·Basic Research·

Gene expression and histopathological evaluation of thiamine pyrophosphate on optic neuropathy induced with ethambutol in rats

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Abstract

• AIM: To compare the effects of thiamine pyrophosphate (TPP) and thiamine (TM) in oxidative optic neuropathy in rats induced by ethambutol.

• METHODS: The animals were divided into four groups: a control group (CG), an ethambutol control (ETC) group, TM plus ethambutol group (TMG), and TPP plus ethambutol group (TPPG). One hour after intraperitoneal administration of TM 20 mg/kg to the TMG group and TPP 20 mg/kg to TPPG group, 30 mg/kg ethambutol was given *via* gavage to all the groups but the CG. This procedure was repeated once daily for 90d. After that period, all rats were exposed to high levels of anaesthesia in order to investigate the gene expression of malondialdehyde and glutathione in removed optic nerve tissue and histopathologically to examine these tissues.

• RESULTS: Malondialdehyde gene expression significantly increased, whereas glutathione gene expression significantly decreased in the ETC group compared to the CG. TM could not prevent the increase of malondialdehyde gene expression and the decrease of glutathione, while TPP significantly could suppress. Histopathologically, significant vacuolization in the optic nerve, single-cell necrosis in the glial cells, and a decrease in oligodendrocytes were observed in the ETC group. Vacuolization in the optic nerve, a decrease in oligodendrocytes and single-cell necrosis were found in the TMG group, while no pathological finding was observed in the TPPG group except for mild vacuolization.

• CONCLUSION: TPP protects the optic nerve against the ethambutol-induced toxicity but TM does not. TPP can be beneficial in prophilaxis of optic neuropathy in ethambutol therapy.

• KEYWORDS: ethambutol; gene expression; optic neuropathy; rat; thiamine pyrophosphate
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INTRODUCTION

Ethambutol is a commonly used drug for the treatment of tuberculosis. Ethambutol often is used in cases with resistance anti-tuberculosis drugs [1]. Therefore, ethambutol usually is required for use against most tuberculosis strains. However, side effects observed during ethambutol therapy remain one of the most important causes of treatment discontinuation. Although side effects such as peripheral nervous system disease, thrombocytopenia and hepatotoxicity are seen with ethambutol use [2-4], its most important side effect is optic neuropathy. Ethambutol has adverse side effects on peripheral nervous system disease, thrombocytopenia, and hepatotoxicity [2-4], but mainly on neuropathy [5]. Optic neuropathy symptoms develop months after initiation of the treatment [6]. However, emerging toxic neuropathies after taking ethambutol also have been reported [7]. Studies have demonstrated that optic neuropathy might occur with ethambutol treatment even at low doses[7-8]. Optic neuropathy connected with ethambutol has been reported to dose dependently range from 3%-10%[9]. This demonstrates that the toxicity of ethambutol in the optic nerve is selective

compared to other tissues. Therefore, elucidation of the toxic effects of ethambutol on the optic nerve and treatment for pathogenesis is important.

There are no studies in the literature elucidating toxic mechanism of ethambutol. However, oxidative stress was observed in patients after medical therapy with ethambutol and other anti-tubercular drugs, suggesting the side effects of these medications in the etiopathogenesis [10]. Sahin *et al* [11] reported that experimentally administered ethambutol increases the amount of malondialdehyde (MDA), a lipid peroxidation product in the optic nerve that decreases activity of the endogenous antioxidant superoxide dysmutase (SOD), producing oxidative damage, whereas Nebbioso *et al*[12] reported that oxidative injury of the optic nerve can be treated with antioxidants. Aditionally, Cinici *et al*[13] reported that thiamine pyrophosphate (TPP) protect the eye from ethambutol toxicity by inhibiting the overproduction of MDA, and preserving the total glutathione (tGSH) level.

There is no data in the literature about protective effects of TPP and TM against ethambutol-induced oxidative optic nerve damage. Therefore, the aim of this study was to investigate the gene expression and histopathological effects of TPP in oxidative optic neuropathy induced with ethambutol in rats and to evaluate these effects in comparison with TM.

