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Moringa (*Moringa oleifera*) leaf powder in reducing the grade of liver injury of wistar rat induced by mancozeb

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ABSTRACT

Background: Mancozeb (Mz) is one of the poisoning chemical substances that cause the liver injury (LI). Moringa leaf (Mo) contains antioxidant to muffle the free radical. The objective of this study is to know the effects of Mo leaf in reducing the level of LI of Wistar rat induced by Mz through an antioxidant mechanism.

Method: It was an experimental study by using 24 randomized Wistar rats. Group I was terminated at the beginning of the study as the basis of necrosis index. Group II was treated as the control group. Group III was exposure with Mz started at day 11 until day 20. Group IV was given Mo started at the day 1 until day 10 and prior to giving Mz until day 20. The overall blood rats in Group II-IV were aspirated prior to and after study in measuring MDA, ALT, GPx, CAT, and SOD. The necrosis index was measured at the end of the study. The data were analyzed by unpaired t-test and linear regression.

Results: There was no LI in the Group I and II. MDA level increase in Group IV (9.67 nmol/ml), but lower than Group III (45.05 nmol/ml; p = 0.006). The decrease of level of GPx, CAT, and SOD in Group IV (0.07 nmol/ml; 0.48 nmol/ml; and 81.5 U/ml), which was lower (p=0.008; p=0.004; and p=0.0001) than Group III (0.23 nmol/ml; 0.86 nmol/ml; and 140.9 U/ml). Necrosis index and ALT level increase in Group IV (4.42; 25.1 u/l) was lower (p=0.04; p=0.0001) than Group III (8.56 and 67.4 u/l). A multivariate test result that SOD was the most beneficial towards the LI status.

Conclusion: Mo can reduce the LI level in Wistar rats induced by Mz through the decrease rate of endogen antioxidant of SOD.

Keywords: *Mancozeb*, *Moringa oleifera*, liver injury, antioxidant, SOD.

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BACKGROUND

Liver injury (LI) is a liver disorder marked by the increase of serum level in alanine aminotransferase (ALT) of the blood as well as the presence of necrosis.[1]

Etiology is divergent including viral or bacterial infections, alcohol poisoning, trauma, drugs or certain chemicals such as pesticides.[2] One of pesticide groups that may cause LI is a carbamate, e.g. mancozeb (Mz).[3,4] The previous study showed that Mz could increase the ALT enzyme levels and trigger oxidative stress. Oxidative stress is characterized with the increase of malondialdehyde (MDA) level and the significant decrease of endogenous antioxidants namely superoxide dismutase (SOD) serum, catalase (CAT) and glutathione peroxidase (GPx).[5]

Considerable farmers remain using pesticides excessively; however, they may have an adverse impact on health. The prevalence of liver injury keeps rising in Brebes, from 90 patients in 2011 to 251 in 2013. There were 96.3% of the patients coming from the onion agricultural centers that considerably use Mz.[4] The farmers in Brebes have a particular habit i.e. storing seeds of onion in the kitchen. The onion's seeds that have been sprinkled with Mz are placed on top of the stove where this habit led to food prepared in the kitchen easily contaminated by Mz. A study conducted by Bertini et al. showed that the toxic effects of Mz increase during cooking or heating process.[6]

The body's physiological system has an ability to reduce cell damages due to oxidative stress.[7] The body needs extra protection mechanisms during its weak condition or too many exposures against reactive oxygen species (ROS). Additional protection can be obtained by consuming antioxidants made from herbal ingredients.[8,9] A lot of herbal ingredients have been developed into a supplement, such as Moringa oleifera (Mo) originally grown in South Asia. Mo is promoted to have a high potential of medicinal value.[10] The purpose of this study is to prove that the grade of LI in Wistar rats induced by Mz can be reduced by giving Mo leaf powder. In addition, this study also aims to know the antioxidants mechanisms of Mo leaf powder.

