

PROTECTIVE EFFECT OF TURMERIC (*Curcuma longa*) ETHANOL EXTRACT TO CATARACT OCCURENCE ON RAT INDUCED BY SODIUM SELENITE

Sapto Yuliani, Annis Widyastuti

Faculty of Pharmacy, University of Ahmad Dahlan
Jl. Prof. Soepomo SH Janturan Yogyakarta, Indonesia

Abstract

Background. One of the causes of cataract formation is oxidative stress. The free radicals can initiate lens opacification. Turmeric (*Curcuma longa*) is one of plants containing curcumin which have antioxidant activity. The objective of this study was to define the protective effect of turmeric ethanol extract to the cataract occurrence on Wistar rat pups induced by sodium selenite.

Method. Cataract was induced in 9-day-old Wistar rat pups. Pups in test group were injected intraperitoneally with 25 μ mol of sodium selenite per kg/BW. Three days before the selenite challenge, rats divided randomly into the following groups (n=5 per group) : the first group was as control group, the second group was injected by the extract solvent, the third group was injected by vitamin E dose 378 IU/kgBW, the fourth-the sixth group was injected by ethanol extract of turmeric dose 125 mg/kgBW, 250 mg/kgBW 500 mg/kg bw respectively.

Results. The cataract were observed in all groups on day 16 of postnatal, when the eyes of the pups first opened. The result showed that the occurrence of cataract is 80% on the rat given ethanol extract dose 250 mg/kgBW and 40% on the 500 mg/kgBW. **Conclusion.** It was suggested that turmeric ethanol extract can decrease the occurrence of cataract on Wistar rats induced by sodium selenite.

Key words : cataract, turmeric (*Curcuma longa*), ethanol extract, sodium selenite

INTRODUCTION

Cataract is an opacity of the eye lens. Cataract remains a major cause of blindness, affecting over 20 million of the nearly 45 million blind people worldwide with the highest incidence in developing countries (Nirmalan et al., 2003). Presently, surgery is the only approach for the treatment of cataract, and while favorable outcomes are quite predictable. The limited number of surgeons in underdeveloped countries and the high cost of surgery have made cataract became a major public health problem (Asbell et al., 2005).

Cataracts are the most frequent cause of treatable blindness worldwide with the majority of cataracts occurring in the elderly. Oxidative damage is currently held to be a major cause of age related nuclear cataract (Truscott, 2005). Extensive oxidation of lens protein and lipid is associated with human cataract. Opacity of the lens is a direct result of oxidative stress.. Free radicals can initiate lens opacification by oxidizing lens protein and lipid (Ishchenko et al., 2003)

Antioxidants are the protective agents that can inactivate reactive oxygen species and therefore significantly delay or prevent oxidative damage (Ahmad et al., 2005) The previous evidence suggested that curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione), a products of turmeric and the dried rhizome of *Curcuma longa*, may be useful for the prevention and/or treatment of some age-related degenerative disorders. Turmeric, commonly known as a yellow curry spice, is used widely among Asians as a food flavoring and preservative and as a herbal remedy for the treatment of coryza, hepatic disorders, and rheumatism (Sharma et al., 2005). Curcumin and other products isolated from turmeric are well known to possess potent antiinflammatory and antioxidant properties (Bisnoi et al., 2008). It inhibits in vitro lipid peroxidation of the brain

scavenges nitric oxidebased radicals and is several times more potent than vitamin E as a free radical scavenger (Dohare et al., 2008) The objective of this study was to define the protective effect of turmeric ethanol extract to the cataract occurrence on Wistar rat pups induced by sodium selenite.

METHOD

2.1. Animal

The nine days old Wistar rats, weighing of 15-20 g were procured from Laboratory of Integrated Testing and Research, Gadjah Mada University. The rats were kept under standart housing condition .

2.2. Materials

Na-Selenite was purchased from Sigma (Jerman). Other chemical such as formaldehyde, hematoxilyne eosin, xylem, ethanol were obtained from Pathology Dept, Veterinary Faculty Gadjah Mada University and Parmacology Dept. Pharmacy Faculty, Ahmad Dahlan University. Turmeric was purchased from PT. Merapi Farma

2.3. Method

2.3.1. Extraction of Turmeric

Identification of the samples was done using standard botanical monographs. The ethanol extract was prepared by maceration of 500 g of turmeric powder (*Curcuma longa* L.) in 1,25 l of 96% ethanol allowed to stand overnight. The solution was filtered, concentrated, dried, weighed and calculated its rendemen.

