

JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

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Original Research Article

Effect Of Zinc Administration in Preventing Ethambutol-Induced Optic Neuropathy in Wistar Rats Model

Disti Hardiyanti^{1,3}, Maharani^{1,3}, Arief Wildan^{1,3}, Arnila Novitasari Saubig^{1,3}, Hermawan Istiadi^{2,3}, Riski Prihatningtias^{1,3}

¹Department of Ophthalmology, Universitas Diponegoro, Indonesia

²Department of Pathology Anatomy, Universitas Diponegoro, Indonesia

³Dr Kariadi General Hospital, Indonesia

Article Info

History

Received: 12 Apr 2023

Accepted: 20 Aug 2023

Available: 31 Aug 2023

Abstract

Background: Ethambutol-induced optic neuropathy (E-TON) is one of the most common optic neuropathies, especially in developing countries with an increasing rate of tuberculosis (TB). Various outcomes of visual impairment that may lead to permanent visual loss makes it imperative to prescribe supplementation to prevent E-TON. Zinc supplementation is suggested as one of many to prevent E-TON since ethambutol is a zinc chelator.

Purpose: To analyze the apoptosis rate of retinal ganglion cell (RGC) in Wistar rats given ethambutol and zinc supplementation.

Methods: A true experimental research, post-test only control group design using wistar rats was done. Subjects were divided into 2 groups, treatment group was given ethambutol and zinc and control group was given ethambutol only for 30 days. Assessment of apoptosis rate from hematoxylin eosin staining specimen was observed by two pathologist using histopathological scoring. Differences between two groups were statistically analyzed using Mann Whitney U test, (significant $p < 0.05$).

Results: The score of apoptosis rate in treatment group was 28.6% and in control group was 71.4%. There was a significantly different in apoptosis retinal ganglion cell between two groups ($p = 0.015$).

Conclusion: The rate of retinal ganglion cell apoptosis was lower in rats treated ethambutol with zinc than ethambutol only.

Keywords: E-TON; zinc; retinal ganglion cell

Permalink/ DOI: <https://doi.org/10.14710/jbtr.v9i2.17973>

INTRODUCTION

Ethambutol-induced optic neuropathy (E-TON) is one of the most common and recognized drug-induced optic neuropathies, especially in developing countries with increasing rate of tuberculosis (TB). Treatment for TB is divided into two phase, intensive and continuation phase. Ethambutol is one of the medications used in the intensive phase.^{1,2}

According to World Health Organization (WHO), the incidence of TB in 2016 is 10.4 million (CI 8.8-12 million) or equivalent to 120 cases in 100.000 population. The incidence of ethambutol-induced optic neuropathy has been reported to range from 1.0% to 22%. The reported incidence of E-TON varies between 18% in patients receiving more than 35 mg/kg per day,

5% to 6% with 25 mg/kg per day, and less than 1% with 15 mg/kg per day of ethambutol. No 'safe dose' of ethambutol has been reported, 0.3% developed E-TON with dose of < 15 mg/kg/day.³⁻⁵

Reports of E-TON in Indonesia were varied in each area. Indrayani et al recorded 5 of 30 patients (16.7%) with TB on ethambutol developed E-TON in Sanglah Hospital Bali. Juzmi et al reported 3 of 119 patients developed E-TON in Wahidin Sudirohusodo Hospital Makassar.

* Corresponding author:

E-mail: riski.dikk.undip@gmail.com

(Riski Prihatningtias)

Table 1. RGC apoptosis between treatment group and control group

Group	Microscopic Apoptosis			Total	p
	No apoptosis	< 25%	25 – 50%		
Treatment	28.6%	71.4%	0%	100%	0.015*
Control	0%	42.9%	457.1%	100%	
Total	14.3%	57.1%	28.6%	100%	

Description: * significant if *p* value < 0,05

Munandar from Cicendo Hospital Bandung, reported 33 patients developed E-TON with various visual disturbances. Based on these data we can conclude that the incidence is low, but because of permanent visual impairment impact of E-TON, it not only need a health concern but also an economic and social problems.⁶⁻⁸

The toxic effect of ethambutol has been recognized as an apoptosis of retinal ganglion cell (RGC) in the excitotoxic glutamate pathway due to zinc chelator in the forming of cytoplasmic vacuole, protein kinase C (PKC) activation, and increase of malondialdehyde (MDA) which results in decrease of endogen antioxidants superoxide dismutase (SOD). Ethambutol binds to cuprum (Cu) and zinc (Zn) in the RGC and optic nerve fibers. Ethylene diimino dibutanol acid is an ethambutol metabolite that strongly binds to Cu and Zn which inhibits the electron transport chain and disrupted oxidative phosphorylation secondary to decreased available copper in human mitochondria or inhibit lysosomal activation due to the chelation of Zn.^{4,9-14}

