

EFFECT OF TURMERIC (*Curcuma Domestica* Val.) RHIZOME ETHANOLIC EXTRACT TO PLASMA LIPID PEROXIDE LEVEL ON WISTAR RAT INDUCED BY TRIMETHYLTIN

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Abstract

Background. Oxidative stress is one cause of death of neurons in the brain. The reduced number of neurons from oxidative stress disrupt memory functions of the brain that causes dementia. Turmeric (*Curcuma domestica* Val.) contains curcumin, which has antioxidant activity.

Objective. This study aims to determine the effect of ethanol extract of turmeric (*Curcuma domestica* Val.) to plasma lipid peroxide level on Wistar rat induced by trimethyltin chloride.

Methods. This study performed 45 male Wistar rats, divided into 6 groups randomly. Group I was a healthy control group, which the rat given CMC-Na solvent. Group I as a negative control was given trimethyltin (TMT) 12.5 mg/kg intraperitoneally on 9th day. Group III as a positive control, was given by Piracetam dose 500 mg/kgBW. The groups IV, V and VI were given turmeric ethanol extract dose 120 mg/kgBW, 240 mg/kgBW and 480 mg/kgBW orally. All treatments carried out for 8 days. On 9th day, groups II, III, IV, V and VI induced by 12.5 mg/kg TMT intraperitoneally. After 24 h of TMT injection, the blood were taken to measure malondialdehyde (MDA) level which is a biomarker of lipid peroxidation using TBARSC₁₈ (Thiobarbituric Acid Reactive Substance C₁₈) method. MDA levels of data were statistically analyzed using the test for normality and homogeneity test followed by ANOVA and Post Hoc Least Significant Difference (LSD).

Outcome measured. MDA levels using TBARSC₁₈ (Thiobarbituric Acid Reactive Substance C₁₈) method

Results. The results showed that administration of ethanol extract of turmeric for 8 days can reduce plasma MDA level on rat induced by TMT. Piracetam dose 500 mg/kg body can lower MDA level 85.53%. While turmeric ethanol extract dose 120, 240 and 480 mg/kgBW lowered MDA levels respectively 46.80%, 61.36% and 81.17%.

Conclusion. Ethanolic extract of turmeric for 8 days can reduce plasma MDA level on rat induced by TMT

Keywords : lipid peroxides, antioxidants, dementia, trimethyltin, ethanol extract of turmeric

INTRODUCTION

Oxidative stress is an important pathogenesis of dementia. Brain cells are particularly vulnerable to oxidative damage because of their high utilization of oxygen and the substantial polyunsaturated fatty acid content, and this organ has limited ability to combat oxidative stress (Halliwell and Gutteridge, 1985; Halliwell, 2001). Oxidative damage to lipid (lipid peroxidation) and protein (protein carbonyl formation) can lead to structural and functional disruption of the cell membrane, inactivation of enzymes, and finally cell death. Oxidative damage to lipid can lead to formation of breakdown products such as malondialdehyde (MDA), 4-hydroxy-2, 3-nonenal (HNE), acrolein, etc. Antioxidant agents from diet have a significant therapeutic influence on various neurodegenerative disorders associated with oxidative stress (Ahmad *et al.*, 2005; Ishrat *et al.*, 2006).

Turmeric contains curcumin which has been known to have antioxidant activity (Rochmaulana, 2009). Curcumin can reduce oxidative stress and amyloid pathology associated with Alzheimer's dementia (Menon and Sudheer, 2007). This study aim is to determine the antioxidant effects of turmeric (*Curcuma domestica* Val.) ethanol extract on MDA plasma levels of rat dementia model induced by trimethyltin.

METHODS

1. Material

Turmeric were obtained from Samigaluh, Kulonprogo, Trimethyltine (TMT) were purchased from Sigma Chem. Other chemicals such as curcumin, H₃PO₄, Tiobaritric Acid, Tetraetoxiprophylene were obtained from Integrated Research and Testing Laboratory, Gadjah Mada University.

