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Comparison Of The Effectiveness Of Alpha-Tocopherol And Ginkgo Biloba Extract On The Prevention Of Ototoxic Kanamycin In The Treatment Of Multiple Drug-Resistant Tuberculosis

Fransiskus Harf Poluan
Medical Faculty, Universitas Kristen Indonesia

ABSTRACT

Introduction: Ototoxic due to kanamycin which resulting sensorineural hearing loss is one of the effects of kanamycin. Antioxidant treatment could prevent the effects of ototoxic. Aim: To compare the effectiveness of Ginkgo Biloba and alpha-tocopherol on prevention incidence of ototoxic due to kanamycin for MDR TB patients. Method: An experimental study in dr. Hasan Sadikin Hospital on July - October 2016, followed by 32 subjects were divided into two groups of patients, 16 subjects were given Ginkgo Biloba (EGB 761), and 16 subjects were given alpha-tocopherol. Examination of ototoxic by using the audiogram and DPOAE. Statistical analysis for this study using the chi-square test. Interpretation of the results of hypothesis testing based on the value of p <0.05. Result: Subjects in group Ginkgo Biloba experience less in hearing loss than alpha-tocopherol group after three weeks post-treatment based on an audiogram (p = 0.048) and DPOAE at a frequency of >8-10 kHz(0.001) and >6-8 kHz (0.014). After four weeks post-treatment group EGB 761 on DPOAE examination less experienced hearing loss at a frequency of >8-10 kHz (0.000) and >6-8 kHz (0.022). Conclusion: Ginkgo Biloba is more effective in preventing ototoxic due to kanamycin in patients with MDR TB than alpha-tocopherol. Giving Ginkgo Biloba can be used as therapy protocols in preventing sensorineural hearing disorders in patients with MDR TB who treated with kanamycin.

Keywords: kanamycin, MDR TB, Ginkgo Biloba, alpha-tocopherol.

1. INTRODUCTION

Tuberculosis (TB) is a disease caused by infection with the bacteria Mycobacterium tuberculosis. Tuberculosis is a significant health problem worldwide and is the second leading cause of death caused by infection. In 2013, the World Health Organization (WHO) estimated that the prevalence of pulmonary TB was 9 million cases with 1.5 million cases resulting in death and the prevalence of TB multidrug-resistant (MDR TB) cases as many as 480,000 cases with an estimated death toll of 210,000 people. In the same year, Indonesia found a prevalence of pulmonary TB cases of 6,800, with 2% of new cases and 12% of MDR TB cases [1; 2]. Multiple drug-resistant tuberculosis is TB that is resistant to rifampin, and isoniazid drugs with or not resistant to first-line pulmonary TB treatment and thus require more complex treatment. MDR TB treatment consists of two stages, namely the initial stage and the advanced stage, using anti-tuberculosis drugs (ATD) which have more side effects

and take around 19-24 months. In the early stages, the patient will receive second-line ATD with at least four types of sensitive drugs, one of which is the administration of kanamycin injection and the advanced stage is that all second-line ATDs used in the early stages are continued except for injection ATD [3; 4; 5; 6]. The remainder of the first-line oral drug should be appropriately combined with additional second-line drugs consisting of injectable aminoglycosides (amikacin, kanamycin, capreomycin), fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gatifloxacin), second-line anti-TB bacteriostatic (ethionamide, protionamide, cycloserine, para-aminosalicylic acid, thiocetazone) and other anti-tuberculosis (clofazimine, amoxicillin/clavulanate, clarithromycin, linezolid).

Ototoxic drugs are defined as those that have the potential to cause structural damage to the inner ear, including the cochlea, vestibule, semicircular canal, and otolith that can result in auditory and accompanying vestibular disturbances resulting in decreased hearing and balance [7; 8]. The ototoxic effect of permanent aminoglycosides can reduce the quality of life of the patient [9; 10]. MDR TB treatment is administered using a residual first-line oral drug combined with the addition of a second-line drug consisting of aminoglycoside injection of which the most commonly used is kanamycin [11; 12].

Kanamycin has a significant side effect on the cochlea resulting in sensorineural hearing loss. The high concentration of kanamycin due to high doses is a contributing factor to cochlear damage. Acoustic trauma, simultaneous use of other ototoxic agents, and a previous history of deafness are additional risk factors. Hearing loss can occur at the start of therapy until several months after completion of therapy. Kanamycin is more likely to cause unilateral hearing loss. Ototoxic drug exposure initially affects the basal area of the cochlea and subsequently causes damage to the apex [13; 14]. A study was conducted on the possibility of ototoxicity as an early step in early detection of ototoxic in the use of kanamycin in MDR TB treatment and concluded that kanamycin ototoxicity in MDR TB patients is likely to occur at the end of the second week in the form of sensorineural hearing loss starting at high frequency [15; 16; 17].