The exact mechanism of this ocular neurotoxic effect proved by in vivo and in vitro in rodent's animal studies have demonstrated ethambutol toxicity in the retinal ganglion neurons of rodents. One of the principal theories for its toxicity has been the zinc-chelating effect of ethambutol and its metabolite. Many postulated biochemical pathways that mediate the toxic damage include downstream effector caspase-3 and caspase-6,8 and an excitotoxic glutamate pathway.^{9,15}

The best management for E-TON is to cease the use of ethambutol.⁵⁻⁷ Previous studies reports Zn therapy in E-TON results in good prognosis. Zn therapy in E-TON may prevent visual field impairment, improve contrast, and prevent progression. These studies reported the effect of Zn after E-TON has developed, but none have suggested supplementary Zn in patients receiving ethambutol before E-TON occurs. Our goal is to research this process.^{3,16-18}

There is currently no effective treatment for E-TON. However, if detected early and with prompt discontinuation of ethambutol, between 30 and 64% of patients show some improvement in their visual disturbances over a period of several months.^{5,19}

MATERIALS AND METHODS

This study was experimental study with post-test only randomized controlled group design using Wistar rats that given ethambutol. Subjects were divided into 2 groups, treatment group was given ethambutol 32 mg/200 gr rat's body weight (BW), equivalent to 25mg/kg human BW and Zn 1.5mg/200 gram rat's BW equivalent to 80 mg in human, and control group was given ethambutol only. Both groups were treated for 30

days. Doses have been converted in accordance to Laurence and Bacharach. This study included 14 Wistar rats, male, age 2-3 months, 200-300 gram of weight. Rats with eye infection and rats that showed no activity within treatment period were excluded.

Rats were acclimatized for 14 days and then divided into 2 groups. After 30 days of treatment period, rats were enucleated, and histopathology examination with hematoxylin eosin was prepared and interpreted by two pathologists. Retinal ganglion cell apoptosis was determined by measuring cell morphology and cell count based on score classification as follows; score 0: no apoptosis, score 1: <25% cell apoptosis, score 2: 25-50% cell apoptosis, score 3: 50-75% cell apoptosis, score 4: >75% cell apoptosis.²⁰ Differences between two groups were statistically analysed using Mann Whitney U test, (significant *p* <0.05).

RESULTS

Seven eyes from seven rats of each group were obtained. No rats died or dropped out during treatment period. RGC apoptosis reliability results were examined by two anatomical pathologist using Cronbach's Alpha and had a 84% Kappa (K), that's means the best inter-rater agreement. (Figure. 1)

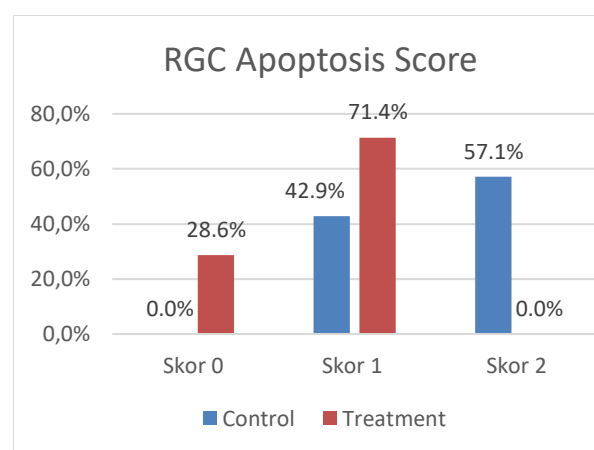


Figure 1. RGC Apoptosis between treatment group and control group

RGC apoptosis in the control group showed none in score 0, 42.9% in score 1, and 57.1% in score 2. Treatment group had 28.6% in score 0, 71.4% in score 1, and nil in score 2. RGC apoptosis between treatment group and control group showed statistical significance (*p*<0.05), in which RGC apoptosis was lower in the treatment group compared to control group (table 1).

Histopathology examination of RGC with HE staining compared in treatment group. Figure 2a represents normal RGC (score 0) with normal, homogenous cells, without apoptotic body. Figure 2b shows wrinkled/smaller, disparate, along with <25% karyorhexis and apoptotic body within 5 high power field.

