

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

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The status of antioxidant superoxide dismutase (SOD) was reported decreased in the liver tissues of diabetic experimental *Macaca fascicularis*. This study observed effect of *Mamordica charantia* on the status of SOD in the liver and kidney of diabetic experimental rats. The SOD was localized using immunohistochemical technique. Male Wistar rats of negative control and diabetes mellitus (DM) group treated with 5 and 10% of *M. charantia* powder for 28 days. The DM condition was achieved by alloxan (110 mg/kg BW) induction. *Charantia* powder increased the status of antioxidant SOD in the liver and kidney of diabetic experimental rats. Application of *M. charantia* powder 10% gave better results than that of 5%. The results suggested that *M. charantia* powder can increase the status of antioxidant in the oxidative stress condition, such as diabetes mellitus.

Key words: Superoxide dismutase (SOD), *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 million and it will be 306 million in 2020 (Mandrup 1998). DM condition decreased the status of antioxidant enzyme and increased tissues alteration. It can lead to atherosclerosis and cataract (Szaleczky *et al.* 1999; Ferrari & Torres 2003). Wagenknecht *et al.* (1998) and Gerrity *et al.* (2001) also reported that DM patient has higher risk 2-6 times to get atherosclerosis than normal condition.

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. 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 & Gutteridge 1995).

Antioxidants play an important role in protection of cells against oxidative stress and maintain 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, as well as catalyzing free radicals by intracellular antioxidant enzymes (Mates *et al.* 1999).

The intracellular antioxidant enzymes comprise catalase, glutathione peroxidase, and three isoforms of SOD (Valko *et al.* 2007); Cu,Zn-SOD, Mn-SOD, and 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 & Makita 1997). SOD was also reported plays important role in physiological processes. Some cases of failed pregnancy in human was caused by the decreasing level of SOD (Sugino *et al.* 2000). Profile of SOD was also reported in pathological condition such as stress, diabetes mellitus, and hypercholesterolemia (Wresdiyati *et al.* 2002; Wresdiyati 2003; Wresdiyati *et al.* 2003; Wresdiyati *et al.* 2006a,b), in neoplastic tissues (Keller *et al.* 1991), and neuron of hippocampus in Alzheimer and Down's syndrome patient (Furuta *et al.* 1995).

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

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

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MATERIALS AND METHODS

Mamordica charantia Powder Production and Analysis. The present study used 18 days old *M. charantia* L. fruit. It was obtained from Balitro Cimanggu, Deptan Bogor. The *M. charantia* were blanched with alcohol at 50 °C for 1 minute, then the seeds were removed. The remain materials were then sliced and dried using cabinet dryer at 60 °C for 16 hours, followed by grinding to obtain *M. charantia* 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 rats were obtained from BPOM Jakarta. 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; (i) negative control group (A), (ii) positive control group/DM (B), (iii) and (iv) DM groups that treated by 5% (C) and 10% (D) of *M. charantia* powder (C). The treatments was done for 28 days. DM condition (>150 mg/dl), except group A, 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. And the tissues were then processed by paraffin standard method. Specimens were cut into 4 mm-thick sections and subjected to immunohistochemical technique for detection of Cu,Zn-SOD.

Immunohistochemistry. SOD was localized immunohistochemically as describe 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 copper zinc-superoxide dismutase (Cu,Zn-SOD) at 4 °C. The tissues were then incubated with enhanced labelled polymer peroxidase (Dako K1491). The reaction product of antigen-antibody was visualized using diaminobenzidine (DAB). The tissue sections were then dehydrated with series of alcohol, and cleared with xylol. 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 qualitatively observation of Cu,Zn-SOD content in the tissues was based on the brown colour intensity and the distribution of the positive reaction product of Cu,Zn-SOD in the liver and kidney tissues. While the quantitatively observation of Cu,Zn-SOD content was done by counted the number of hepatocytes, as well as renal tubule cells of kidneys that give different level of brown colour intensity.

RESULTS

Mamordica charantia Powder Analysis. *M. charantia* powder contain a large amount of soluble (25.38%) and insoluble (39.73%) dietary fibers, and carbohydrate 86.2% of dry weight (Table 1).

Blood Glucose Levels. *M. charantia* powder showed decreased blood glucose level of diabetic rats (Table 2). The 10% of *M. charantia* powder treatment decreased blood glucose to the normal level at 7 days after treatment, while the dose of 5% *M. charantia* powder treatment needs 14 days treatment to reach normal blood glucose level.