The use of aminoglycosides generates free radicals in the inner ear which cause permanent damage to sensory cells and neurons, resulting in permanent hearing loss. Histopathology suggests that outer hair cells are more sensitive to ototoxic injury than inner hair cells [18; 19] Therapeutic approaches to the prevention of hearing loss have long been established. Currently, most efforts have been made to prevent the formation of reactive oxygen species (ROS) which are suspected of causing toxicity and resulting in cell death. -methionine, salicylate, and traditional medicines have been widely used. These antioxidants are useful in the prevention of ototoxic administration of aminoglycosides, including gentamicin, neomycin, kanamycin, streptomycin, amikacin, and tobramycin [20; 21].

Alpha-tocopherol administration in mice given gentamicin was reported to be effective in preventing ototoxic effects. Alpha-tocopherol is fat-soluble, has natural antioxidant properties found in cells, prevents the formation of free radicals due to peroxidation that occurs in cell membranes and low-density lipoprotein particles so that it can regulate mitochondria against free radicals. Miman et al. in mouse experiments suggested that there was an improvement in preventing the ototoxic effects of amikacin by administering Ginkgo Biloba extract (EGb 761) [22; 23].

Yang et al. experimented on pigs and explained that Ginkgo Biloba (EGb 761) has a protective effect against ototoxic gentamicin by inhibiting the formation of ROS and nitric oxide (NO), thus preventing cell death Ginkgo Biloba extract has a good effect on free radical activity by activating superoxide radical anion (O2-), which is produced as the main product of membrane activity is oxidized. Ginkgo biloba extract has good vasoprotective properties

and improves blood vessel tone so that it can improve tinnitus and hearing loss [24; 25]. Until now, there is no therapeutic protocol to prevent ototoxicity due to administration of kanamycin in MDR TB patients. Based on the description above, further research is needed to prevent or reduce ototoxic effects in MDR TB patients receiving kanamycin. The problem that will be answered in this study is "how is the effectiveness of alpha-tocopherol and Ginkgo Biloba in preventing ototoxic kanamycin in MDR-TB patients?", With the aim of the study to compare the effectiveness of alpha-tocopherol and Ginkgo Biloba in preventing the ototoxic effects of kanamycin in MDR TB patients.

2. LITERATURE REVIEW

Multiple drug-resistant tuberculosis (MDR TB) is a state of M. tuberculosis that is resistant to rifampin and isoniazid with or without other ATDs. Treatment basically depends on the results of resistance tests using a minimum of 2 3 sensitive ATD and other additional drugs that can be used, namely the fluoroquinolone group (ofloxacin and ciprofloxacin), aminoglycosides (amikacin, kanamycin and capreomycin), ethionamide, cycloserine, clofazimine, amoxicillin plus clavulanic acid. Treatment of multiple resistant tuberculosis is complicated and requires a long time, namely at least 12 months, even up to 24 months. One of the drugs recommended by WHO for MDR TB treatment is the aminoglycoside group, namely kanamycin [26; 27].

Aminoglycosides have polycationic characteristics (positive charge) and are strongly polar. Aminoglycosides are antibiotics that have a bactericidal effect and act to inhibit bacterial protein synthesis by binding to the ribosome 30s subunit [28]. A single injection of aminoglycosides reaches peak serum values at one hour, then becomes insignificant after six hours. Perilymph levels peak slowly over three to six hours, and remain in perilymph for an extended period, then reach a minimum level within 24 to 36 hours. The aminoglycoside class includes amikacin, gentamicin, neomycin, netilmicin, streptomycin and tobramycin. Kanamycin has a significant side effect on the cochlea, producing characteristics of neurological hearing loss [29; 30; 31; 32].

The cochlea is shaped like a cochlea with a length of 3-3.5 cm from the base to the top which is the auditory sensory organ and functions to translate a series of auditory events into a pattern of nerve stimulation that provides an accurate description of the sound stimulus [33]. The function of the cochlea can be further divided into a) micromechanical function [34]; b) micromechanical function [35]. Hair cells are found in the membrane labyrinth, which is an essential receptor in the hearing process where a transduction process occurs, which converts sound energy into electrical energy in the form of nerve impulses. Hair cells are of two types, inner hair cells and outer hair cells [36]. Hair cells are mechanical receptors on which there are several stereocilia of varying length. The deflection of stereocilia towards the longest stereocilia (chinosilia) produces stimulation of sensory cells and produces auditory impulses [37].

