



Effect of Mixture of Mengkudu and Temulawak Juices on MDA Levels and SOD Activity in Streptozotocin-induced Diabetic Rats Liver

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Abstract

Antioxidants are widely used to repair damage to the organs in the body. This research aims to look at the effect of the mixture of *mengkudu* fruit juice and *temulawak* rhizome juice on MDA levels and SOD activity in diabetic rat liver induced by streptozotocin. The mixed juice is given to white male Wistar strain rats for 28 days after the rats induced by streptozotocin and found diabetes. The results showed that there were differences between the rats which were given preferential treatment with rats which were not given the treatment. The conclusion is the giving of mixture juices of *mengkudu* fruit and *temulawak* rhizome can decrease the MDA levels and increase the SOD activity of rat liver induced by streptozotocin.

Key Words: *mengkudu*, *temulawak*, MDA, SOD

INTRODUCTION

Morinda citrifolia is a plant in the family Rubiaceae (Backer and Brink, 1980), in Indonesia known as *mengkudu*. This plant is still widely used traditionally as a remedy by the public. Parts of the plant can be used as medicine is the fruit, leaves, roots, stems and tree bark (Lim, 2013). Scopoletin is the main content, used as a marker for *Morinda citrifolia* (Wang *et al.*, 2002). In previous research, *Morinda citrifolia* can be used to improve the condition of some diseases which are antibacterial, antiviral, antifungal, antitumor, antihypertensive, anthelmintic, analgesic, anti-inflammatory, and immune enhancing effects (Kamiya *et al.*, 2008).

Curcuma xanthorrhiza is a plant in the family Zingiberaceae (Backer and Brink, 1980). Native plant to Indonesia was known as *temulawak* (Yasni *et al.*, 1991), usually used as a traditional medicine to help the recovery in hepatitis, liver complaints, and diabetes (Devaraj *et al.*, 2014). Curcumin is the main content which is used as a marker for *Curcuma xanthorrhiza* (Rafi *et al.*, 2011). Previous research on *Curcuma xanthorrhiza* showed antioxidant activity, anti-inflammatory, antimicrobial, hepatoprotective and anticarcinogenic, nephroprotective, antirheumatic, and hypoglycemic (Ambarsari *et al.*, 2014).

Jamu is traditional medicine in Indonesia which is derived from plants. *Jamu* is still widely used traditionally in Indonesia that is by boiled, squeezed, or made into juice. The word herbal medicine '*Jamu*' has become the brand image of Indonesia nation which must be maintained and preserved (Elfahmi *et al.*, 2014).

Based on the above reasons, then the researcher is inspired to make a combination or a mixture of traditional medicine from the *mengkudu* fruit and *temulawak* rhizome in form of juice as an antioxidant, which is used to fight free radicals and enhance antioxidative status of the body. The parameters used to see antioxidant activity of mixture *mengkudu* and *temulawak* juices in this research are the MDA levels and SOD activity in rat liver.

MATERIALS AND METHODS

Plant

Mengkudu fruit and *temulawak* rhizome obtained from Balai Materia Medika Batu, Malang, Indonesia. *Mengkudu* fruit is taken from the age of 3-4 months, whereas *temulawak* is taken at age 9-10 months.

Process of Making Mengkudu and Temulawak Juices

Fresh *Mengkudu* fruit and *temulawak* rhizome sliced with a knife. Then, *mengkudu* fruit is inserted into the juice extractor to the brim. Run juice extractor and then we will get lumpy noni juice which has been separated with the dregs. The treatment is also done on the *temulawak* rhizome.

Testing on Rats

The 21 rats that are used in the test, first adapted for 1 week, then a total of 18 rats induced with single dose of 60 mg/kg BW streptozotocin (Nugroho, 2006). Four days later, the rats blood sugar levels is checked, the rats is found diabetes after blood sugar levels more than 200 mg/dl (Muhtadi *et al.*, 2015).

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After that, the rats were divided into 7 groups namely: normal rats (N), diabetic rats (NC), diabetic rats given glibenklamid medicine (PC), diabetic rats given mixed juices of *mengkudu* fruit 3.6 ml/kg BW/day + *temulawak* rhizome 10 ml/kg BW/day (MT1), diabetic rats given mixed juices of *mengkudu* fruit 1.8 ml/kg BW/day + *temulawak* rhizome 5 ml/kg BW/day (MT2), diabetic rats given mixed juices of *mengkudu* fruit 0.9 ml/kg BW/day + *temulawak* rhizome 2.5 ml/kg BW/day (MT3) and diabetic rats given mixed juices of *mengkudu* fruit 0.45 ml/kg BW/day + *temulawak* 1.25 ml/kg BW/day (MT4). After 28 days of treatment, the rats of each group is euthanized to analyze the MDA levels and SOD activity of liver (Akhgari *et al.*, 2003).

Sample Preparation of Liver

The sample preparation of liver made in this research are as follows (Murthy *et al.*, 2002): 1.25 g of liver minced in cold conditions in 5 ml of PBS (phosphat buffer saline) containing 11.5 g/L KCl. The homogenate is then centrifuged to obtain a clear supernatant. Supernatant is then used to measure the MDA levels and SOD activity using a spectrophotometer.