MATERIALS AND METHODS

Animals Animals used in this study were supplied by the Recep Tayyip Erdogan University, Medical Experimental Application & Research Center. A total of 48 male albino Wistar rats weighing 340-350 g were used in the experiment. Animals were housed and fed at room temperature (22℃) in the pharmacology laboratory for 1wk before the experiment. Animal experiments were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals and approved by the Local Animal Ethics Committee of Recep Tayyip Erdogan University, Rize, Turkey (ethics committee number: 2015/10; dated: 2015-02-20).

Chemical Agents Sodium thiopental was obtained from Ibrahim Etem Ulagay, Turkey; thiamine (TM) and TPP from Biopharma, Russia; and ethambutol from Unipharm Drug, Turkey.

Experimental Procedure Rats used in this study were divided into four groups, a control group (CG), an ethambutol control (ETC) group, a TM plus ethambutol group (TMG), and a TPP plus ethambutol group (TPPG). An injection was administered i.p. to the TMG (n=12) group and 20 mg/kg TPP to TPPG (n=12) group. Ethambutol was given *via* oral gavage to the TMG, TPPG, and ETC (n=12) rat groups one hour after the original drug administration. Distilled water was administered as a solvent at the same volume in the CG (n=12) group. This procedure was repeated once a day for 90d [13]. After the period was over, all the rats were killed with a high dose administration of thiopental sodium anaesthesia and gene expression of MDA and glutathione (GSH) were determined in the removed optic nerve tissues. In addition, tissue samples were examined histopathologically. Gene expression and histopathological outcomes obtained with the TMG, TPPG, and CG groups were evaluated in comparison with outcomes of the ETC group.

Gene Expression of Malondialdehyde and Glutathione RNA isolation RNA was isolated from the homogenizated optic nerve samples using the Roche Magna Pure Compact LC device (Meinheim, Germany) with MagNA Pure LC RNA Kit (Roche Diagnostics, Germany). The quantity and quality of the isolated RNA was assessed with a nucleic acid measurement device (Maestro Nano, USA). A total of 50 μL of RNA samples were stored at -80℃.

cDNA synthesis The cDNA was synthesized from the isolated RNA samples using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics). For each subject, 1 μL ddH_2O, 10 μL RNA, and 2 μL random primer were combined and incubated in a thermal cycler for 10min at 65℃. After incubation, 4 μL reaction buffer, 0.5 μL RNAase, 2 μL deoxynucleotide mix, and 0.5 μL reverse transciptase were added, and the reactions were incubated for 10min at 25℃, 30min at 55℃, and 5min at 85℃, then held at 4℃.

Quantitative gene expression evaluation by real-time polymerase chain reaction For each cDNA sample, gene expression of MDA and GSH, and thereference gene (G6PD) were analyzed using the Roche LightCycler 480 II real-time polymerase chain reaction (PCR) instrument (Meinheim, Germany). The PCR in a final volume of 20 μL: 5 μL cDNA, 3 μL distilled water, 10 μL LightCycler 480 Probes Master (Roche Diagnostics, Germany) and 2 μL primer-probe set (Real-Time Ready single assay-Roche, Germany). Cycle conditions of the relative quantitative polymerase chain reaction (qPCR) were preincubation at 95℃ for 10min, followed by 45 amplification cycles of 95℃ for 10min, 6℃ for 30s, 72℃ for 1min, followed by cooling at 40℃ for 30min. A qPCR analysis and calculation of the quantification cycle (Cq) values for relative quantification were performed with the LightCycler 480 Software, Version 1.5 (Roche Diagnostics). Relative quantitative amounts were calculated by dividing the target genes by the expression level of the reference gene. The reference gene was used for normalization of target gene expression.