Aminoglycoside (kanamycin) bonds with iron compounds to form iron aminoglycoside complexes resulting in redox formation which reacts with unsaturated fatty acids to form superoxide radicals (O2-) and lipid peroxides.

The reduced endogenous antioxidants, along with the formation of excess free radicals, can result in cell death. Aminoglycosides activate nitric oxide synthase increasing nitric oxide. Under these conditions, O2 reacts with available nitrate oxidants to form destructive radical peroxynitrite to initiate cell death [38; 39]. Aminoglycoside exposure causes ROS and stress kinase activation damages the mitochondria by activating the c Jun N terminal kinase (JNK)

pathway, which interferes with protein synthesis, causing the release of cytochromes that trigger apoptosis. Mitochondrial damage results in decreased ATP synthesis resulting in reduced ion pump activity and hypoxia in the vascular stria, thus progressively lowering the endococcal potential. The accumulation of aminoglycosides enters the hair cells through mechanotransduction channels, thereby increasing the susceptibility of hair cells to a greater extent than other cells. Outer hair cell damage often occurs due to the lower antioxidant capacity of other cells [40].

The three main approaches to audiological monitoring for ototoxicity are pure tone audiometry, high-frequency audiometry, and autoacoustic emission (EOA). All of these approaches can be used separately or in combination [41]. Pure tone audiometry is a basic hearing test that can determine the type and degree of hearing loss. The hearing threshold is defined as the lowest (softest) sound that can be heard or detected by fifty per cent at a single examination. Information about the hearing threshold can be known in the form of a graph known as Autoacoustic Emission (EOA) is an objective audiometric method used to determine the function of cochlear hair cells quickly and objectively. Autoacoustic emissions are produced by normal outer cochlear hair cells which disappear when the outer cochlear hair cells are damaged so that it can be used as a tool to evaluate the cochlear function, especially cochlear amplifier function [42]. Antioxidants inhibit the formation of free radicals by preventing the oxidation process that removes free radicals through the catalyzation process [43].

Antioxidants consist of exogenous antioxidants and endogenous antioxidants, while endogenous antioxidants consist of primary and secondary antioxidants. Superoxide dismutase (SOD), catalase and glutathione peroxidase are primary antioxidant enzymes. Primary antioxidants also consist of water-soluble antioxidants (ascorbate, glutathione, uric acid) and fat (alpha-tocopherol, ubiquinols and carotenoids). Secondary antioxidants consist of glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione-S-transferase and ubiquinone. Exogenous antioxidants come from foods like herbs, spices, vitamins, foods, vegetables etc. Flavonoids (Ginkgo Biloba), isoflavones, flavones, etc., can be found in natural foods called phytochemicals [44].

Alpha-tocopherol is a radical chain breaker, is hydrophobic and fat-soluble. The effect of alpha-tocopherol as an antioxidant has a direct action on membranes and lipoproteins. Only one structure of tocopherol is essential for humans, namely alpha-tocopherol, because other forms of vitamin E are not metabolized in the liver [45; 46]. In cell membrane lipids, vitamin E prevents fat oxidation, especially Poly Unsaturated Fatty Acid (PUFA). Due to its function as an antioxidant, vitamin E is one of the primary defences against destroying oxygen, lipid peroxide, and free radicals and stops chain reactions of free radicals [21; 25]. Side effects of vitamin E use in humans are infrequent, and the American Food and Nutrition Board has set an upper limit for vitamin E administration of 1000 mg per day [21].

The Ginkgo Biloba plant belongs to the gingkoaceae family, which is the oldest plant [22; 23; 24]. Ginkgo biloba indirectly inhibits the formation of free radicals and increases the activity of antioxidant enzymes. Ginkgo biloba leaf extract inhibits the formation of reactive oxygen species (ROS) and increases the activity of antioxidant enzymes. Flavonoids capture hydroxyl and peroxyl radicals and inhibit lipid peroxidase. Flavonoids also play a role in vasodilation of blood vessels [47]. Side effects that can occur with excessive consumption of Ginkgo Biloba leaf extract include bleeding, allergic skin reactions, indigestion, headaches, dizziness, and occasional anaphylactic reactions. At doses from 120 to 240 mg/day, Ginkgo Biloba has no bleeding effect.