2. Animal Treatment

Male adult Wistar rats of 100–20 g BW were used for all experiments. They were housed

in separate cages under 12 h light and 12 h dark periods. Rats had free access to standard food and water ad libitum. 45 male Wistar rats, divided into 6 groups randomly. Group I was a healthy control group, which the rat given CMC-Na solvent. Group I as a negative control was given trimethyltin (TMT) 12.5 mg/kg intraperitoneally on 9th day. Group III as a positive control, was given by Piracetam dose 500 mg/kgBW. The groups IV, V and VI were given turmeric ethanol extract dose 120 mg/kgBW, 240 mg/kgBW and 480 mg/kgBW orally. All treatments carried out for 8 days. On 9th day, groups II, III, IV, V and VI induced by 12.5 mg/kg TMT intraperitoneally. After 24 h of TMT injection, the blood were taken to measure malondialdehyde (MDA) level which is a biomarker of lipid peroxidation using TBARSC₁₈ (*Thiobarbituric Acid Reactive Substance C₁₈*) method

3. Extraction of turmeric

A total of 250 grams of turmeric powder were weighed and then put into a stirred maserator electric, added 1.0 liters of 96% ethanol, stirred for 3 hours, allowed to stand for 24 hours. The filtrate was evaporated, weighed, and then counted its yield.

4. TLC densitometry-test

TLC test aims to ensure the presence of curcumin in turmeric extract and determine levels of curcumin in the extract. Previously made standard stock solution of curcumin concentration of 1 mg / ml. The standard stock solution was diluted to 0.125; 0.25, 0.5, 1, 2, 4 mg / ml. Sample solution was prepared by dissolving 100 mg of the extract into 10 ml of 96% ethanol. Standard solution and the sample spotted on the fixed phase 60 F₂₅₄ silica gel and eluted with mobile phase chloroform: methanol with a ratio of 9: 1. Paches were read by TLC-densitometer at a wavelength of 426 nm.

5. Determiration of MDA Level

Levels of MDA was determined using the or Reactive Substance C₁₈ (TBARSC₁₈) method (Wuryastuti, 2000). Lipid peroxide precipitation is done by mixing 0.75 m H₃PO₄, TBA 0.25 mL, 0.05 mL of sample / blank / standard TEP and 0.45 mL H₂O with a vortex for 2 minutes, then heated in a waterbath for 60 min at 100 ° C. The mixture then was cooled for 1-2 hours until the temperature reaches 30°C. The mixture was eluted on C₁₈ column, then placed in a 1 cm cuvette for absorbance read with a spectrophotometer at a wavelength of 532 nm. MDA levels is in units (mmol / L)

RESULT AND DISCUSSION

The yield of 96% ethanol extract of the rhizome of turmeric (*Curcuma domestica* Val.) suggests that as many as 1000 g powder turmeric extract obtained weighing 297.7 g extract. While based on the TLC test showed spots of compounds curcumin extract is at Rf 0.65 and

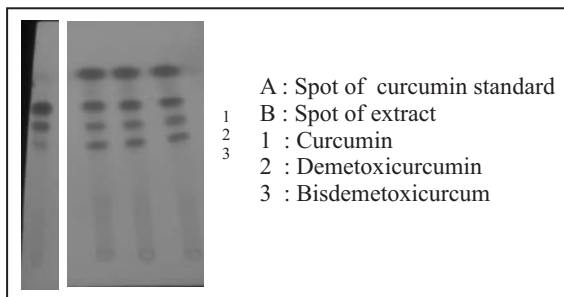


Figure 1. The Profil of Thin Layer Chromatography of Turmeric Extract

turmeric extract containing curcumin compound, with curcumin levels were obtained at 15.78% w / w.

The level of MDA was determined by TBARSC₁₈ method using a spectrophotometer with a maximum wavelength of 532 nm. Principle of the assay by reading the absorbance of the complex MDA-TBA₂ (pink). The complex formation reaction MDA-TBA₂ was shown in Figure 2.