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

Table 1. Data of proximate analysis of *Mamordica charantia* powder

Component (%)	Contents (% dry weight)
Soluble dietary fiber	25.38
Insoluble dietary fiber	39.37
Total dietary fiber	65.12
Moister	10.60
Ash	11.50
Protein	1.20
Lipid	1.40
Carbohydrate	86.20

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

Days	Blood glucose (mg/dl)			
	N	P	McP5%	McP10%
0	108.50 $\pm$ 9.09	600.00 $\pm$ 0.00	436.00 $\pm$ 15.56	407.00 $\pm$ 23.04
7	98.00 $\pm$ 6.97	557.50 $\pm$ 60.10	377.00 $\pm$ 31.37	139.00 $\pm$ 16.97
14	97.50 $\pm$ 3.54	346.00 $\pm$ 66.88	169.00 $\pm$ 32.53	179.50 $\pm$ 17.68
21	100.00 $\pm$ 5.66	600.00 $\pm$ 0.00	99.50 $\pm$ 4.95	136.00 $\pm$ 21.21
28	81.00 $\pm$ 9.90	600.00 $\pm$ 0.00	94.00 $\pm$ 8.38	110.50 $\pm$ 17.68

N: Negative control group; P: Positive control group (DM); MCP5%: DM+5% of *Mamordica charantia* powder; MCP10%: DM+10% of *Mamordica charantia* powder.

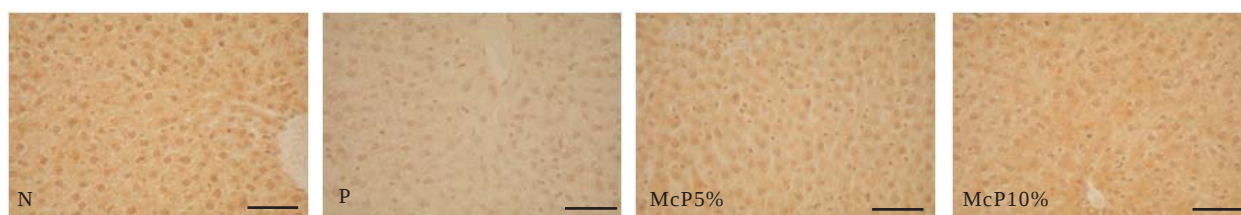


Figure 1. Photomicrographs of immunohistochemical localization of Cu,Zn-SOD in the rat liver tissues. N: negative control group, P: positive control group (DM), McP5%: DM+5% of *Mamordica charantia* powder, McP10%: DM+10% of *Mamordica charantia* powder. Scale = 50 μ m.

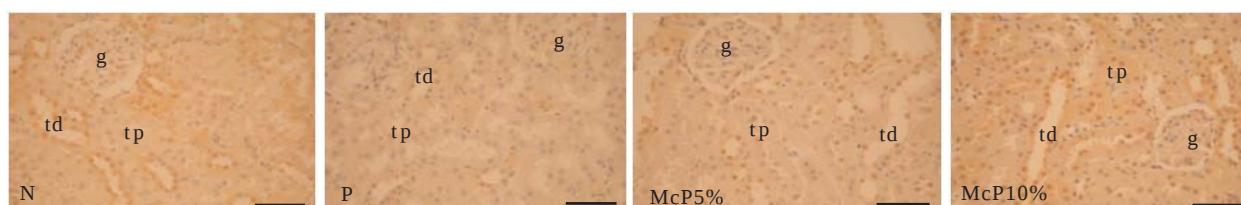


Figure 2. Photomicrographs of immunohistochemical localization of Cu,Zn-SOD in the rat kidney tissues. N: negative control group, P: positive control group (DM), McP5%: DM+5% of *Mamordica charantia* powder, McP10%: DM+10% of *Mamordica charantia* powder, g: glomerulus, tp: tubuli proximalis, td: tubuli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

Qualitatively observation of Cu,Zn-SOD in the tissues of positive control (DM) group, showed the enzyme content decreased compare to the negative control group (Figure 1 & 2). It was shown by the intensity of brown colour reaction product in the positive control group lower than that of negative control group. The content of Cu,Zn-SOD in the DM groups treated with *M. charantia* powder increased compare to the positive control group. The treatment of 5% *M. charantia* powder gave slightly increasing on the content of Cu,Zn-SOD, while 10% *M. charantia* 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 (Figure 1 & 2). It showed by increased the brown colour intensity of positive reaction product to the intracellular antioxidant Cu,Zn-SOD.

Quantitatively observation of Cu,Zn-SOD in the liver and kidney tissues also showed that the enzyme content in the DM is the lowest compared to other groups. It was shown by the number of hepatocytes and renal tubule cells that showed strong positive (+++) reaction to Cu,Zn-SOD in the DM group was significantly ($P < 0.05$) the smallest, while that of showed negative (-) reaction was significantly ($P < 0.05$) the biggest number (Table 3 & 4). The content of Cu,Zn-SOD in the liver and kidney of DM groups treated with 5 and 10% of *M. charantia* powder increased compare to the DM. It was shown by the number of hepatocytes that showed strong positive (+++) reaction to Cu,Zn-SOD increased significantly ($P < 0.05$) compared to that of positive control group. However, the number is still smaller compared to that of negative control group. It was also shown by the number of renal tubule cells that showed negative (-) reaction to Cu,Zn-SOD decreased significantly ($P < 0.05$) compared to that of positive control group. Both in liver and kidney tissues, the content of antioxidant Cu,Zn-SOD increased more remarkably in the DM group that treated with 10% of *M. charantia* powder than that of 5%.