3. RESEARCH METHODS

This research is an experimental clinical trial with a single-blind randomized clinical trial design. The research subjects were MDR TB patients with kanamycin given alpha-tocopherol or Ginkgo Biloba extract. A sampling of research subjects was carried out by consecutive sampling into one group. The research group was divided into two groups, namely the treatment group I and the control group II. The sample size calculation is carried out with the number of each group being equal, and then a random code sequence is generated. Code I or II put in a closed envelope. The envelopes were given serial numbers 1 to 32. The subjects of the study were all MDR TB patients in the *RSHS Internal Medicine MDR TB polyclinic* who received kanamycin therapy according to the inclusion and exclusion criteria and were willing to participate in the study by signing informed consent. The sample size is based on the formula to test the difference between the two means, namely:

$$n = \frac{2S^2 (Z\alpha + Z_\beta)^2}{d^2}$$

$$n = \frac{2(0,96)^2 (1,64+1,28)^2}{(1)^2}$$

n= 16 per group

The data collection techniques of this study were before the patient underwent kanamycin therapy in the form of ENT-TLC physical examination, tympanometry, pure tone audiometry, and DPOAE. Patients who were included in the inclusion criteria and not included in the exclusion criteria received informed consent and signed their consent to participate in the study. Then randomization was carried out by giving the antioxidant drug Ginkgo Biloba and alpha-tocopherol randomly to two groups. The following data taken is the result of pure tone audiometric examination and DPOAE after 2, 3, and 4 weeks of kanamycin. The study was conducted after obtaining ethical approval from the Research Ethics Committee of *FK UNPAD No: 408/UN6.C1.3.2/KEPK/PN/2018* and research permit Number: LB.02.01/C02/9939/VII/2016. Samples came from patients undergoing multiple drug resistance treatment in the Pulmonology subdivision of Internal Medicine, *RSHS*. Hearing function examination was carried out at the Hearing and Speech Disorders Clinic, ENT-KL Health Sciences Section *RSHS*. The research period was carried out from July to October 2016.

4. RESULT AND DISCUSSION

A study on the comparison of the effectiveness between alpha-tocopherol and Ginkgo Biloba in 32 patients undergoing treatment for dual drug-resistant tuberculosis has been conducted. The subjects of the study were all MDR TB patients at the MDR TB clinic in Internal Medicine Hasan Sadikin Hospital who received kanamycin therapy according to the inclusion criteria and were willing to take part in the study by getting an explanation and signing the consent form during the period June - October 2016. General characteristics of MDR TB sufferers who are the subject of research including age and gender, which can be seen in table 1. Table 1 describes the comparison of age and sex in the two groups. Based on age, it was obtained an age range of 15-53 years in the Ginkgo Biloba group and 23-55 years in the alpha-tocopherol group, but statistically, this comparison was not significant ($p > 0.05$). Based on gender, there were more men than women in both groups ($p > 0.05$). However, this difference is not significant.

Table 1.
Characteristics of Research Subjects

Variable	**Group**		**p**
	Ginkgo biloba n = 16	Alpha Tocopherol n = 16	
Usia			
Mean	30	41	0.053*
Range (min-max)	15-53	23-55	
Jenis Kelamin			
Laki-laki	13 (81.3%)	12 (75.0%)	0.669**
Perempuan	3 (18.8%)	4 (25.0%)	

*Note: n: Total STD: Standard deviation * Unpaired T-test ** Fisher's Exact test*

Based on the characteristic analysis in table 1, the analysis of all variables shows a p value> 0.05, so it is not statistically significant or homogeneous. So that the two groups, both in terms of age and sex, both showed no difference. So it can be concluded that the two groups are the same or homogeneous so that it is feasible to be compared and further statistical hypothesis testing can be done.
Table 2, none of the subjects in the two groups experienced hearing loss two weeks after therapy.

Table 2.
The audiogram and two-week DPOAE examination after Kanamycin therapy

Variable		**Group**		**p**
		Ginkgo biloba n = 16	**Alpha Tocopherol n = 16**	
Audiogram				
Normal		16 (100%)	16 (100%)	
SNHL Lightweight		0(0%)	0(0%)	1.00
SNHL Medium		0(0%)	0(0%)	
DPOAE				
> 8-10 kHz	Pass	16 (100%)	16 (100%)	1.00
	Refer	0(0%)	0(0%)	
>6-8 kHz	Pass	16 (100%)	16 (100%)	1.00
	Refer	0(0%)	0(0%)	
4-6 kHz	Pass	16 (100%)	16 (100%)	1.00
	Refer	0(0%)	0(0%)	

Description: SNHL: *Sensorineural hearing loss*
DPOAE: *Distortion product otoacoustic emission*

In Table 3, based on the audiogram, subjects in the Ginkgo Biloba group experienced less sensorineural hearing loss than subjects in the alpha-tocopherol group with p-value <0.05. On the DPOAE examination, subjects in the Ginkgo Biloba group experienced fewer referrals than subjects in the alpha-tocopherol group at frequencies> 8-10 kHz, frequencies> 6-8 kHz and 4-6 kHz. This difference was statistically significant at frequencies> 8-10 and> 6-8 kHz with each p-value <0.005, but at a frequency of 4-6 kHz, the difference was not significant (p = 0.144).