Histopathologic examination Enucleaction materials removed from the rats were fixed in a 10% formalin solution, and 5 μm sections were obtained from the paraffin blocks after the routine tissue monitoring process and stained using haematoxylin and eosin (H&E). All the sections were coded and examined under a light microscope (Olympus BX 51,

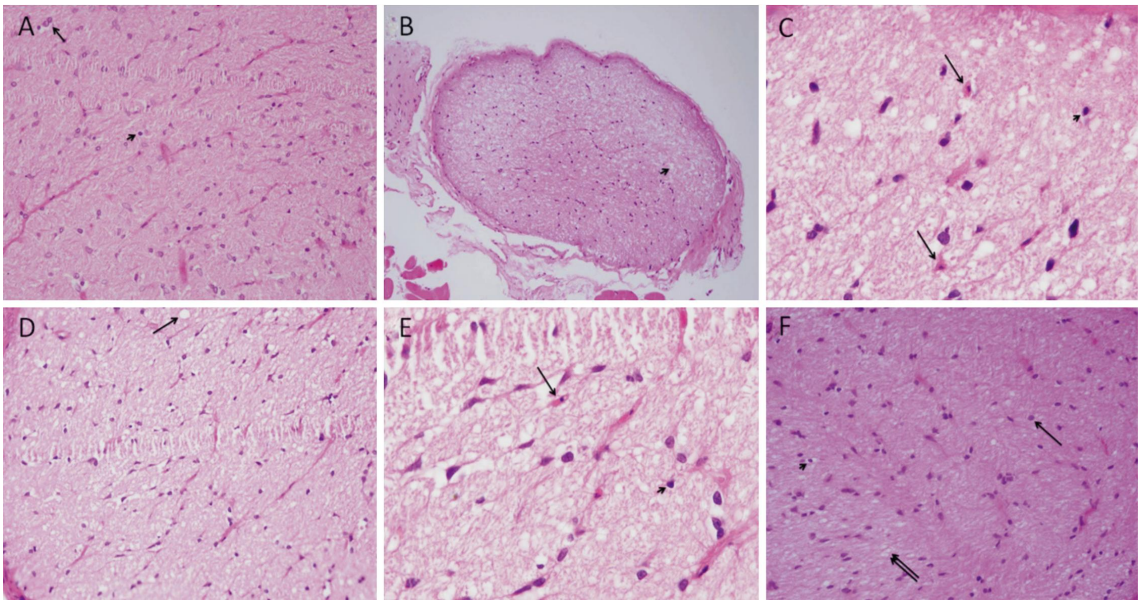

Figure 1 Light microscopic view of the study groups A: CG normal histopathologic view; B: ETC vacuolization (arrow); C: ETC single-cell necrosis in the glial cells (long arrow) and decrease in the oligodendrocytes (short arrow); D: The TMG vacuolization (arrow); E: TMG decrease in the oligodendrocytes (short arrow) and single-cell necrosis (long arrow); F: The TTPG groups normal numbers of astrocytes (long arrow), oligodendrocytes (short arrow) and mild vacuolization (double black arrow).

Tokyo, Japan) by two independent pathologists who were blind to the treatments applied and the images were taken with a digital camera (Olympus DP 71).

Statistical Analysis Statistical analyses were carried out using the Statistical Package for Social Sciences, Windows version 18.0 (SPSS, Chicago, IL, USA). Descriptive statistics for each variable were determined. Normality of the data distribution was assessed with the Kolmogorov-Smirnov test. Results for continuous variables were demonstrated as mean± standard error of the mean (mean±SEM). The significance of differences between the groups was determined using the one-way ANOVA test followed by Fisher's post-hoc LSD (least significant differences) analysis. A *P* value less than 0.05 was considered significant.

RESULTS

Histopathologic Findings In Figure 1A, normal histopathologic view of astrocyte (long arrow) and oligodendrocytes (short arrow) of the optic nerve in the CG group was monitored under a light microscope (H&E, ×40). All of the TMG and ETC group which examined the histopathology have been found with obvious signs of optic neuropathy. However, only 33% of TPPG group was seen mild vacuolization. Vacuolization (arrow) was marked with the small magnification of the optic nerve in the ETC group, which received only ethambutol (H&E, ×10; Figure 1B). Whereas in the large magnification, marked single-cell necrosis in the glial cells (long arrow) and a decrease in the oligodendrocytes (short arrow) were monitored with a light microscope (H&E, ×100; Figure 1C). Vacuolization (arrow) was seen in the optic nerve of the TMG group (H&E, ×40; Figure 1D). In the large magnification, a marked decrease in the oligodendrocytes (short arrow) and single-cell necrosis (long arrow) (H&E, ×100; Figure 1E). Normal numbers of astrocytes (long arrow) and oligodendrocytes (short arrow) and mild vacuolization (double arrow) were observed in the optic nerves of the TPPG group (H&E, ×40; Figure 1F).