MATERIAL AND METHOD

It was an experimental study with Randomized Pretest-Posttest Parallel Control Group Design which conducted in Unit IV Integrated Research and Experiment Laboratory, since September-December 2015. The Wistar rats with 300 to 400 g

bodyweight and 12 to 16 weeks-old were adapted for 1 week. The sample size was in accordance with the requirements of the WHO in which each group has 6 mice. 24 healthy mice were randomly divided into 4 groups. The Group I as a baseline was terminated on the first day. Group II as a control group was given only water and pellets ad libitum until day 20. Group III, in the first day till day 10, just got water + pellets ad libitum; meanwhile, on days 11 to 20, they got the exposure of Mz at 313 mg/kg of bodyweight. Group IV, in the first day until day 10, was given Mo 500 mg/kg of bodyweight followed by the exposure of Mz at 313 mg/kg of bodyweight until day 20. The Mo leaf powder was purchased from the industry of Indonesia Moringa, Blora. Mz of Dithane M-45® trademark was acquired from a pesticide store in Brebes. Mo leaf powder and Mz were dissolved in distilled water and then given to experimental animals using a stomach sonde.

The Group I samples was terminated under anesthesia either at the beginning of the study. Their liver tissues were taken out and examined as the baseline data of necrosis index. The blood of all samples of Group II, III and IV was aspirated to measure the levels of MDA, SOD, CAT, GPx and ALT. The overall blood samples that could survive until the end of the study were aspirated to measure the variables aforementioned. Afterward, all samples were terminated to take out and examine their liver tissue in the laboratory of Anatomy Pathology, Faculty of Veterinary Medicine, UGM Yogyakarta. The indicators of antioxidant status were MDA, SOD, GPx and CAT. MDA level was measured using a spectrophotometer and observed using a light microscope. The levels of SOD, GPx and CAT were measured using Enzyme Linked Immuno-Sorbent Assay (ELISA). LI indicator was the necrosis index and ALT level. The necrosis index was examined by histological examination using H & E staining method. Meanwhile, the ALT enzyme level was measured using Vitros 250.

This unpaired t-test was applied in this study to test the hypothesis; meanwhile, the paired t-test was used to analyze the pretest and posttest. Whereas, the multivariate test was used to test the linear regression. The Health Research Ethics Committee has approved this study registered in number; 458/EC/FK-RSDK/2015, Medical Faculty of Diponegoro University, Dr. Kariadi Hospital, Semarang, Indonesia.

Table 1 The results of Research Variable Measurement

Variables and Groups	Pretest	Posttest	p^*	Delta (Δ)	$p^\dagger$
MDA Level (nmol/mL)					
Group III	12.00 ± 1.78	57.03 ± 18.43	0.002	45.04 ± 19.51	0.006
Group IV	12.22 ± 1.57	21.89 ± 6.19	0.008	9.67 ± 5.61	-
GPx Level (Umol/mL)					
Group III	2.09 ± 0.07	1.86 ± 0.10	0.001	-0.228 ± 0.08	0.009
Group IV	2.09 ± 0.08	2.02 ± 0.05	0.115	-0.068 ± 0.09	-
CAT Level (nmol/mL)					
Group III	2.100 ± 0.84	1.18 ± 0.04	0.0001	-0.86 ± 0.0.05	0.008
Group IV	2.097 ± 0.19	1.62 ± 0.39	0.003	-0.48 ± 0.22	-
SOD Level (U/mL)					
Group III	203.4 ± 4.9	62.5 ± 1.4	0.0001	-140.9 ± 5.8	0.0001
Group IV	203.5 ± 5.5	122.0 ± 11.0	0.0001	-81.5 ± 7.0	-
ALT Level (U/L)					
Group III	22.2 ± 6.8	89.6 ± 11.3	0.0001	67.4 ± 14.6	0.0001
Group IV	22.3 ± 6.5	47.4 ± 7.6	0.003	25.1 ± 11.1	-
Necrosis Index					
Group III	0	2.67 ± 1.5	0.007	2.67 ± 1.5	0.040
Group IV	0	0.83 ± 1.2	0.141	0.83 ± 1.2	-

The result is average ± SD;
* The comparison result of pretest-posttest and paired t-test;
† The comparison result of delta and Unpaired t test.