Table 3
. The audiogram and Three Weeks Post-Therapy DPOAE Examination

Variable	**Group**		**p**
	Ginkgo biloba n = 16	**Alpha Tocopherol n = 16**	
Audiogram			
Normal	15 (93.8%)	9 (56.3%)	
SNHL Lightweight	1 (6.3%)	6 (37.5%)	0.048
SNHL Medium	0 (0,0%)	1 (6.3%)	
DPOAE			
>8-10kHz Pass	13 (81.3%)	4 (25.0%)	
Refer	3 (18.8%)	12 (75.0%)	0.001
>6-8 kHz Pass	15 (93.8%)	9 (56.3%)	
Refer	1 (6.3%)	7 (43.8%)	0.014
4-6 kHz Pass	16 (100%)	14 (87.5%)	
Refer	0 (0.0%)	2 (12.5%)	0.144

Description: SNHL: *Sensorineural hearing loss*
DPOAE: *Distortion product otoacoustic emission*

In table 4, based on the audiogram description, the subjects in the Ginkgo Biloba group experienced less sensorineural hearing loss than the alpha-tocopherol group subjects, but this condition was not statistically significant (p = 0.063). In the DPOAE examination, subjects in the Ginkgo Biloba group experienced fewer referrals than subjects in the alpha-tocopherol group, namely at frequencies> 8-10 kHz, frequencies> 6-8 kHz and 4-6 kHz. This difference was statistically significant at frequencies> 8-10 and> 6-8 kHz with p values of 0.000 and 0.022, respectively, but at frequencies of 4-6 kHz, the difference was not significant (p = 0.144).

Table 4.
The audiogram and Four-Week Post-Therapy DPOAE examination

Variable	**Group**		**P**
	Ginkgo biloba n = 16	**Alpha Tocopherol n = 16**	
Audiogram			
Normal	14 (87.5%)	8 (50.0%)	

SNHL Lightweight		1 (6.3%)	6 (37.5%)	**0.063**
SNHL Medium		1 (6.3%)	2 (12.5%)	
DPOAE				
>8-10 kHz	*Pass*	13 (81.3%)	3 (18.8%)	**0.000**
	Refer	3 (18.8%)	13 (81.3%)	
>6-8 kHz	*Pass*	14 (87.5%)	8 (50.0%)	**0.022**
	Refer	2 (12.5%)	8 (50.0%)	
4-6 kHz	*Pass*	16 (100%)	14 (87.5%)	**0.144**
	Refer	0 (0,0%)	2 (12.5%)	

Table 5 shows that the subjects in the Ginkgo Biloba group had a smaller percentage of ototoxic events than subjects in the alpha-tocopherol group with a p-value = 0.049, which indicates that the difference is statistically significant.

Table 5.
Comparison of Otomycosis between Ginkgo biloba and Alpha-Tocopherol Groups

Variable	**Ototoxic**		**P-Value**
	Yes n=9	**No n=23**	
Ototokxic			**0,049**
Ginkgo Biloba	2 (22.2%)	14(60.9%)	
Alfa Tokoferol	7 (77.8%)	9 (39.1%)	

In this study, MDR TB patients suffered more from men than women. It is by a study reported by Duggal that more men (60.9%) suffered from MDR TB than women (39.1%) [7]. Rafique, in another study, reported that MDR TB patients who received aminoglycoside therapy were 60.5% male and 39.5% female.29 According to Rahmawati, 57.8% MDR TB patients who received kanamycin therapy and 42 women, 2%. This difference is probably because men are less adherent in first- or second-line TB treatment than women, resulting in multiple drug resistance [5]. Based on age, the results of this study indicate that MDR TB sufferers are experienced by subjects in the age range of 15-55 years. It is consistent with Reviono's research which reported that MDR TB sufferers were suffered by subjects aged 20-62 years [33]. Nizamuddin reported that MDR TB patients who received aminoglycoside therapy occurred in the age range 15 - 55 years [30]. Rahmawati reported that most of the MDR TB study subjects who received kanamycin therapy were aged 16-58 years who were in the productive age group. that age has high activity, which causes high exposure to pollution to the environment and reduces adherence to TB treatment, leading to drug resistance [5].