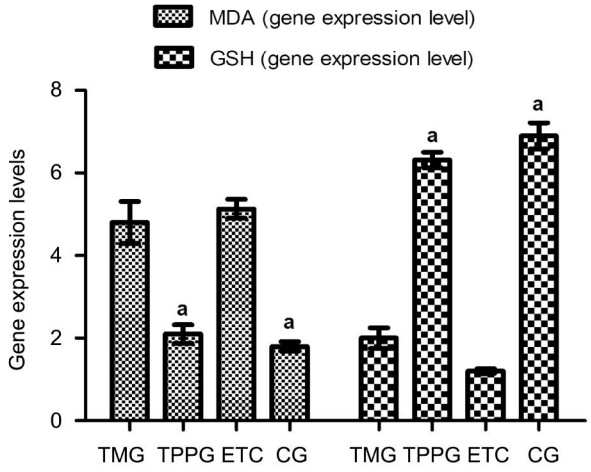

Figure 2 The effects of TMG and TPPG on MDA and GSH gene expression levels against optic neuropathy induced with ethambutol in rats [a]*P* <0.0001, according to ETC group.

Gene Expression As seen in Figure 2, MDA gene expression levels in the optic nerve tissue of the CG, ETC, TMG, and TPPG groups were found to be 1.8±0.28, 5.1±0.56, 4.8±1.25, and 2.1±0.55, respectively. The increase in the MDA gene expression was significantly greater in the optic nerve tissue of the ETC group compared to the CG group (*P*<0.0001). The increase in the ethambutol-induced MDA gene expression was not prevented by TM (*P*>0.05), whereas TPP significantly prevent the increase (*P*<0.0001).

GSH gene expression levels in the optic nerve tissue of the CG, ETC, TMG, and TPPG groups were found 6.9±0.78, 1.2±0.14, 2.0±0.61, and 6.3±0.46, respectively (Figure 2). Ethambutol led to a decrease of GSH gene expression in the optic nerve tissue of rats (P<0.0001). TPP prevented the decrease in GSH gene expression due to ethambutol (P< 0.0001), whereas TM was insufficient to prevent of the decrease (P >0.05) (Figure 2).

DISCUSSION

In this study, the effect of TPP on oxidative optic neuropathy induced with ethambutol in rats was investigated through gene expression and histopathologic examination and evaluated in comparison with TM. The reason for the comparison of TPP to TM was that some drugs inhibit the transformation of TM to TPP, (the active form of TM) by inhibiting the thiamine pyrophosphokinase (TPK) enzyme[14-15]. This causes the reduction of activity of TM. Therefore, TPP compared with TM activity was evaluated. The study also evaluated the relationship between the inhibition of TPK and the toxic effects of ethambutol on the optic nerve. This study investigated the protective effects of TPP against optic neuropathy induced with ethambutol, an active form of TM[16]. TPP was demonstrated to protect the tissue against oxidative stress by decreasing the amount of oxidant increase and alteration of endogenous antioxidants [17]. It has been reported in a previous study that ethambutol causes damage in retinal tissue, increasing oxidant parameters and reducing antioxidant parameters. Also, TPP inhibits the ethambutol-induced oxidative retinal damage, while TM does not, as evidenced by the histopathological findings [13]. This suggests TPP may be useful in prevention and treatment of oxidative optic neuropathy due to ethambutol. Results showed that MDA gene expression increased and GSH gene expression decreased in rat optic nerve tissues administered with ethambutol compared to healthy tissue. It has been reported that ethambutol increases MDA and total oxidant capacity but decreases the antioxidant capacity in the optic nerve tissue of animals [11]. Ethambutol increases the amount of MDA and reduces the amount of GSH in the retinal tissue[13]. The literature review and results of this study indicate that oxidative stress develops in the optic nerve tissue of the ethambutol-administered animals. According to Williams[18], oxidative stress plays a role in the pathogenesis of many eye-disease's stress such as conjunctiva, cornea, uvea inflammation, lens cataract, glaucoma, and optic neuritis. Izzotti *et al*[19] suggested that optic nerve damage result from oxidative stress. Optic neuropathy is a well-known complication of ethambutol therapy [20] and several studies in the literature have demonstrated that antioxidants such as N-acetylcysteine and SOD are useful in the prevention of oxidative injury in the optic nerve[12].