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RESULTS

The overall rats remain survived during the study in Group II-IV. The invalidity sources such as history, maturation, co-interventions, survival, mortality and contamination in this study were controlled. There is no liver tissue changes found on microscopic observation in the Group I and II. Meanwhile, the liver injury (LI) was suggested in the Group III and IV after Mz exposure. In addition, the changes of MDA, GPx, CAT and SOD levels at the beginning and the end of the study was not significant and did not affect the liver cells in Group II. It can be said that the component of time (maturation) did not play any role in liver damage. Therefore, subsequent analysis to assess the effectiveness of Mo would merely compare Group III and IV. The results of variable measurement are presented in Table 1.

This study showed that the increase of the MDA level in Group IV was lower than Group III. The increase of necrosis index in Group IV was lower than Group III and not significant;y different (P = 0.115). The increase of ALT level in Group IV was lower than Group III and significantly different ($P < 0.001$). The decrease of GPx level in Group IV was lower than Group III. The decrease CAT level in Group IV was lower than Group III. The decline of SOD level in Group IV was lower than Group III and significantly different ($P < 0.001$).

The effect of Mo antioxidant in preventing liver injury among the Wistar rats can be found out through the analysis of the antioxidant status in relation to LI status. The linear regression analysis was conducted with the previous method. There is a strong and significant correlation between antioxidant status and each LI variable status, as presented in Table 2.

This study showed variable of ALT level were positively correlated to MDA level and the necrosis index. The correlation of ALT to GPx level, CAT and SOD was negative. The results of linear regression between ALT variable and each intermediator variables indicated that the most influential variable was SOD (ANOVA, p=0.0001) with the equation ALT=3.04 - 0.41 SOD. Adjusted R^2=0.707 meant that SOD variable had 70.7% influence towards the change of ALT variable. Other Mo antioxidant contents such as flavonoids, vitamin C, E, and A possibly affected changes in ALT variable, beyond the SOD variable.

DISCUSSION

The MDA level increased due to exposure of Mz. These results are consistent with previous studies which stated that MDA level increased due to exposure of Mz on rat liver.[3,11,12] This can happen since Mz is decomposed into CS_2 and ETU which is pro-oxidant; therefore, it can trigger the formation of free radicals such as H_2S_2 and CS_2.[13,14] The free radicals formed will be neutralized by the endogenous antioxidant as the liver defense. However, when the free radical exceeds the capability of liver defense, the oxidative stress occurs and MDA level increases.[15]

Giving Mo can significantly inhibit the increase of MDA level. These results are consistent with some previous studies stating that Mo has antioxidant activity that can inhibit the increase of MDA level among the mice exposed to cigarette smoke, acetaminophen or CCl_4.[16,17,18]

The contents of Mo antioxidant, phenolic compounds, various minerals, and vitamins, along with endogenous antioxidant system may inhibit the increase of MDA level.[3,18,19,20] The phenolic

Table 2 The correlation of ALT variable and intermediator variable

		ALT Delta	MDA Delta	GPx Delta	CAT Delta	SOD Delta	Nekr Delt
ALT Delta	P. Correlation	1	0.629	-0.692	-0.609	-0.851	0.654
	p		0.005	0.001	0.007	0.000	0.003
MDA Delta	P. Correlation	0.629	1	-0.645	-0.549	-0.727	0.505
	p	0.005		0.004	0.018	0.001	0.033
GPx Delta	P. Correlation	-0.692	-0.645	1	0.719	0.713	-0.563
	p	0.001	0.004		0.001	0.001	0.015
CAT Delta	P. Correlation	-0.609	-0.549	0.719	1	0.740	-0.624
	p	0.007	0.018	0.001		0.000	0.006
SOD Delta	P. Correlation	-0.851	-0.727	0.713	0.740	1	-0.706
	p	0.000	0.001	0.001	0.000		0.001
Nekr Delta	P. Correlation	0.654	0.505	-0.563	-0.624	-0.706	1
	p	0.003	0.033	0.015	0.006	0.001	