Audiometry examination and DPOAE one and two weeks, post-therapy had not shown a hearing loss in all subjects in both groups, but after three and four weeks post-therapy, mild and moderate sensorineural hearing loss was found. It is consistent with a study conducted by Rahmawati which stated that the chances of ototoxic events in MDR TB patients who received kanamycin therapy began to occur on days 19-21 [5]. In this study, hearing loss began in the third week at a frequency of> 8 - 10 kHz and> 6 - 8 kHz in both groups. It is consistent with a study conducted by Rahmawati which stated that the chances of ototoxic

events in MDR TB patients who received kanamycin therapy occurred starting on days 19-21 at a frequency of 10000 Hz and days 25-27 at a frequency of 8000 Hz [5].

Audiological monitoring in patients experiencing ototoxicity can be done through three main approaches, namely pure tone audiometry, high-frequency audiometry (HFA) and OAE. All of these approaches can be used separately or in combination [9]. Audiological monitoring of ototoxicity has two main objectives, they are early detection of hearing loss and audiological intervention if hearing loss has occurred. Autoacoustic emission is one objective audiometric method that can be used to determine cochlear hair cell function quickly and objectively. OAE examination can be used in patients who on audiometric examination are questionable [6; 8]. In diagnostic DPOAEs can measure at a frequency of 8-20 kHz so as to know the incidence of hearing loss at high tones [10; 29; 31; 32]. In this study, pure tone audiometry and DPOAE were used for monitoring and evaluation of ototoxicity in MDR TB patients receiving kanamycin therapy. Martin et al. stated that audiological examination in patients with ototoxicity could be carried out by pure tone audiometry, but the best used are HFA (8-14 kHz). Autoacoustic emissions to measure the incidence of sensorineural hearing loss and the degree of ototoxicity [31; 34; 35]. The same thing was stated by Ribeiro and Javadi who explained that audiometric examinations with a frequency of more than 8000 Hz were more sensitive in assessing ototoxic incidence in patients who had multiple drug resistance TB [36; 37; 38]. In this study, to detect the presence of ototoxic due to kanamycin in MDR TB patients, a pure tone audiometry examination of 125 Hz-8 kHz and DPOAE frequencies of 4-10 kHz was used.

Based on the American Academy of Audiology, follow-up examination as an evaluation or monitoring of audiology on the use of aminoglycosides should be done once a week or two weeks. Monitoring can be continued even after discontinuation of use because aminoglycosides can cause hearing loss that is delayed up to several months after stopping treatment. According to Konrad-Martin et al., evaluation of audiological monitoring is performed once or twice a week in individuals receiving ototoxic drugs. Audiological monitoring and evaluation should also be performed for any patient who complains of hearing loss or symptoms such as tinnitus and dizziness. Monitoring can be continued after administration of the TB regimen or when there are symptoms such as hearing loss which can be carried out for up to six months after TB therapy. Ototoxicity can occur as early as 72 hours after administration of aminoglycosides. So, it is the basis that evaluation can be carried out within 72 hours of aminoglycoside administration [7; 8; 35]. In this study, audiological monitoring was carried out once a week after administration of kanamycin for one month and could detect ototoxic events by audiometry and DPOAE in the third week after kanamycin therapy in MDR TB patients. Sharma and Nizamuddin stated that audiometric examination in patients receiving aminoglycoside therapy had a pattern of hearing loss in the frequency range 4 kHz - 8 kHz [6; 8; 30; 31; 34; 35]. In this study, sensorineural hearing loss was found in the range of 4 kHz-8 kHz in both groups of subjects after three and four weeks of post-therapy.

Aminoglycosides inhibit the mechanotransduction channels, causing the disturbance in sensory cells resulting in ototoxic. If the sensory cells are lost, regeneration will not occur and is followed by worsening degeneration of the auditory nerve, causing irreversible hearing loss [29; 30; 39]. In the study, there was a decrease in sensorineural hearing in both groups and to find out whether there was an irreversible decrease or not, monitoring and evaluation were necessary for six months or more. Aminoglycosides more frequently damage the outer hair cells of the tertiary organs and type 1 hair cells in the ampullar crest. Sensory epithelial damage to the utriculus and sacculus can occur. Support cells and hair cells in the tertiary

organs are not affected. Degeneration of nerves, spiral ganglion neurons and supporting cells in further processing can be damaged. One of the possible causes of hair cell damage is the antioxidant content. The levels of glutathione, an endogenous intracellular antioxidant, in outer hair cells are lower than in support cells and in hair cells. Besides, there is a gradient of glutathione levels from basal to cochlear apex, where the outer hair cells in the cochlear apex have higher glutathione levels than outer hair cells in the cochlear basal [37]. With increased doses and prolonged exposure, external hair cell damage develops starting from the basal cochlea, where high-frequency sounds are processed, which further development can occur at the apex, where low-frequency sounds are processed at high frequencies detected on the DPOAE probe at frequencies> 8-10 kHz.