Measurement of MDA Levels

Measurement of MDA conducted in this study are as follows (Murthy *et al.*, 2002): 0.5 ml of the supernatant liver plus 2.0 ml of cold HCl 0.25 N containing 15% TCA, 0.38% TBA and 0.5% BHT. The mixture is heated to 80°C for 1 hour. Once its cool, the mixture is centrifuged for 10 minutes. The absorbance of the supernatant was measured at a wavelength of 532 nm. As the standard solution used TEP.

Measurement of SOD Activity

The measurement of the SOD activity conducted in this study are as follows (Murthy *et al.*, 2002): 0.5 ml of the supernatant liver and 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 µM NBT, and 0.2 ml of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm followed by recording the absorbance after 5 min at 25°C. The control was simultaneously run without supernatant liver. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units per milligram of proteins.

RESULTS AND DISCUSSION

This study aims to determine the giving effect of mixed *mengkudu* and *temulawak* juices to MDA levels and SOD activity of diabetic rat liver induced by STZ. In this study also examined blood glucose levels of rats before induced by STZ and after 4 days induced by so the rats can be found in diabetes condition or not. The results obtained in the measurement of MDA levels and SOD

activity in rat liver can be seen in the following Tables 1 and 2 below:

Table 1 MDA levels in Rats Liver

	Mean (nmol/g)	Std. Deviation	Std. Error
N	2.79	0.32	0.19
PC	4.25	0.54	0.31
NC	7.86	0.10	0.06
MT1	4.14	0.51	0.30
MT2	4.91	0.43	0.25
MT3	5.68	1.17	0.68
MT4	5.72	0.30	0.17

Based on table 1. in the above, it appears that the average levels of liver MDA group NC is the highest 7.86 ± 0.10 nmol/g, while the average MDA levels of liver of group N is the lowest with 2.79 ± 0.32 nmol/g. The average MDA levels of liver of group PC was 4.25 ± 0.54 nmol/g, whereas the average MDA levels of liver of group combination MT1 is lower than the positive control (PC) with 4.14 ± 0.51 nmol/g. Based on a statistical analysis of Anova test with a 95% degree of confidence ($p < 0.05$) indicates that there is a significant difference between the MDA levels of liver in research that was followed by Post Hoc Tests Multiple Comparison (LSD). The results showed a significant difference between the group of normal rats with rats induced by STZ, there was a significant difference between the Group of NC with all groups, while in the PC group there was no significant difference with the MT1 and MT2.

Table 2 SOD Activity in Rats Liver

	Mean (%)	Std. Deviation	Std. Error
N	75.00	3.57	2.06
PC	53.57	10.71	6.19
NC	13.69	2.73	1.58
MT1	56.55	4.49	2.59
MT2	46.43	6.44	3.72
MT3	30.95	15.39	8.89
MT4	33.93	6.44	3.72

Based on table 2. in the above, it appears that the average SOD activity of liver of NC group is the lowest $13.69 \pm 2.73\%$, while the average SOD activity of liver of N group is the highest that is $75.00 \pm 3.57\%$. The average SOD activity of liver of PC group was $53.57 \pm 10.71\%$, while the average SOD activity of liver of MT1 group higher than group PC with $56.55 \pm 4.49\%$. Based on a statistical analysis of Anova test with a 95% degree of confidence ($p < 0.05$) indicates that there is a significant difference in the SOD activity of liver between research groups thus continued with the Post Hoc Tests Multiple Comparison (LSD). The results showed a significant difference between group of normal rats with rats induced by STZ. In the PC group there was no significant difference with the MT1 and MT2.

The data in table 1. indicates that the giving of mixed *mengkudu* and *temulawak* juices MT1 can decreases the MDA levels by 47.33% and MT2 by 37.53%, allegedly

because of the nature of the antioxidative of the mixed *mengkudu* and *temulawak* juices containing scopoletin and curcumin or other substances which are antioxidants that can work as a chain breaker works by donating electrons to free radicals. This MDA levels decrease will cause the oxidative damage caused by free radicals will be decreased so that the condition of the liver is expected to be better, like the research ever done before saying that decreased levels of MDA decrease the oxidative damage (Mateos *et al.*, 2005). MDA is a product of lipid peroxidation levels can be reduced by the presence of antioxidant compounds that can be said that the low levels of MDA showed inhibition of lipid oxidation by an antioxidant, and vice versa (Liu *et al.*, 2000).

The vulnerability of an organ toward oxidative damage depending on antioxidant enzymes. SOD enzymes are enzymes that play a role in protecting tissue oxidation by means of neutralizing superoxide radicals (Décordé *et al.*, 2010). Superoxide radical is dismuted by SOD into H₂O₂ that they are toxic and then H₂O₂ degraded to products that are not toxic (da Silva *et al.*, 2013). In this study, an increase the SOD activity of liver after the addition of mixed *mengkudu* and *temulawak* juices compared to NC. There is an increase SOD activity in MT1 which is enormous i.e. to 313.07% so it is possible the occurrence of dismutation in superoxide radical and H₂O₂ decomposition more than before. This will make the liver organs became more protected against oxidative damage, such research has been done before as saying that the increased SOD activity can reduce oxidative damage in rat liver (Wresdiyati *et al.*, 2006).

CONCLUSION

The giving of mixed *mengkudu* and *temulawak* juices in this study can increase the antioxidative status of the body, this is indicated by a decrease in liver MDA levels of up to 47.33% and an increase in liver SOD activity of up to 313.07% compared to the negative control group.

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