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The Effect of Black Cumin (*Nigella sativa*) On Body Weight, Otoacoustic Emission Examination and Histopathology Examination of Organ Corti Male White Rats (*Rattus norvegicus*) Inducted by Streptomycin

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Abstract. Streptomycin is an aminoglycoside type of antibiotics. It is used as a first line treatment for tuberculosis (TB). Streptomycin also cause ototoxicity to its long term user as a fatal side effect. Black cumin (N. sativa) is an herbal plant that is widely consumed and contains thymoquinine as an active compound. Thymoquinine can reduce oxidative stress and increase antioxidant defences in the body. To evaluate the relationship and differences of administration of black cumin (N. sativa) to body weight, optoacoustic emission test and the degree of the Organ Corti damage induced by streptomycin in Wistar rats using histopathological examination. In-vivo experimental method using 25 wistar rats (Rattus norvegicus) with 5 treatments group, group K0(control), P1 (Streptomycin 20mg/kgBW/day IM), P2 (Streptomycin IM with low-dose black cumin), P3 (Streptomycin IM with medium-dose black cumin), P4 Streptomycin IM with high-dose black cumin). The experiment was conducted as a post-test only experimental group design and assessed body weight, SNR values on OAE examination and the degree of damage through Haematoxylin-eosin staining histopathological examination. There were no significant differences in post-experimental weight ($p > 0.05$) with a p-value: 0.549 between every group (K0 to P4) and there were significant differences between SNR values in OAE examination and the degree of damage to Organ of Corti between every group ($p<0.005$) with a p: 0.000. There was a strong relationship between OAE examination and the degree of damage to Organ of Corti with p-value: 0.001 and r-value: 0.635. There was a moderate relationship between the treatment group and the degree of Organ Corti damage with p-value: 0.036 and r-value: 0.421. There were also no relationship between body weight and the degree of Organ Corti damage (p: 0.725), between the OAE treatment and treatment groups (p: 0.780), between the treatment group and body weight (p: 0.491) and between body weight and OAE examination (p: 0.465). The hypothesis is proven on the strong relationship between OAE examination and degree of Organ Corti damage and also on the moderate relationship between treatment groups and the degree of Organ Corti damage.

Kata Kunci: Streptomycin, N. sativa, Black cumin, Aminoglycoside ototoxicity, Body weight examination, OAE examination, Degree of Organ Corti damage, Histopathological examination.

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1 Introduction

Streptomycin is one of the aminoglycoside (AGs) antibiotics. Aminoglycosides (AGs) were first discovered in 1940. Streptomycin is used as a first-line treatment for tuberculosis (TB).[1] Although aminoglycosides (AGs) are broad-spectrum antibiotics, their use is limited and some are only used topically because the side effects are permanent ototoxic and reversible nephrotoxic.[2] The therapeutic action of aminoglycosides (AGs) depends on the dose level. In some studies, the ototoxic ratio released by AG is in around 2% to 25%.[3]

Aminoglycosides (AGs) may be cochleotoxic and vestibulotoxic. There are the types of AGs, such as streptomycin and gentamicin are more vestibulotoxic, whereas amikacin, neomycin and kanamycin are more cocleotoxic. [4]

The symptoms of cochleotoxic are hearing loss and / or tinnitus while the symptoms of vestibulotoxic are disequilibrium and dizziness. These symptoms may not be detected until in acute phase of severe infection and the diagnosis may be too late by the time the manifestation of the symptoms. AG cochleotoxicity is usually more likely to be bilateral which affects at high frequencies first compared to low frequencies and is more susceptible to high dose usage. [5]

Drug that is ototoxic can result in dysfunction of the auditory system or vestibular system which causes hearing loss or disequilibrium reversibly or permanently. Hearing loss or deafness is one of the factors that cause a decrease in the quality of life of a person and one of the problems that the prevalence is increasing in all countries in the world.