Factors that influence the increased susceptibility of patients to ototoxic aminoglycosides include drug dosage, frequency of drug administration, and duration of treatment. According to Ribeiro et al., genetic disorders in the form of mitochondrial DNA mutation A1555G in 12S ribosomal RNA is one of the risk factors associated with deafness so that an increase in susceptibility to aminoglycosides is obtained which results in hearing loss. These mutations can result in an increased risk of ototoxicity in patients receiving aminoglycoside therapy. Genetic screening is necessary to exclude these patients from receiving aminoglycoside therapy. Despite an increased susceptibility to aminoglycosides producing hearing loss due to mitochondrial mutations, there is little or no vestibular disturbance [8]. In this study, genetic screening was not carried out as an inclusion criterion, and further research is needed on the role of antioxidant interventions with these genetic disorders.

Aminoglycosides induce the formation of ROS, which is thought to be the primary mechanism underlying cell death. Under physiological conditions, various enzymatic processes produce ROS as primary metabolic products and physiological mediators such as nitric oxide, or as byproducts such as superoxide. The detrimental effects of ROS can be neutralized by the cellular antioxidant system, thereby maintaining a redox balance. However, excess production of ROS disrupts the balance and results in highly reactive oxidative reactions such as hydrogen radicals or peroxynitrite with the consequence of damaging cell DNA and proteins that ultimately lead to cell death [15]. Aminoglycosides can accelerate the formation of ROS through the Fe complex through redox reactions and result in enzymatic reactions in cells. Metabolic disorders can occur due to mitochondrial damage due to ROS or activation of the NADPH oxidase complex via the Rac / Rho pathway, which is the primary source of superoxide radical formation [8]. One study stated that cell death involved c-Jun N terminal kinase (JNK) and caspase cascade, nuclear translocation endonuclease G (Endo G) and μ-calpain activation, as well as concurrent activation of the cell death pathway due to aminoglycosides inhibiting cell survival pathways, such as the PI 3-kinase / Akt signal [8]. Ototoxic side effects on the cochlea are characteristic of neurological hearing loss, but vestibular disturbances are rare. Hearing loss due to kanamycin administration may occur after three to four days of administration or can be delayed days, weeks or months after completion of therapy [8; 9]. In this study, it was found that hearing loss occurred at three weeks after therapy.

Aminoglycosides consist of amino sugars that are linked to other parts by means of glycoside bonds. Variations in chemical configuration affect the toxicity as well as antibiotic activity. Aminoglycosides are antibiotics that have a bactericidal effect and act to inhibit bacterial protein synthesis by binding to the ribosome 30s subunit. The aminoglycoside class includes amikacin, gentamicin, neomycin, netilmicin, streptomycin and tobramycin. Kanamycin destroys outer hair cells (OHC), but most support cells and inner hair cells (IHC) are not affected. Apart from differences in antioxidant levels, this is also due to increased activation

and translocation of the kB pathway (NF) in IHC nuclei and support cells that have a signalling role that induces ROS. Hence, outer hair cells have a tendency to breakdown earlier [9]. A recent study confirmed that the high accumulation of ROS is a critical initial stage of gentamicin-induced hair cell damage and that differences in the sensitivity of hair cells in the Corti organs are closely related to the difference in the high accumulation of ROS. It is consistent with the fact that hair cells are more susceptible to ROS damage than support cells and basal hair cells are more susceptible than apical hair cells [8; 11; 14; 40]. In this study, it was found that there was a decrease in the sensorineural hearing at high frequencies. There is currently no therapy to cure the damage caused by ototoxic drugs. Handling of hearing disorders and balance is emphasized on prevention because most hearing disorders are irreversible.8 In this study Ginkgo Biloba and alpha-tocopherol were given as antioxidants to prevent ototoxic events.

At three weeks post-therapy, there was a change in the form of a decrease in sensorineural hearing in the Ginkgo Biloba group which had a slight decrease in the mild sensorineural hearing by 6.3% compared to the alpha-tocopherol group who experienced a mild sensorineural hearing loss of 37.5% to a moderate degree of 6, 3%. The DPOAE examination three weeks after therapy was obtained at a frequency of> 8-10 kHz, the Ginkgo Biloba pass group was 81.3%, and the referral was 18.8%, while the alpha-tocopherol pass group was 25% and 75% referral. At a frequency> 6-8 kHz, the Ginkgo Biloba pass group was 93.8%, and the referral was 6.3% in the alpha-tocopherol pass group at 56.3% and the referral 43.8%. Based on the audiogram and DPOAE examination, it showed that the Ginkgo Biloba group was more effective in preventing a decrease in the degree of sensorineural hearing loss than the alpha-tocopherol group.