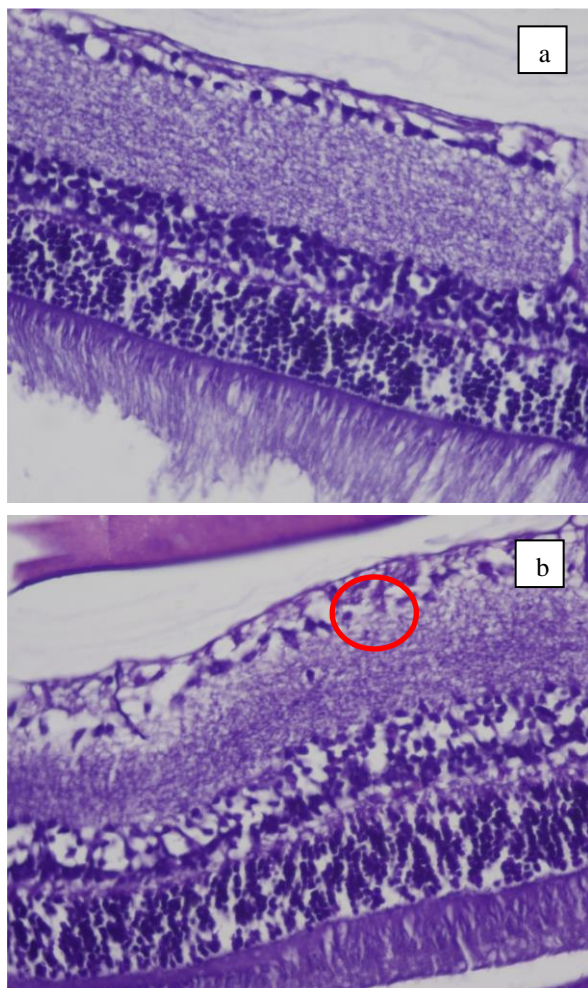


Figure 2. RGC in treatment group, (a). without apoptosis /score 0, (b). apoptosis < 25% / score 1

Figure 3 represents histopathology examination of RGC with HE in the control group. Figure 3a shows wrinkled, smaller, disparate RGC, along <25% of karyorhexis and apoptotic body within 5 high power field. Figure 3b depicts 25-50% / score 2 and show cell loss.

DISCUSSION

This study presents statistically significant differences in RGC apoptosis between the treatment group and control group ($p = 0.015$). RGC apoptosis in treatment group is lower compared to control group. Forty-two-point nine percent of treatment group had apoptotic score 1 and 57.1% had apoptotic score 2.

Based on the theory as a metal chelator, ethambutol interferes with oxidative phosphorylation and inhibits lysosomal activation due to Zn chelators, resulting in decreased mitochondrial function by interfering with complex I (Fe/Zn) and complex IV (Cu). Other studies suggest the damaging effects caused by ethambutol are

due to formation of cytoplasmic vacuoles in RGC which in turn will decrease intracellular Zn.⁹⁻¹⁴

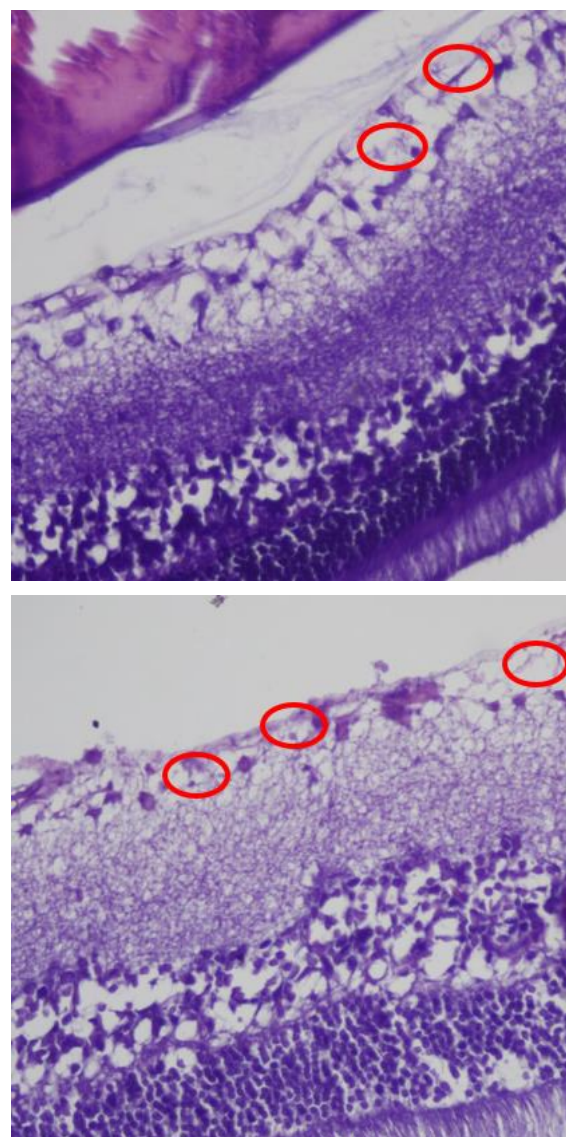


Figure 3. RGC control group, (a) apoptosis <25 % /score 1, (b) apoptosis 25-50% /score 2