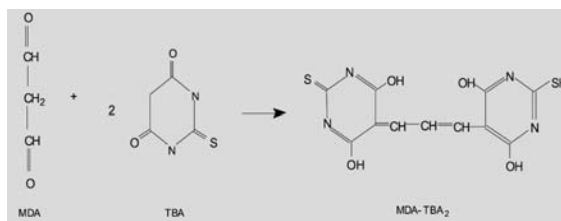


Figure 2. Reaction between MDA and MDA-TBA into TBA₂ (Grotto *et al.*, 2009)

The reaction between one molecule of malondialdehyde (MDA) and two molecules tiobarbiturat acid (TBA) as a nucleophilic attack involving all five of TBA carbon and carbon to one of MDA followed by dehydration and the same reaction occurs in the molecule into two TBA-MDA complex formed TBA₂ pink (Grotto *et al.*, 2009).

Based on statistical tests showed that trimethyltin administration can significantly increase plasma levels of MDA. TMT selectively

Table I. The average of MDA level in all groups

Group	Treatment	Average of MDA levels (mmol/L) ± SD
I	Control	0,0808 ± 0,0392
II	TMT group	2,0742 ± 0,0996
III	Pirasetam group	0,3000 ± 0,1095*
IV	Extract 120 mg/kgBB	1,1035 ± 0,0860*
V	Extract 240 mg/kgBB	0,8014 ± 0,0752*
VI	Extract 480 mg/kgBB	0,3905 ± 0,0643*

*difference significant with TMT group (p<0,05)

standard curcumin is at Rf 0.65. It is proved that can reduce the population of neurons in the brain,

especially the hippocampus formation that plays a role in the memory process. Trimethyltin lead to activation of NMDA receptors and cainic acid toxin that produces a number of free radicals or reactive oxygen species ROS group (Shin *et al.*, 2005). NMDA receptor activation causes the increasing of ROS. ROS are free radicals of oxygen reactive group that includes the triplet ($3O_2$), single (singlet/ $1O_2$), superoxide anion (O_2^-), hydroxyl radical ($-OH$), nitric oxide (NO), peroxy nitrite ($ONOO^-$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), alkoxy radical (LO $^-$), and peroxy radicals (LO-2) (Hadyathma, 2010). Free radicals can attack polyunsaturated fatty acids (PUFA) in the nerve cell membrane and induce lipid peroxidation resulting in increased lipid peroxidation product such as malondialdehyde. The increasing of malondialdehyde has been reported to occur in patients with Alzheimer's dementia (Devore *et al.*, 2010).

The groups administered by turmeric extract dose 120 mg / kg, 240 mg / kg, 480 mg / kg can decrease MDA levels as many 46.80%, 61.36%, 81.17% respectively compared with TMT group.

Turmeric extract can reduce levels of MDA plasma. The largest decrease was shown in the group given 480 mg kg / BW of turmeric extract. Turmeric containing curcumin known as an antioxidant. Curcumin is a lipophilic compound that can enter the blood-brain barrier. The antioxidant activity of curcumin the methoxy group and phenols that can capture free radicals, such as superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals (Chattopadhyay *et al.*, 2004). Binding of free radicals by curcumin produce more stable

products such as vanillin, ferulic acid and curcumin dimer (Fujisawa *et al.*, 2004). Decrease the amount of free radicals by curcumin prevents oxidative stress resulting in lower risk of lipid peroxidation and increased lipid peroxidation products such as malondialdehyde. Therefore, turmeric is expected to be used for the treatment of dementia.

In the group given piracetam indicate that piracetam can also reduce levels of MDA. Piracetam (2-oxo-pyrrolidone) is nootropic drug which is structurally related to GABA. Piracetam have various effects on glutamate neurotransmission at micromolar levels while also piracetam potentiate potassium-induced release of glutamate from hippocampal nerve. Piracetam have an effect on the subunits of glutamate NMDA receptors are involved in learning and memory. Piracetam can give comprehensive effect on brain neurotransmission via modulation of ion channels (ie, Na $^+$, K $^+$). Additionally, piracetam protects neurons against oxidative stress by normalizing activity associated cell membrane (Alkuraishy *et al.*, 2012).