The fourth week of post-therapy based on the audiogram image, giving Ginkgo Biloba showed a mild and moderate sensorineural hearing loss of 6.3% respectively, while the administration of alpha-tocopherol decreased mild sensorineural hearing by 37.5% and a moderate degree of 12.5%. The four-week post-therapy DPOAE examination was obtained at a frequency of> 8-10 kHz, the Ginkgo Biloba pass group was 81.3%, and the referral was 18.8%, while the alpha-tocopherol pass group was 18.8% and the referral 81.3%. At a frequency of> 6-8 kHz, the Ginkgo Biloba pass group was 87.5%, and the referral was 12.5% in the alpha-tocopherol pass group at 50% and referral at 50%. Based on the DPOAE examination, it showed that the Ginkgo Biloba group was more effective in preventing a decrease in the degree of sensorineural hearing loss than the alpha-tocopherol group. The insignificant results on the audiogram image were caused by the pure tone audiometry examination in this study only assessing the frequency of 125 Hz - 8 kHz, while the DPOAE used in this study was able to measure frequencies up to 10 kHz. Martin and Duggal stated that the DPOAE test has the speciality of being able to measure more specific frequencies and to assess higher frequencies and to detect early ototoxic events due to cochlear damage [34; 41; 42].

In this study, it was found that the ototoxic incidence in the alpha-tocopherol group was more significant than the Ginkgo Biloba group (EGb761), so that Ginkgo Biloba was more effective in preventing the ototoxic effects of MDR TB kanamycin. Ginkgo biloba and alpha-tocopherol are antioxidants that prevent ototoxicity. Ginkgo Biloba is more effective in preventing ototoxic due to several factors, namely inhibiting the formation of ROS and preventing the formation of NO synthesis, preventing the formation of lipid peroxidase and peroxynitrite radicals, preventing apoptosis, and as a vasodilator thereby increasing blood flow or microcirculation in the cochlea. In contrast, alpha-tocopherol only works to inhibit the formation of lipid peroxidase, thus preventing cell death. The content of Ginkgo Biloba

includes flavonoids which are known to capture hydroxyl radicals, peroxyl radicals, and inhibit lipid peroxidase, which is a complex process due to the reaction of polyunsaturated fatty acids that make up cell membrane phospholipids with Reactive Oxygen Species (ROS), forming hydroperoxides. Ginkgo Biloba also plays a role in vasodilation of blood vessels and antioxidants. Another content of Ginkgo Biloba, namely bilobalide, increases the activity of antioxidant enzymes (SOD and catalase) and increases cell viability. Ginkgo biloba also prevents damage to cell membranes due to free radicals because it functions to increase the activity of the Na K-ATPase enzyme so that it restores the balance of Na and K ions, and can also function to repair Ca-channels so that the concentration of Ca ions in the cochlea becomes normal. Ginkgo biloba has the ability to increase blood flow to the brain, improve peripheral circulation, and is a platelet-activating factor inhibitor [21; 22; 23; 24; 25].
This study hypothesises that Ginkgo Biloba is more effective than alpha-tocopherol in preventing hearing and neurological disorders in MDR TB patients receiving kanamycin therapy. Based on the chi-square test in table 3, the subjects in the Ginkgo Biloba group had less hearing loss than the alpha-tocopherol group after three weeks post-therapy based on the audiogram ($p = 0.048$) and DPOAE examination at a frequency of> 8-10 kHz and> 6-8 kHz. With each p-value of 0.001 and 0.014. Based on the chi-square test in Table 4, the subjects in the Ginkgo Biloba group on the DPOAE examination experienced less hearing loss at frequencies> 8-10 kHz and> 6-8 kHz with p values of 0.000 and 0.022, respectively. Based on table 5, the ototoxic incidence in the Ginkgo Biloba group was as many as two people, and the alpha-tocopherol was seven people. Based on the chi-square test, it was statistically significant ($p = 0.049$). Based on the statistical test above, the research hypothesis is accepted

5. CONCLUSION

Ginkgo Biloba therapy is more effective than alpha-tocopherol in preventing ototoxicity due to kanamycin in MDR TB patients. MDR TB sufferers are more common in men, MDR TB sufferers occur in the age range 15-55 years, hearing loss begins to occur three weeks after administration of kanamycin in MDR TB patients. Administration of EGb 761 can be used as a therapeutic protocol in preventing sensorineural hearing loss in MDR TB patients receiving kanamycin therapy. Further research is needed to determine the ototoxic incidence due to the use of kanamycin in MDR TB using a subjective High-Frequency Audiometry (HFA) examination tool for monitoring or detection of ototoxicity due to aminoglycoside administration.

6. REFERENCES

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