Table 3 The results of linear regression test of delta-dependent variable of ALT and intermediator variables (MDA, Necrosis Index, GPx, CAT and SOD)

Delta ALT														
R	R^2	*Adjusted* R^2	*p Anova*	C	MDA	*p Coeff*	Necrosis Index	*p Coeff*	GPx	*p Coeff*	CAT	*p Coeff*	SOD	*p Coeff*
0.865	0.748	0.644	0.003	6.92	-0.05	0.87	1.17	0.79	-54.25	0.33	13.79	0.52	-0.37	0.02
0.865	0.748	0.670	0.001	6.77	-	-	1.19	0.78	-51.86	0.31	13.52	0.51	-0.36	0.01
0.864	0.746	0.692	0.0001	6.33	-	-	-	-	-51.07	0.30	11.99	0.10	-0.38	0.003
0.859	0.739	0.704	0.0001	3.49	-	-	-	-	-38.74	0.37	-	-	-0.35	0.002
0.851	0.724	0.707	0.0001	3.04	-	-	-	-	-	-	-	-	-0.41	0.0001

Equivalence → ALT = 3.04 – 0.41 SOD

compounds can act as a reductant through the capture of singlet oxygen and hydrogen atom donor. The content of vitamins in Mo can directly bind or neutralize the free radicals as the metabolism results of Mz.[3,21]

In this study, giving Mo inhibits the decrease of levels of GPx, CAT, and SOD. These results are consistent with previous studies stating that the antioxidant content of Mo leaf powder can inhibit the GPx, CAT, and SOD level reduction in mice exposed with acetaminophen or CCl_4.[17,18] This can occur due to the antioxidant activity of Mo in the form of minerals can help activation of SOD, GPx, and CAT.[21]

Giving Mo inhibits the increase of necrosis index significantly. These results are consistent with results of previous studies stating that the antioxidant activity of Mo can inhibit histological changes of rat liver induced by acetaminophen or CCl_4.[17,18] This occurs since the antioxidant activity of Mo along with endogenous antioxidant system are able to reduce the oxidative stress.[3] The free radicals bound and suppressed by antioxidants of Mo can prevent damage to DNA and proteins, and further can prevent the mitochondrial dysfunction and necrosis of liver cells.[3,21]

Giving Mo inhibits the increase of ALT level significantly. These results are consistent with the results of previous studies stating that Mo can inhibit the increase of ALT levels in mice induced by Acetaminophen or Diclofenac. This phenomenon occurs because the antioxidant activity of Mo along with endogenous antioxidants can reduce the oxidative stress. Therefore, damage to the cell membrane and mitochondrial dysfunction in liver cells can be minimized. The decrease in cell membrane damage will reduce the release of ALT into the blood.[8,24,25]

Mo can lessen the level of LI through its antioxidant activity along with endogenous antioxidant by reducing oxidative stress.[3] The content of Mo in the form of phenolic compounds and vitamin plays a role in the direct defense mechanisms of the liver by binding or neutralizing the free radicals.[19,20] The mineral content of Mo acts indirectly by activating the endogenous antioxidant.[21] The role of endogenous antioxidants is to reduce the free radicals and prevent the formation of new free radicals. SOD can convert superoxide anion into H_2O_2 by the cytosol and mitochondria. Hydrogen peroxide (H_2O_2) formed is reduced into water by CAT in peroxisomes and GPx in the cytosol and mitochondria.[15,21]

The multivariate test indicated that the most influential variable on the dependent variable, ALT, was SOD (70.7%). In order to work, SOD which has been present in the body requires minerals activation such as manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu). Mo contains those minerals altogether, so that the activation of SOD enzyme is more maximal than the endogenous antioxidants such as GPx or CAT.[15] The role of antioxidants in addition to endogenous antioxidants SOD, in preventing LI has not been investigated; therefore, it is not well described yet.