The cochlea is an organ that works continuously so that it is ready to carry out the hearing process for 24 hours, receiving microvascularization from the cochlear artery without the collateral system and the stria vascularis which is a source of energy for hair cells in organ corti that are end entry. Organ corti consists of outer hair cells and inner hair cells, function as auditory sensory organs. Damage to the blood vessels of the organ corti causes impaired vascularization, supply of nutrients, and oxygen to cells in the organ corti which results in impaired sensory function of hearing. The disorder is one of the causes of sensorineural deafness. [6]

Jintan Hitam, Habattus Sauda, Black Seed, Black Cumin, Kalunji, Nutmeg Flower, Kalajira, Fennel flower, and Roman Coriander are common names for *Nigella sativa L*. [7] This plant belongs to the family Ranunculaceae and genus Nigella, is a plant that originated in the Mediterranean and East Asia. [8]

Research in animal experiments shows black cumin (*Nigella sativa*) containing thymoquinone can reduce oxidative stress and increase antioxidant defenses in the body. The decrease in malondialdehyde and other oxidative stress biomarkers occurs in parallel with an increase in total thiol and glutathione levels as a result of treatment using thymoquinone. Pharmacological properties of N. sativa including immune stimulants, hypotension, anti-inflammatory, anti-cancer, antioxidants, hypoglycemic, spasmolytic and bronchodilators have been shown. [8] *N. sativa* contains a variety of bioactive substance such as *thymoquinone, p-cymene, ciscarveol, thymol, a-phellandrene, α-pinene, β-pinene, trans-anethole, alongipinene, dan longifolene*. [9] Various protective effects and benefits that can be produced by black cumin (*Nigella sativa*) on the body, such as the respiratory system, digestive tract, kidney and liver function, cardiovascular system, and immune system support, as well as for general well-being. [10] *N. sativa* is also found to relieve symptoms or cure patients with several diseases, such as hypertension, dyslipidemia, metabolic syndrome, diabetes, asthma, seizures, and natural and chemical toxicity. In addition, several studies mention that the use of N. sativa and thymoquinone can prevent many disorders, including neurobehavioral, kidney, and liver disorders

Research from experimental studies on cochlea from 28 adult Dunkin-Hartley male guinea pigs divided into four groups, control and three groups given gentamicin and black cumin, showed that black cumin significantly reduced the integrity damage from the cochlea histology caused by aminoglycoside uptake. [12] There is research on the protective effect issued by black cumin against amikacin-induced ototoxic ototoxicity, showed that groups of amikacin-induced rats given black cumin did not apply significant changes to the auditory brainstem response (ABR) threshold and distortion values for otoacoustic emissions (DPOAE) while those without given cumin black applies significant changes to the ABR and DPOAE threshold values. [13] Aksoy also states that the total oxidant status value and the oxidative stress index value play a role in causing ototoxicity.

Based on the above background, researcher are interested in examining the relationship and differences effect of Black Cumin (Nigella sativa) on body weight examination, otoacoustic emission examination and the degree of damage to the integrity of the white rat cochlear organ corti tissue (Rattus norvegicus) induced by Streptomycin through histopathological observation.

2 Method

Ethical Clearance

The study was conducted in line with the Guide for the Care and Use of Laboratory Animals and approved by ethical committee of Fakultas Kedokteran, Universitas Sumatera Utara/RSUP H. Adam Malik, Medan with document number: 118 / TGL / KEPK / FK USU-RSUP HAM / 2019.

Experimental Design

The research type is in vivo experimental studies work in the laboratory with a research design of post test only group laboratory experimental design. The study conducted in Pharmacology laboratory of Universitas Sumatera Utara dan Pathology Anatomy of Medicine Faculty Universitas Sumatera Utara.

Sample and Population

For the purpose of the study, the population is male adult Wistar albino rats of 2-5 months with 100-300 gram. 25 rats have been used for this study with simple random sampling method from population that meet the inclusion and exclusion criteria and there are comparative and control group and classified as double blind.

Experimental Variable

Dependent variable for this study is body weight examination, otoacoustic emission (OAE) examination and degree of damage to the integrity of the white rat cochlear organ corti tissue through histopathological observation. Independent variable for this study is dosage of Black cumin given through oral for 30 days and dosage of Streptomycin 20 mg/kg/day given through intramuscular for 30 days. Constant variable for this study is pre-experimental hearing screening.

Operational Definition of Variable

Pre-experimental hearing screening. Evaluate the condition of hearing function of the sample by detecting sound waves generated from outer hair cells.