2.3.2. Experimental design

Cataract was induced in 9-day-old Wistar rat pups. Pups in test group were injected intraperitoneally with 25 μ mol of sodium

selenite per kg/BW. Three days before the selenite challenge, rats divided randomly into the following groups (n=5 per group) : the first group was as control group, the second group was injected by the extract solvent, the third group

RESULTS AND DISCUSSION

Previously, we did pre-research to determine a single dose of sodium selenite that can induce cataract formation. The pre-research result is showed as following Table 1 :

Tabel I. Various dose to determine a single dose sodium selenite

No.	Dose 15 µ mol/ kg BW		Dosis 25 µ mol/ kg BW		Dosis 50 µ mol/ kg BW	
	right	left	right	left	right	left
1.	-	-	cataract	cataract	death	death
2.	-	cataract	cataract	cataract	death	death
3.	-	-	cataract	cataract	death	death



Figure 1. Macroscopic figure, normal eye (A) and cataract eye (B)

was injected by vitamin E dose 378 IU/kgBW, the fourth-the sixth group was injected by ethanol extract of turmeric dose 125 mg/kgBW, 250 mg/kgBW 500 mg/ kg bw respectively. Then, it is calculated percentage of cataract occurrence as the follow:

% of cataract occurrence =

$$\frac{\text{Number of rats with cataract in one group}}{\text{Number of entire rats in one group}} \times 100\%$$

Finally the rats were executed, and their eyes were taken for lens histopathological analyses with hematoxiline eosin staining and observed under microscop.

From Table 1 shows that if the rats were injected a single dose 15 µmole/kg BW, there were no rat with cataract formation. However, If the rats were injected by a single dose 50 µmole/kg BW, all rats were death. The cataract formation occur on all rats induced sodium selenite dose 25 µmole/kg BW, thus in this study we used a single dose 25 µmole/kg BW sodium selenite. Selenite-induced cataractogenesis in young rats has been shown to mimic human senile cataract with respect to several morphologic and biochemical changes in the lens. This model, which was being reproducible, has been used extensively to evaluate the anticataract potential of different test agents (Gupta et al., 2003).

The mammalian lens are composed of approximately 65% water and 35% organic compounds, mainly water-soluble structural proteins such as K-, L- and Q-crystallins. The arrangement of the crystallins as well as the interaction between water and the crystallins within the lens is believed to contribute significantly to the maintenance of lens transparency. In general, a disorder in the arrangement of the crystallins brings about lens opacification. In cataract, a general name for those diseases in which the eye lens becomes opaque, lens protein aggregates are always formed (Pande et al., 2001). It is therefore very

important to elucidate the mechanism of the protein aggregates in cataractous lenses.

A single dose of selenite administration leads to impaired oxidative defense, membrane damage, and cataract formation. Oxidation of the critical sulfhydryl groups of Ca⁺²-ATPase on lens epithelial membrane, influx of calcium from the aqueous humor, activation of calpain, cleavage of N-terminal extensions of α -crystallins of the lens, interaction between exposed charged groups, and the formation of insoluble protein aggregates are some of the steps leading to the development of opacification (Gupta et al., 2003).

products isolated from turmeric are well known to possess potent antiinflammatory and antioxidant properties (Bala et al., 2006; Kuhad and Chopra, 2007). It inhibits in vitro lipid peroxidation scavenges nitric oxidebased radicals and is several times more potent than vitamin E as a free radical scavenger (Perluigi et al., 2006) Antioxidant are protective agents that inactivate reactive oxygen species and therefore significantly delay or prevent oxidative damage such as cataract.

The administration of selenite seems to disrupt oxidative defense mechanisms, as well as elevate calcium and turn on degradative enzymes

Table II. Percentage of cataract occurrence in all group

Group	Percentage of cataract occurrence
Group I (health control)	0%
Group II (vehicle control)	100%
Group III (Vit. E 378 IU/kg bw)	0%
Group IV Ethanol extract dose 125 mg/kgbw	100%
Group V Ethanol extract dose 250 mg/kgbw	80%
Group V Ethanol extract dose 500 mg/kgbw	40%