Antioxidant activity of MO, as well as endogenous antioxidants, can prevent the oxidative stress and further can prevent mitochondrial dysfunction and inhibit liver cell necrosis. The under-controlled necrosis can prevent LI.[3,21]

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CONCLUSIONS AND RECOMMENDATIONS

Giving Mo leaf powder is proven to reduce the level of LI among the Wistar rats induced by Mz through activation of endogenous antioxidants SOD. The results of this study showed that the effect of Mo leaf powder in reducing the level of LI indirectly through the activation of endogenous antioxidants

SOD was 70.7%. The direct mechanism for the other 20.3% is probably the direct effect of antioxidants contained in Mo such as vitamin C, A, E, and flavonoids. Antioxidant activity of Mo has not been indirectly studied; therefore, it needs further research to explain the role of the contents.

REFERENCES

1. Mukherjee S, Gollan JL. Assessment of Liver Function. In: Sherlock S, (ed). Diseases of the Liver and Biliary System. 12th Ed. Oxford: Blackwell Scientific; 2011: 20-34.
2. Lindseth GN. Disorders of Liver, Gall Bladder and Pancreas. In: Sylvia AP, Lorraine MW, (Eds). Pathophysiology, clinical of disease process. 6th Ed. Jakarta: EGC. 2006: 472-513.
3. Gu X, Manautou JE. Molecular mechanisms underlying chemical liver injury. Expert Rev Mol Med. 2013; 14(4): 1-25. DOI: 10.1017/S1462399411002110.
4. Siwiendrayanti A, Suhartono, Nur EW. Hubungan Riwayat Pajanan Pestisida Dengan Kejadian Gangguan Fungsi Hati (Studi pada Wanita Usia Subur di Kecamatan Kersana Kabupaten Brebes). Jurnal Kesehatan Lingkungan Indonesia. 2012; 11(1): 9-14.
5. Sakr SA. Ameliorative effect of ginger (Zingiber officinale) on mancozeb fungicide induced liver injury in albino rats. Australian Journal of Basic and Applied Science. 2007; 1(4): 650-56.
6. Bertini S, Carratore RD, Giorgi M, Bronzetti G, Croce CL. Genotoxic and mono-oxygenase system effects of the fungicide maneb. Springer-Verlag, Arch Toxicol. 2000; 74: 415-20. DOI: 10.1007/S002040000152.
7. Nugraha AS, Hadi NS, Siwi SU. Efek Hepatoprotektif Ekstrak Buah Merah (Pandanus conoideus Lam) pada Hati Mencit Jantan Galur Swiss induksi dengan CCl_4. Journal Natur Indonesia. 2008; 1(1): 24-30.
8. Hossain MB, Brunton NP, Barry-Ryan C, Martin-Diana AB, Wilkinson M. Antioxidant activity of spices extracts and phenolics in comparison to synthetic antioxidants. Rasayan J Chem. 2008; Vol 1 (4):751–56.
9. Sreelatha S, Patma PR. Antioxidant Activity and Total Phenolic Content of Moringa oleifera Leaves in Two Stages of Maturity. Plant Foods Hum Nutr. 2009; 64:303–11. DOI: 10.1007/s11130-009-0141-0.
10. Sharifudin SA, Fakurazi S, Hidayat MT, Hairuszah I, Moklas MAM, Arulselvan P. Therapeutic potential of Moringa oleifera extracts against acetaminophen-induced hepatotoxicity in rats. Pharmaceutical Biology. 2013; 51(3):279-88. DOI:10.3109/13880209.2012.720993