Table 3. The number of hepatocyte at different degree of antioxidant Cu,Zn-SOD content in liver tissues of rats (per view of 20 magnification)

Group	Number of hepatocytes		
	+++	++	+/-
N	54.0 $\pm$ 3.54d	41.7 $\pm$ 2.91c	32.0 $\pm$ 1.87a
P	1.2 $\pm$ 1.09a	7.0 $\pm$ 2.57a	101.7 $\pm$ 6.83d
McP5%	14.8 $\pm$ 1.96b	32.1 $\pm$ 4.04b	79.0 $\pm$ 6.69c
McP10%	34.7 $\pm$ 4.24c	40.7 $\pm$ 3.02c	52.5 $\pm$ 4.30b

N: negative control group, P: positive control group (DM), McP5%: DM+5% of *Mamordica charantia* powder, McP10%: DM+10% of *Mamordica charantia* powder. Strong positive (+++), moderate positive (++), and weak positive (+/-) reaction to the Cu,Zn-SOD.

DISCUSSION

The content of Cu,Zn-SOD in the liver and kidney tissues of DM groups showed the lowest level compared to other groups (Tables 3 & 4; Figures 1 & 2). 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). Under fasting stress, the number of peroxisomes increased 2.8 fold in the renal tubule cell of Japanese monkeys (Wresdiyati & Makita 1995). These conditions also increased cytochrome P-450 for fatty acid oxidation (Orellana *et al.* 1992). All those oxidations will create more free radicals as a side effects, so the conditions needs more antioxidant to neutralize free radicals. Subsequently the status of antioxidant especially Cu,Zn-SOD 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.

Mamordica charantia powder treatment showed increased the antioxidant status in the liver and kidney tissues of diabetic rats (Figure 1 & 2; Table 3 & 4). The *M. charantia* powder may contain flavonoid and

Table 4. The number of renal tubule cells at different degree of antioxidant Cu,Zn-SOD content in kidney tissues of rats (per view of 20 magnification)

Group	Number of renal tubule cells			
	+++	++	+/-	-
N	57.33 ± 2.52b	52.57 ± 6.65b	107.33 ± 1.15a	11.33 ± 0.57a
P	18.33 ± 3.15a	26.67 ± 1.53a	114.33 ± 5.03a	80.33 ± 1.53c
McP5%	20.67 ± 1.51a	24.67 ± 1.53a	147.67 ± 11.54b	23.33 ± 6.35b
McP10%	54.67 ± 5.77b	60.00 ± 1.00c	116.00 ± 6.55a	21.33 ± 1.15ab

N: negative control group, P: positive control group (DM), McP5%: DM+*Mamordica charantia* powder 5%, McP10%: DM+*Mamordica charantia* powder 10%. Strong positive (+++), moderate positive (++), weak positive (+/-), and negative (-) reaction to the Cu,Zn-SOD.

polyphenol that scavenged free radicals (Halliwell & Gutteridge 1995), then cellular antioxidant Cu,Zn-SOD can be saved and subsequently the status in the tissues increased compared to that of DM group without treatment of *M. charantia* powder.

The other pathway is *M. charantia* powder treatment decreased blood glucose level. This effect was caused by a large amount of dietary fiber content in *M. charantia* powder (Table 1). It was reported that dietary fiber could maintain degenerative diseases such as diabetes, heart disease, and cancer (Thompson 1988). In this diabetes case, dietary fibers directly bind glucose in intestine and inhibit absorption of glucose. Beside of dietary fiber content of *M. charantia* powder, which able to decreased blood glucose level, it may also contain bioactive compound-*charantin* that has a hypoglycemic effect (Taylor 2002). Wresdiyati et al. (2008) also reported that *M. charantia* powder treatment could inhibit the rate of pancreatic β -cells damage in rats. Comprehensively, *M. charantia* powder treatment could maintain blood glucose level by insulin secretion of β -cells and it's dietary fiber content. Subsequently, all oxidations reactions that create energy can be decreased, such as fatty acid oxidation. Then free radical production, as a side effect from these oxidations, decreased. This condition can also save the utility of intracellular antioxidant especially Cu,Zn-SOD to neutralize the free radicals, and subsequently the status in the tissues increased as shown in the liver and kidney of DM group that were treated with 5 and 10% of *M. charantia* powder. The dose of 10% *M. charantia* powder gave better results because the dose contains more dietary fibers, flavonoid, and polyphenol.

The study concluded that *M. charantia* powder decreased blood glucose level and increased the antioxidant status, especially Cu,Zn-SOD in the liver and kidney tissues of diabetic experimental rats. These results suggested that *Mamordica charantia* powder can be used as an alternative substitution material in order to produce some functional foods for maintain antioxidant status of diabetes mellitus patients.

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