In our study, the MDA and GSH expression levels for the TPPG group were closest to the CG group, and mild vacuolization was observed in the optic nerve tissue. However, marked vacuolization, single-cell necrosis in glial cells and oligodendrocytes decrease were observed in the TMG and ETC groups, having high MDA and low GSH gene expressions. There are several studies showing the significant vacuolization in optic nerve damage [21]. Also, some experimental studies have demonstrated that vacuolization may be caused by the toxic effects of medications [22]. Zubair *et al* [23] histopathologically demonstrated that vacuolization develops in the optic nerve of ehtambutol-administered animals shown in vacuolization caused by ethambutol in retinal tissue [24]. Again single-cell necrosis was found in glial cells of the TMG and ETC groups, while single-cell necrosis was not observed in the TTP administered animals. Single-cell necrosis developed in the optic nerve induced by ethambutol, consistent with the literature. The same finding was reported by Kinoshita *et al* [25]. The negative effect of ethambutol on glial cells was determined by Gong and Amemiya[26], it was expressed as the cause of the reduction of zinc. In addition, TTP prevented a decrease in oligodendrocytes caused by ethambutol. It is understood from some studies that oxidative stress plays a role in pathogenesis decrease in oligodendrocytes [27]. In a study conducted by El-Sayyad *et al* [28] with animals, demyelination decrease in oligodendrocytes and astrocytes were argued to be a result of oxidative stress. As is known, the astrocytes are a type of glial cell and the myelin is produced by oligodendrocytes. This information from the literature is consistent with our histopathological results. The literature reports that oxidative stress in oligodendrocytes depends on the use of ethambutol or B-group vitamin deficiency [29]. This is consistent with our experimental results. In our study, we determined that the TPP protects against the toxic effects of ethambutol on the optic nerve tissue, but TM doesn't protect. The previous study showed that the TPP protects against the toxic effects of ethambutol on the retinal tissue, but TM was unable to protect [13]. As is known, TM can not enough transformation to TPP in cells leads to the functions and organic disorders in the tissue [30]. In the TTP deficiency, pyruvate and lactic acid accumulates in the blood and tissues [31]. The accumulation of lactic acid and pyruvate leads to tissue damage in the eye's retina tissue [32]. According to Polat *et al* [15], TPP prevented oxidative damage induced by doxorubicin, while TM could not; in addition, it was argued in the same study that doxorubicin prevented TPP production by inhibition of the enzyme expression of TPK and therefore, externally administered TM could not prevent cardiotoxicity caused by doxorubicin. It was stated again in another study that, although normal levels of TM were found in the blood, TM deficiency indications in the tissue also may be seen [33]. This

information suggests that ethambutol might lead to a decrease in TPP production and neuropathy in optic nerve tissue by inhibition of TPK enzyme activity since it is argued that some medications inhibit TPK, preventing its conversion to TPP, an active form of TM and lead to vitamin deficiency[14,34-35]. In the future this study will be examine with more animals and more detailed by adding the ERG and electron microscopy evaluation.

In conclusion, oxidative damage in rat optic nerves caused by ethambutol was demonstrated based on the MDA and GSH gene expression and histopathological findings. TPP was understood to protect optic nerve tissue against the toxic effect of ethambutol. However, TM could not protect the optic nerve against the toxicity of ethambutol. Therefore, it is thought that TPP may be useful in optic neuropathy caused by ethambutol.

However, in order to explain why the presence of TM is ineffective against ethambutol toxicity, detailed research should be performed later to determine the ethambutol-inhibitory effect on the TPK enzyme.

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REFERENCES

1 Chan ED, Laurel V, Strand MJ, Chan JF, Huynh M-LN, Goble M, Iseman MD. Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis. *Am J Respir Crit Care Med* 2004;169 (10):1103-1109.