11. Sakr SA, Abel-Samie HA. Apoptosis Related Protein Bax In Liver of Metalaxyl Fungicide –Treated Mice: The Effect of Antox. Ozean Journal of Applied Sciences. 2008; 1(1): 17-27.
12. Sakr SA, Mahran HA, Abo-Elyazid SM. Effect of Ddb on Mancozeb Fungicide Induced Ultrastructural and Biochemical Changes in The Liver of Albino Mice. Proceedings of the 9th International Conference on Environmental Science and Technology. 1-3 September 2005; B809:B816.
13. Hochstein C, Arnesen S, Goshorn J. Environmental health and toxicology resources of the United States National Library of Medicine. Medical Reference Services Quarterly. 2007; 26(3): 21–45. DOI:10.1300/J115v26n03_02
14. Montesano MA, Wang R. Biomonitoring of Contemporary Pesticides: Ethylenethiourea in Occupational Settings. In; Stoytcheva M, (Ed). Pesticides in the Modern World - Effects of Pesticides Exposure. Croatia: Intech. 2011: 167-80. ISBN 978-953-307-454-2.
15. Winarsi H. Antioksidan Alami dan Radikal Bebas: Potensi dan Aplikasinya dalam Kesehatan. Kanisius. 2011: 26-273.
16. Sumarno, Theresia P, Wahyuningsih R. Peran Antioksidan Pada Ekstrak Tepung Daun Kelor (Moringa Oleifera) Terhadap Kadar MDA (Hepar) Pada Tikus 'Rattus Novergicus Strain Wistar' Yang Dipapari Asap Rokok Akut. E Lebery Universitas Brawijaya Malang. 2011: 1-11.
17. Fakurazi S, Sharifudin SA, Arulselvan P. Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules, 2012; 17: 8334–8350. DOI:10.3390/molecules17078334.
18. El-bakry K, Toson E, Serag M, Aboser M. Hepatoprotective Effect of Moringa Oleifera Leaves Extract Against Carbon Tetrachloride- Induced Liver Damage In Rats. 2016; 5(5): 76-89. DOI: 10.20959/wjpps20165-6638.
19. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. Journal of Ethnopharmacology. 2014; 155(2): 857–924.
20. Karthivashan G, Tangestani FM, Arulselvan P, Abas F, Fakurazi S. Identification of bioactive candidate compounds responsible for oxidative challenge from hydroethanolic extract of Moringa oleifera leaves. Journal of Food Science. 2013; 78(9): 1368–375. DOI: 10.1111/1750-3841.12233.
21. Ferrer-Sueta G, Radi R. Chemical biology of peroxynitrite: kinetics, diffusion, and radicals. ACS Chemical Biology. 2009; 4:161–77. DOI: 10.1021/cb800 279q.
22. Samani MA, Farkhad NK, Azimi N, Fasihi A, Ahandani EA, Kopaei MR. Medicinal plants with hepatoprotective activity in Iranian folk medicine. Asian Pac J Trop Biomed. 2015; 5(2): 146–57.
23. Taha NR, Rabah SO, Shaker SA, Mograby MM. Effect of Moringa oleifera Leaves on Diclofenac Sodium Induced Hepatic Injury in Albino Rats: Ultrastructural and Immunohistochemical Studies. J Cytol Histol. 2015; 6:315. DOI:10.4172/2157-7099.1000315
24. Pessayre D, Mansouri A, Berson A, Fromenty B. Mitochondrial involvement in drug-induced liver injury. Handbook of Experimental Pharmacology. 2010; 196:311–65. DOI: 10.1007/978-3-642-00663-0_11.
25. Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, Li CYJ. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature. 2001; 410:549–554.