Dosage of Streptomycin. Treatment was given dose of Streptomycin at 20mg /kg / day to 4 treatment groups.

Dosage of Black Cumin. Doses used for the treatment is 0g/kg/day for group P1, 0,1g/kg/day for group P2, 0,5g/kg/day for group P3, 1,0g/kg/day for group P4.

Degree of damage to the integrity of cochlear organ corti tissue through histopathological observation. Observe the damage structure and the degree of damage at 400x and 1000x magnification.

Body weight examination. Evaluate weight gain or loss after treatment.

Otoacoustic emission (OAE) examination. Evaluate the condition of hearing function of the sample by detecting sound waves generated from outer hair cells.

Anesthesia

All rats were anesthetized with 100 mg *ketamine hydrochloride* mixwith 20 mg *xylazine* and diluted by 7 ml aquadest. The mixture given with a dose of 0.1 ml/ 20 gram body weight by intramuscular.

Surgical Procedure

All procedures were carried out in hygienic but nonsterile environments. On the 31th day, the rats were terminated using neck dislocation method while under anesthesia. The head separated from the body, and continued by removing the skin and lower jaw. Then, the temporal bones cut and remove the brain and muscle from the bone. Finally, the temporal bones contained cochlea were fixed in 10% buffered formalin and ready for tissue preparation.

Tissue Preparation

Bone tissue is fixed 24 hours after surgery. After 24 hours, the tissue was softened in a decalcification solution with a ratio of tissue and histo-decalsifier solution of 1:20 for 24 hours. After finishing the process of fixation and decalcification, the tissue washed with clean water for 24 hours and carried out a gradual dehydration process and followed by a vacuum and paraffin block molding process. The paraffin block is cut using a microtome machine with a thickness of 3-4 μm. Next, the process of hematoxylin-eosin (H&E) staining is done and covered with a glass cover. The results of staining can be seen under a light microscope

Statistical analysis

Data analysed were performed with SPSS 25.0 version for Windows. Results undergo univariate analysis in form of mean, standard deviation from the body weight examination, otoacoustic emission (OAE) examination and degree of damage to the integrity of the white rat cochlear organ corti tissue through histopathological observation. Results also undergo bivariate analysis with Saphiro-Wilk test and Levene test. The data is not normally distributed so analysis using Kruskall-Wallis test and post hoc test to analyse the differences between group. Results were also analysed by Spearman correlation to analyse the correlation between variable.

3 Result

Body weight examination

The highest average weight gain was 54.2 grams in the K0 group (control) and the lowest average weight gain was 26.6 grams in the P4 group (Table 1 and Figure 1). The results of the analysis found differences in mean post-experimental body weight values were not significant $\rho > 0.05$ with p values: 0.549 in each treatment group (K0 to P4).

Table 1. Distribution of Means and Standard Deviation of Body Weight in Pre and Post Experiment Examination

GROUP	MEANS (GRAM)		
	Pre-experiment	***Post*-experiment**	**Differences of body weight (post-pre experiment)**
K0	176.2±12.8	230.4±17.4	54.2
P1	170±11.1	214.2±18.9	44.2
P2	197.2±3.7	234.8±12.9	37.6
P3	188.2 ±48.3	220.4±57.0	32.2
P4	192 ±16.2	218.6±27.2	26.6