2 Younossian AB, Rochat T, Ketterer JP, Wacker J, Janssens JP. High hepatotoxicity of pyrazinamide and ethambutol for treatment of latent tuberculosis. *Eur Respir J* 2005;26(3):462-464.

3 Uzar E, Varol S, Acar A, Firat U, Basarslan SK, Evliyaoglu O, Yucel Y, Alp H, Gökalp O. Assesment the role of oxidative stres and efficacy of caffeic acid phenethyl ester (CAPE) on neurotoxicity induced by isoniazid and ethambutol in a rat model. *Eur Rev Med Pharmacol Sci* 2014;18(19): 2953-2959.

4 Kant S, Natu NK, Mahajan V. Rifampicin, ethambutol and pyrazinamide-induced thrombocytopenia. *Int J Clin Pharmacol Ther* 2008;46 (8): 440-442.

5 Lim SA. Ethambutol-associated optic neuropathy. *Ann Acad Med Singapore* 2006;35(4):274.

6 Chuenkongkaew W, Samsen P, Thanasombatsakul N. Ethambutol and optic neuropathy. *J Med Assoc Thai* 2003;86(7):622-625.

7 Karnik AM, Al-Shamali MA, Fenech F. A case of ocular toxicity to ethambutol--an idiosyncratic reaction? *Postgrad Med J* 1985;61 (719): 811-813.

8 Choi SY, Hwang J-M. Optic neuropathy associated with ethambutol in Koreans. *Korean J Ophthalmol* 1997;11(2):106-110.

9 Aouam K, Chaabane A, Loussaief C, Ben Romdhane F, Boughattas NA, Chakroun, M. Adverse effects of antitubercular drugs: epidemiology, mechanisms, and patient management. *Med Mal Infect* 2007;37 (5): 253-261.

10 Plit M, Theron AJ, Fickl H, van Rensburg CE, Pendel S, Anderson R. Influence of antimicrobial chemotherapy and smoking status on the plasma concentrations of vitamin C, vitamin E, β-carotene, acute phase reactants, iron and lipid peroxides in patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1998;2(7):590-596.

11 Sahin A, Kursad Cingu A, Kaya S, Turkcu G, Arı S, Evliyaoglu O, Cinar Y, Turkcu FM, Yuksel H, Murat M, Caca I, Gokalp O.The protective effects of caffeic acid phenethyl ester in isoniazid and ethambutol-induced ocular toxicity of rats. *Cutan Ocul Toxicol* 2013;32(3):228-233.

12 Nebbioso M, Scarsella G, Tafani M, Pescosolido N. Mechanisms of ocular neuroprotection by antioxidant molecules in animal models. *J Biol Regul Homeost Agents* 2013;27(1):197-209.

13 Cinici E, Cetin N, Ahiskali I, Suleyman B, Altuner D, Alp HH, Sener E, Calik I, Suleyman H. The effect of thiamine pyrophosphate on ethambutol-induced ocular toxicity. *Cutan Ocul Toxicol* 2016;35 (3): 222-227.

14 Gastaldi G, Casirola D, Patrini C, Ricci V, Laforenza U, Ferrari G, Rindi G. Intestinal transport of thiamin and thiamin monophosphate in rat everted jejunal sacs: a comparative study using some potential inhibitors. *Arch Int Physiol Biochim* 1988;96(5):223-230.

15 Polat B, Suleyman H, Sener E, Akcay F. Examination of the effects of thiamine and thiamine pyrophosphate on doxorubicin-induced experimental cardiotoxicity. *J Cardiovasc Pharmacolo Ther* 2015;20 (2): 221-229.

16 Sica DA. Loop diuretic therapy, thiamine balance, and heart failure. *Congest Heart Fail* 2007;13(4):244-247.

17 Turan MI, Cayir A, Cetin N, Suleyman H, Siltelioglu Turan I, Tan H. An investigation of the effect of thiamine pyrophosphate on cisplatin-induced oxidative stress and DNA damage in rat brain tissue compared with thiamine:thiamine and thiamine pyrophosphate effects on cisplatin neurotoxicity. *Hum Exp Toxicol* 2014;33(1):14-21.

