis in human and animals (Halliwell and Gutteridge 1995).

Antioxidant plays an important role in protection cells against oxidative stress and maintains a balance between the various toxic oxygen species (Touati 1992). The protection can be done by several ways such as prevention, stopping or decreasing of oxidations (Schuler 1990), as well as catalyzing free radicals by intracellular antioxidant enzymes (Mates *et al.* 1999).

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The intracellular antioxidant enzymes comprise catalase, glutathione peroxidase, and three isoforms of superoxide dismutase (SOD); copper, zinc (Cu,Zn)-SOD, manganese (Mn)-SOD, and iron (Fe)-SOD. The SOD provides a primary defense against superoxide anion radical generated intracellularly. It was reported that SOD was immunohistochemically and immunocytochemically localized in the human and rat tissues (Dobashi *et al.* 1989; Wresdiyati and Makita 1997). SOD was also reported plays important role in physiological processes. Some cases of failed pregnancy in human were caused by the decreasing level of SOD (Sugino *et al.* 2000). Profile of SOD was also reported in pathological condition such as stress, DM, and hypercholesterolemia (Wresdiyati *et al.* 2002; Wresdiyati 2003; Wresdiyati *et al.* 2003; Wresdiyati *et al.* 2006a; Wresdiyati *et al.* 2006b), in neoplastic tissues (Keller *et al.* 1991), and neuron of hypocampus in Alzheimer and Down's syndrome patients (Furuta *et al.* 1995).

Pare (*Mamordica charantia*) was reported to have hypoglychemic effects (Taylor 2002) and inhibit the decreasing number of beta cells in the Langerhans islets of diabetic experimental rats (Wresdiyati *et al.* 2008). While the effect of *pare* on antioxidant status in liver and kidney of diabetic condition is remain to be elucidated.

The present study was conducted to observe the effect of *pare* powder on intracellular antioxidant copper,zinc-superoxide dismutase (Cu,Zn-SOD) in liver and kidney tissues of diabetic rats using immunohistochemical technique.

METHODS

Pare Powder Preparation and Analysis.

The present study used 18-day old *pare*. The fruit of *pare* was treated with alcohol at 50°C for 1 minute, and then the seeds were removed. The remaining materials were then sliced and dried using cabinet dryer at 60C for 16 hours, followed by grinding to obtain powder. Proximate analysis was done to the powder (AOAC 1995).

Treatment of Animals and Tissues Preparation.

A total of 20 male Wistar rats (250 5 g BW) were used for this study. The animals were adapted to the situation and conditions of the animal laboratory for 2 weeks, and then blood glucose was analysis before treatment. The rats were then randomly divided into four groups: negative control group (N), positive control group/DM (P), DM groups treated by 5% (McP5%) and DM group treated by 10% *pare* powder (McP10%). The treatments were done for 28 days. DM condition (>150 mg/dL) was achieved by alloxan induction in dose of 110 mg/kg BW. Drinking water was provided *ad libitum.*

Blood glucose analysis was done once a week using glucometer. Tissues sampling was carried out at the end of each treatment. Following cervical dislocation, liver and kidney tissues were collected from each animal in all groups. The liver and kidney tissues were then processed by paraffin standard method. Specimens were cut into 4 m-thick sections and subjected to immunohistochemical technique for detection of Cu,Zn-SOD.

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Immunohistochemistry.

SOD was localized immunohistochemically as described previously (Wresdiyati *et al.* 2003). The tissue sections were washed for 15 min with 3 changes of PBS between each step. After deparaffinization and rehydration, the tissue sections were exposed to 3% H_2O_2 for 10 min to inactivate endogenous peroxidase activity and then to 10% normal goat serum to block nonspecific binding. Following rinsing with PBS, the tissue sections were incubated in primary antibody of Cu,Zn-SOD at 4C. The tissues were then incubated with enhanced labeled polymer peroxidase (Dako K1491). The reaction product of antigen-antibody was visualized using diamino benzidine (DAB). The tissue sections were then dehydrated with series of alcohol, and cleared with xylene. Finally, the sections were mounted with entelan. As control of staining, tissue sections were incubated with PBS instead of Cu,Zn-SOD antibody. The tissue sections of control staining showed negative reaction with minimal background staining.

Observation and Data Analysis.

The immunoreaction products of the Cu,Zn-SOD were observed by using a light microscope. The observation of Cu,Zn-SOD in the tissues was based on the brown color intensity in the cells and the distribution of the reaction product. The qualitatively observation of Cu,Zn-SOD reaction product was done to the nucleus and cytoplasm of hepatocytes, as well as to the renal tubule cells of kidneys.

RESULTS AND DISCUSSION

***Pare* Powder Characteristics.**

The results of *pare* powder analysis are showed in Table 1. The powder contains a large amount of soluble (25%) and insoluble (40%) dietary fibers, and carbohydrate 86% of dry weight. It was reported that dietary fiber could maintain degenerative diseases such as diabetes, heart disease, and cancer (Thompson 1988). In diabetes case, dietary fiber directly binds glucose in intestine and inhibits absorption of glucose.

Blood Glucose Levels.

The results of blood glucose levels in each week during 4 weeks observation are showed in Table 2. *Pare* powder decreased blood glucose level of diabetic rats. The 10% of *pare* powder decreased blood glucose to the normal level at 7 days after treatment, while the dose of 5% treatment needed 14 days treatment to reach the normal blood glucose level. Beside the dietary fiber content of *pare* powder, which able to decrease blood glucose level, the powder may also contain bioactive compound *charantin* that has a hypoglychemic effect (Taylor 2002).