Reported cases of E-TON in the population is between 0.1-22%. Based on the dosing that is equivalent to this study (25mg/kgBW), the incidence of E-TON is reported to be 5-6%. However, 100% of rats receiving ethambutol in this study suffered mild-moderate RGC apoptosis. Subclinical E-TON may progress in the early stages in humans. Although incidence of E-TON is reported to be very low, subclinical alterations can be detrimental. Studies evaluating visual evoked potential (VEP) and optical coherence tomography (OCT) reports increased VEP latency, decrease in retinal nerve fiber layer (RNFL) and ganglion cell inner plexiform layer (GCL-IPL) thickness upon follow-up despite no complaints or visual deterioration. Mandal et al. reported the incidence of symptomatic E-TON with a dose of 15-20 mg/kg/day is < 2%. However, 46% had increased VEP latency and decrease in RNFL and GCIPL thickness in asymptomatic patients. This is consistent with a study by Yan Sheng et al that suggest RGC damage after ethambutol treatment although no visual disturbance was

present. Regrettably, functional examination cannot be performed in E-TON rat models.^{21,22}

This study reports no apoptosis amongst 28% of wistar rats that received ethambutol and Zn, mild apoptosis (<25%) in 71.4% rats. This signifies the effect of Zn in reducing the incidence of RGC apoptosis. A study by Hasan suggested declining Zn serum in TB patients under ethambutol treatment may return to its normal limits by cessation of ethambutol and consumption of healthy foods. This is due to ethylene diimino dibutanol which is an ethambutol metabolite that strongly binds to RGC cells. Free ZN (Zn^{2+}) is required as a cofactor for cytochrome c oxidase, the main enzyme for transport chain and cellular oxidase metabolism in the mitochondria. Furthermore, Zn^{2+} serves as a disregulator for RGC and glial cells in the events of nerve injury. Binding of Zn^{2+} will cause disruption of ATP production and ultimately damage the ATP-dependent axonal transport system.^{10,22,23}

This study still reports apoptosis in groups receiving ethambutol and Zn (treatment group) though to a lesser degree. This is due to apoptosis occurring in normal cells to maintain hemostasis. Typically, cells will carry out a regeneration process along with cell death called apoptosis.

Zinc supplementation may ameliorate declining Zn serum caused by this mechanism. Daniati suggested Zn supplementation in E-TON patients may prevent visual field abnormalities. Similarly, Rindawati reported a significant difference in contrast sensitivity between patients with and without ethambutol. A study by Yulianti et al shows that Zn supplementation serves as a neuroprotector against toxic effects of ethambutol upon VEP examination. Another study by Fitirah presented decrease of RNFL thickness in the temporal quadrant but none in the nasal, superior, and inferior quadrant that measured using spectral domain optical coherence tomography (SD-OCT).^{16-18,24}

Yudapratwi reported no significance in RGC density between treatment and control groups in rats given ethambutol, 75.5% and 76% respectively. However, histopathology findings presents an orderly arranged cell structure amongst groups given Zn supplementation. A difference in research results is due to interpretation readings where 1 field of view with 400x magnification was performed while our study examined 5 field of view which represents a broad view of RGC. Both Yudapratwi and our study used the same dose, equivalent to 25mg/kgBW in humans for 30 days.²⁵

Zn supplementation in TB patients provides many benefits, not only does it improve RGC structure, boosts the immune system, but also accelerates the conversion to negative sputum after 2 months of Zn supplementation. Given the many advantages of Zn supplementation, it is favourable to prescribe it as a prevention to E-TON, specifically subclinical E-TON.²⁴⁻²⁶

Limitation of this study was indistinction between ethambutol related apoptosis or normal apoptosis that occurs at the cellular level.

CONCLUSION

Analysis of both groups presents lower RGC apoptosis in rats given ethambutol and Zn. Further

research is necessary to evaluate mediating and confounding factors that may influence RGC apoptosis. Additional studies on humans in association with internists to provide a larger sample is essential. The authors expect Zn to be a obligatory supplement in patients receiving ethambutol.

ACKNOWLEDGMENTS

The authors do not have a proprietary interest in any of the materials used in this study. This research was approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Diponegoro according to recommendations of the Association for Research and Vision in Ophthalmology (ARVO).

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