18 Williams DL. Oxidative stress and the eye. *Vet Clin North Am Small Anim Pract* 2008;38(1):179-192.

19 Izzotti A, Bagnis A, Saccá SC. The role of oxidative stress in glaucoma. *Mutat Res* 2006;612(2):105-114.

20 Talbert Estlin KA, Sadun AA. Risk factors for ethambutol optic toxicity. *Int Ophthalmol* 2010;30(1):63-72.

21 Pelit A, Haciyakupoglu G, Zorludemir S, Mete U, Daglioglu K, Kaya M. Preventative effect of deferoxamine on degenerative changes in the optic nerve in experimental retrobulbar haematoma. *Clin Exp Ophthalmol* 2003; 31(1):66-72.

22 van der Lugt JJ, Venter I. Myelin vacuolation, optic neuropathy and retinal degeneration after closantel overdosage in sheep and in a goat. *J Comp Pathol* 2007;136(2-3):87-95.

23 Zubair M, Tahir M, Sheikh TH, Samee KLW, Munir B. Prevention of ethambutol induced changes by memantine in optic nerve of rabbit. *Biomedica* 2009;25:19-23.

24 TsaiRK, He MS, Chen ZY, Wu WC, Wu WS. PKCδ-dependent signaling mediates ethambutol-induced ioxic effects on human retinal pigment cells. *Mol Vis* 2011;17:1564-1576.

25 Kinoshita J, Iwata N, Maejima T, Kimotsuki T, Yasuda M. Retinal function and morphology in monkeys with ethambutol-induced optic neuropathy. *Invest Ophthalmol Vis Sci* 2012;53(11):7052-7062.

26 Gong H, Amemiya T. Optic nerve changes in zinc-deficient rats. *Exp Eye Res* 2001;72(4):363-369.

27 González-Fernández E, Sánchez-Gómez MV, Pérez-Samartín A, Arellano RO, Matute C. A3 Adenosine receptors mediate oligodendrocyte death and ischemic damage to optic nerve. *Glia* 2014;62(2):199-216.

28 El-Sayyad HI, Khalifa SA, El-Sayyad FI, AL-Gebaly AS, El-Mansy AA, Mohammed EA. Aging-related changes of optic nerve of Wistar albino rats. *Age (Dordr)* 2014;36(2):519-532.

29 Carelli V, Ross-Cisneros FN, Sadun AA. Optic nerve degeneration and mitochondrial dysfunction: genetic and acquired optic neuropathies. *Neurochem Int* 2002;40(6):573-584.

30 Soukoulis V, Dihu JB, Sole M, Anker SD, Cleland J, Fonarow GC, Metra M, Pasini E, Strzelczyk T, Taegtmeyer H, Gheorghiade M. Micronutrient deficiencies: an unmet need in heart failure. *J Am Coll Cardiol* 2009;54 (18):1660-1673.

31 Brown G. Defects of thiamine transport and metabolism. *J Inherited Metab Dis* 2014;37(4):577-585.

32 Nyengaard JR, Ido Y, Kilo C, Williamson, JR. Interactions between hyperglycemia and hypoxia: implications for diabetic retinopathy. *Diabetes* 2004;53(11):2931-2938.

33 Sasaki T, Yukizane T, Atsuta H, Ishikawa H, Yoshiike T, Takeuchi T, Oshima K, Yamamoto N, Kurumaji A, Nishikawa T. A case of thiamine deficiency with psychotic symptoms--blood concentration of thiamine and response to therapy. *Seishin Shinkeigaku Zasshi* 2010;112(2):97-110.

34 Hanninen SA, Darling PB, Sole MJ, Barr A, Keith ME. The prevalence of thiamin deficiency in hospitalized patients with congestive heart failure. *J Am Coll Cardiol* 2006;47(2):354-361.

35 Subramanian VS, Subramanya SB, Tsukamoto H, Said HM. Effect of chronic alcohol feeding on physiological and molecular parameters of renal thiamin transport. *Am J Physiol Renal Physiol* 2010;299(1): 28-34.