TablE.1 The results of proximate analysis of *pare* powder

Component	Contents (% dry weight)
Soluble dietary fiber	25.38
Insoluble dietary fiber	39.73
Total dietary fiber	65.12
Moister	10.6
Ash	11.5
Protein	1.2
Lipid	1.4
Carbohydrate	86.2

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Table 2. Blood glucose levels of diabetic rats treated with *pare* powder for 28 days

Days	Blood glucose (mg/dL)			
	N	P	McP5%	McP10%
0	108.5	600.0	436.0	407.0
7	98.0	557.5	377.0	139.0
14	97.5	346.0	169.0	179.5
21	100.0	600.0	99.5	136.0
28	81.0	600.0	94.0	110.5

N : Negative control group McP 5% : DM+*Pare* powder 5%
P : Positive control group (DM) McP10% : DM+*Pare* powder 10%

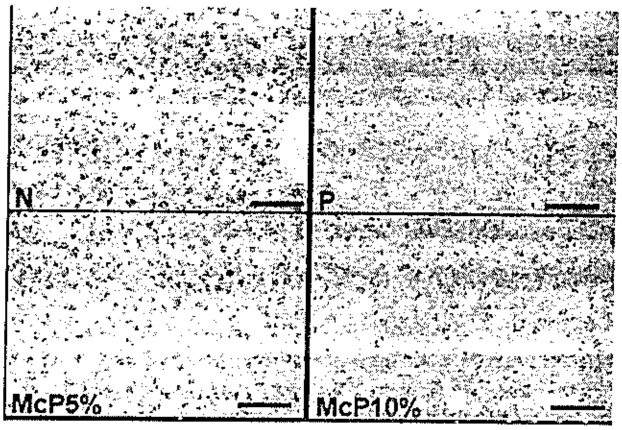

Figure . 1 Photomicrograph of immunohistochemical localization of Cu,Zn-SOD in the rat liver tissues. N: negative control group, P: positive control group (DM), McP5%: DM+*pare* powder 5%, McP10%: DM+*pare* powder 10%. Scale = 50 µm.

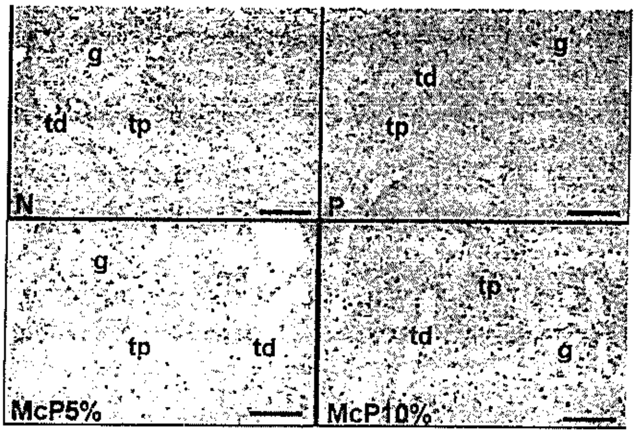

Figure 2. Photomicrograph of immunohistochemical localization of Cu,Zn-SOD in the rat kidney tissues. N: negative control group, P: positive control group (DM), McP5%: DM+*pare* powder 5%, McP10%: DM+*pare* powder 10%, g: glomerulus, tp: tubuli proximalis, td: tubuli distalis. Scale = 50 µm.

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Immunohistochemistry.

Cu,Zn-SOD was immunohistochemically localized in the nuclei and cytoplasm of the hepatocytes, as well as renal tubule cells (Figures 1 and 2). The positive reaction product of the enzyme in the liver and kidney tissues is shown as brown color in the tissues. The distribution and frequency of positive reaction product on the tissues of control group were compared qualitatively with those of the treatment groups. The observation of Cu,Zn-SOD in the tissues was based on the brown color intensity in the cells and the distribution of the reaction product.

The qualitative observation of Cu,Zn-SOD in the tissues of positive control (DM) group showed that the enzyme content decreased as compared to the negative control group (Figures 1 and 2). It is shown by the intensity of brown color reaction product in the positive control group which is lower than that of negative control group. The content of Cu,Zn-SOD in the DM groups treated with *pare* powder increased as compare to that of the positive control group. The treatment of 5% *pare* powder gave slightly increase on the content of Cu,Zn-SOD, while 10% powder treatment showed remarkable increased of Cu,Zn-SOD content in the liver and kidney tissues of rats compared to that of positive control group.

It was reported that lipid beta oxidation, which take place in cellular peroxisomes, increased at fasting and diabetes conditions (Nilson *et al.* 1987; Hawkins *et al.* 1987). These conditions also increased cytochrome P-450 for fatty acid oxidation (Orellana *et al.* 1992). All those oxidations will create more free radicals as side effects, so the condition needs more antioxidant to neutralize the free radicals. Subsequently the status of antioxidant decreased in the liver tissues of diabetic *Macaca fascicularis* (Wresdiyati *et al.* 2003), in the pancreas tissues of diabetic rats (Wresdiyati *et al.* 2008), and in the liver and kidney tissues of diabetic rats in the present study.

Pare powder treatment showed increased the antioxidant status in the liver and kidney tissues of diabetic rats. The *pare* powder may contain flavonoids and polyphenols that scavenged free radicals, then cellular antioxidant Cu,Zn-SOD can be saved and subsequently the status in the tissues increased as compared to that of DM group without treatment of *pare* powder. Comprehensively, the dose of 10% *pare* powder gave better results because the dose contains more dietary fibers, flavonoids, and polyphenols.

The study concluded that *pare* powder decreased blood glucose level and increased the antioxidant status, especially Cu,Zn-SOD in the liver and kidney tissues of diabetic experimental rats. The dose of 10% *pare* powder gave better results than that of 5%. These results suggested that *pare* powder can be used as alternative substitution materials to produce some functional foods for maintaining antioxidant status of DM patients.

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