CONCLUSION

The results showed that administration of e turmeric ethanol extract for 8 days can reduce plasma MDA level on rat induced by TMT. Piracetam dose 500 mg/kg body can lower MDA level 85.53%. While turmeric ethanol extract dose 120, 240 and 480 mg/kgBW lowered MDA levels respectively 46.80%, 61.36% and 81.17% compared with TMT group.

Table II. The percentage of the decreasing of MDA levels

Group	Treatment	The percentage of decreasing MDA levels
III	Piracetam group	85,5%
IV	Extract 120 mg/kgBB	45,80%
V	Extract 240 mg/kgBB	61,36%
VI	Extract 480 mg/kgBB	81,1%

REFERENCES

- Ahmad, R., Ali, A.M., Israf, D.A., Ismail, N.H., Shaari, K., and Lajis, N.H., 2005, Antioxidant, radical-scavenging, anti-inflammatory, cytotoxic and antibacterial activities of methanolic extracts of some *Hedyotis species*, *Life Sci*, 76(17): 1953-1964.
- Alkuraishy H. M., Mahdi A. H., Ashoor A. W., 2012, *Piracetam (2-Oxo-Pyrrolidone) Advance And Modulate Central Processing Reaction Time in Normal Healthy Volunteers: Randomized Experimental Study; Single Blind Clinical Trail*, Webmed Central : Journal of Clinical research and healthcare management, 3(2): WMC003109.
- Chattopadhyay, I., Biswas, K., Bandyopadhyay, U., and Banerjee, R.K., 2004, Turmeric and curcumin: Biological actions and medicinal applications, *Current Science*, Vol. 87, No. 1.
- Fujisawa, S., Atsumi, T., Ishihara, M. and Kadoma, Y., 2004, Cytotoxicity, ROS-generation Activity and Radical-scavenging Activity of Curcumin and Related Compounds, *Anticancer Research*, 24: 563-570.
- Grotto, D., Maria, L. S., Valentini, J., Paniz, C., Schmitt, G., Garcia, S.C., Pomblum, V.J., Rocha, J.B.T., Farina, M., 2009, Importance of the Lipid Peroxidation Biomarkers and Methodological Aspects for Malondialdehyde Quantification, *Quim. Nova*, Vol. 32, No. 1, 169-174.
- Hadyathma, R.I., 2010, Gambaran perilaku para pekerja jalan raya tentang penggunaan antioksidan dan tindakan pencegahan dalam menangkal radikal bebas di Kecamatan Medan Amplas, *Laporan Penelitian*, Fakultas kedokteran Universitas Sumatera Utara.
- Ishrat, T., Khan, M.B., Hoda, M.N., Yousuf, S., Ahmad, M., Anshari, M.A., Ahmad, A.S., and Islam, F., 2006, Coenzyme Q10 modulates cognitive impairment against intracerebroventricular injection of streptozotocin in rats, *Behav Brain Res*, 171(1): 9-16.
- Menon, V.P., and Sudheer, A.R., 2007, *Antioxidant and Anti-Inflammatory Properties of Curcumin*, 595 :105-125, Springer Science+Business Media, LLC, Spring Street, New York, USA.
- Rochmaulana, R.A dan Wahyudi, A., 2009, Sintesis Senyawa Turunan Kurkumin dan Uji Aktivitas Antioksidan dengan Metode Bleaching â-Karoten, *Prosiding Skripsi Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Teknologi Sepuluh Nopember*, Surabaya.
- Shin, E.J., Suh, S.K., Lim, Y.K, Jhoo, W.K., Hjelle, O.P., Ottersen, O.P., Shin, C.Y., Kim, D.S., Chun, W., Ali, S., Kim, H.C., 2005, Ascorbate Attenuate Timethyltin-Induced Oxidative Burden and Neuronal Degeration in The Rat Hippocampus by Maintaining Glutathione Homeostatis, *Neuroscience*, 133: 715-727.
- Wuryastuti, H, 2000, The Influence of Dietary Protein and Fats on Plasma Lipid in Sprague-Dawley rats, *Indonesian food and Nutrition mProgress*; 7(2):38.