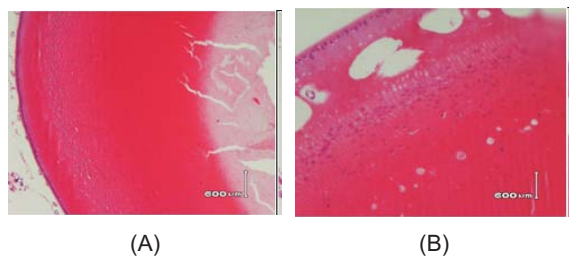


Figure 2. Microscopic figure of (A) normal lens, (B) vacuolization lens

Table II show that the higher dose consecutive of turmeric ethanol extract the lower percentage of occurrence cataract. Turmeric has antioxidant activity. Indeed, curcumin and other

(Shearer et al., 1997). The mechanism of damage is being actively investigated and may be directly relevant to human cataract formation. However, because of the method of initiation and variable response to selenite in different species, it is likely that other animal models that generate ROS as the major source of oxidative damage may be more suitable for the study of human age-related cataracts

CONCLUSION

Extract ethanol of turmeric has the protective effect to cataract occurrence on Wistar rats pups induced by sodium selenite.

REFERENCES

- Asbell, P.A., Dualan, J. Mindel, D. Brocks, M. Ahmad, S. Epstein, Agerelated cataract, *Lancet* 365 (2005) 599–609.
- Ahmad, M., Saleem, S., Ahmad, A.S., Yousuf, S., Ansari, M.A., Khan, M.B., Ishrat, T., Chaturvedi, R.K., Agrawal, A.K., Islam, F., 2005. Ginkgo biloba affords dose-dependent protection against 6-hydroxydopamine-induced parkinsonism in rats: neurobehavioural, neurochemical and immunohistochemical evidences. *J. Neurochem.* 93, 94–104
- Bala, K., Tripathy, B.C., Sharma, D., 2006. Neuroprotective and antiageing effects of curcumin in aged rat brain regions. *Biogerontology* 7, 81–89.
- Bishnoi, M., Chopra, K., Kulkarni, S.K., 2008. Protective effect of curcumin, the active principle of turmeric (*Curcuma longa*) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain. *Pharmacol. Biochem. Behav.* 88, 511–522.
- Dohare, P., Garg, P., Jain, V., Nath, C., Ray, M., 2008. Dose dependence and therapeutic window for the neuroprotective effects of curcumin in thromboembolic model of rat. *Behav. Brain Res.* 193, 289–297.
- Gupta, S.K., Trivedi, D., Srivastava, S., Joshi, S., Halder, N., and Verma, S.D., 2003. Lycopene attenuates oxidative stress induced experimental cataract development an in vitro and in vivo study. Basic Naturally Investigation. University of Maryland. Collage Park. Maryland. USA
- Kuhad, A., Chopra, K., 2007. Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. *Eur. J. Pharmacol.* 576, 34–42.
- Nirmalan, P.K., Krishnadas, R., Tamakrishman, R., Thulasiraj, R., Katz, J., Tielsch, J.M., Robin, A.I., 2003. Lens opacities in a rural population of southern India: the Aravind Comprehensive Eye Study. *Invest. Ophthalmol. Vis. Sci.* 44, 4639–4643.
- Pande, A., Pande, J., Asherie, N., Lomakin, A., Ogun, O., King, J., Benedek, G.B., 2001. Crystal cataracts: human genetic cataract caused by protein crystallization. *Proc. Natl Acad. Sci.* 98, 6116–6120.
- Perluigi, M., Joshi, G., Sultana, R., Calabrese, V., De Marco, C., Coccia, R., Cini, C., Butterfield, D.A., 2006. In vivo protective effects of ferulic acid ethyl ester against amyloid-beta peptide 1-42-induced oxidative stress. *J. Neurosci. Res.* 84, 418–426.
- Sharma, R.A., Gescher, A.J., Steward, W.P., 2005. Curcumin: the story so far (Review). *Eur. J. Cancer* 41, 1955–1968.
- Shearer, T.R., Ma, H., Fukiage, C., Azuma, M., 1997. Selenite nuclear cataract: review of the model. *Mol. Vis.* 3, 8.
- Truscott, R.J., 2000. Age-related nuclear cataract: a lens transport problem. *Ophthalmic Res.* 32, 185–194.
- Ishchenko, A., Sinitsyna, O., Krysanova, Z., Vasyunina, E., Sapparbaev, M., Sidorkina, O., Nevinsky, G., 2003. Age-ependent increase of 8-oxoguanine-, hypoxanthine-, and uracil- DNA glycosylate activities in liver extracts from OXYS rats with inherited overgenration of free radicals and Wistar rats. *Med. Sci. Monit.* 9, 16–24.