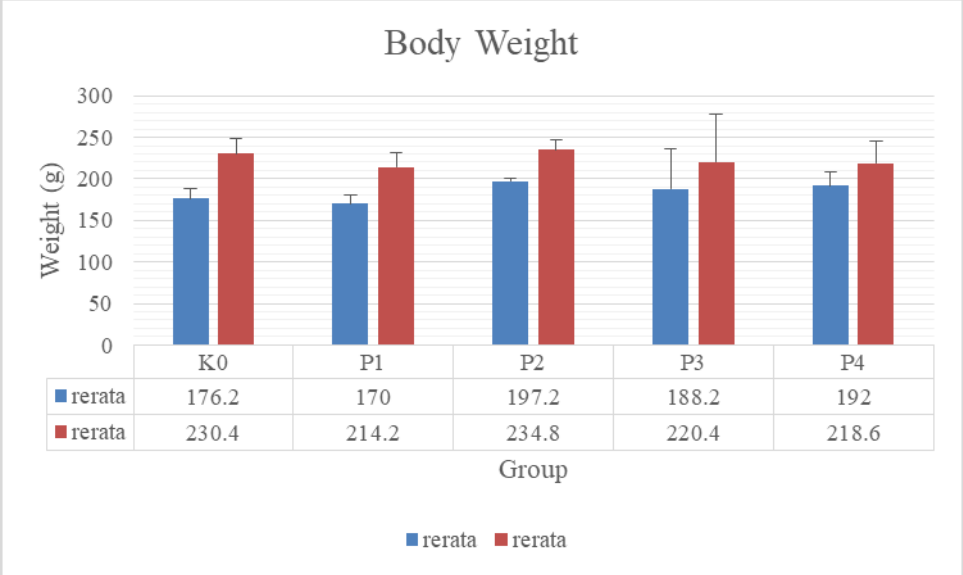


Figure 1 Table of Mean Values of Body Weight in Pre and Post Experiment Examination

Otoacoustic Emission Examination (OAE)

Table 2. Distribusi Between SNR Values and Treatment Groups in Pre and Post-Experiments OAE Examination

Group		SNR values				Total	
		Refer		*Pass*			
		Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
K0	**Pre**	0	0	5	20	5	20
	Post	0	0	5	20	5	20
P1	**Pre**	0	0	5	20	5	20
	Post	5	20	0	0	5	20
P2	**Pre**	0	0	5	20	5	20
	Post	0	0	5	20	5	20

P3	**Pre**	0	0	5	20	5	20
	Post	2	8	3	12	5	20
P4	**Pre**	0	0	5	20	5	20
	Post	2	8	3	12	5	20
Total	**Pre**	0	0	25	100	25	100
	Post	9	36	16	64	25	100

A total of 16 rats (64%) got pass in SNR results and 9 rats (36%) got refer in SNR results on post experimental OAE examinations. Furthermore, from the 16 study subjects (64%) who obtained the pass in SNR results on the post-experimental OAE examination, 5 rats (20%) from the K0 group, 5 rats (20%) from the P2 group, 3 rats (12%) from the P3 group and 3 rats (12%) from the P4 group. From the 9 rats (36%) who obtained the refer in SNR results in the post experimental OAE examination, 5 rats (20%) from the P1 group, 2 rats (8%) from the P3 group, 2 rats (8%) from group P4 (table 2).

Table 3. Distribution of Means Value and Standard Deviation of SNR Value in Pre and Post OAE Experiment Examinations

GROUP		SNR VALUE					
		1000 Hz	**1500 Hz**	**2000 Hz**	**3000 Hz**	**4000 Hz**	**5000 Hz**
K0	**Pre**	7±1.4	19±12.7	10±0	14±2.8	17±1.4	19±12.7
	Post	4±1.4	7.5±2.1	10±1.4	16.5±5.0	17.5±0.7	22.5±7.8
P1	**Pre**	13.8±9.1	16.5±11.0	13.5±2.7	14±2.8	22.1±4.3	25.7±9.3
	Post	14.6±10.1	9.5±0.8	8.5±1.7	13.5±1.6	20.2±2.7	30.5±3.0
P2	**Pre**	13.2±8.1	12.9±9.1	11.5±1.5	15.1±2.9	19.9±2.6	25.9±7.0
	Post	13.5±11.0	9.8±1.6	16.6±2.6	16.6±3.2	23±4.5	32.9±5.9
P3	**Pre**	14.6±9.1	14±12.6	11.2±3.8	15.9±2.8	24.9±7.5	27.9±10.7
	Post	3.6± 14.7	14.5±10.8	10±1.2	14.1±2.6	18.4±4.0	22.1±8.9
P4	**Pre**	22.5±5.9	9.7±3.1	13.5±5.5	16.7±6.3	24.2±6.6	27.1±10.5
	Post	50.5±13.5	10.8±9.4	9±0.9	19.2±1.7	20±2.9	23±8.2

The lowest SNR mean value on the pre-experimental OAE examination for 1000 Hz frequency was 7 ± 1.4 in the K0 group (Control) and the highest mean value was 22.5 ± 5.9 in the P4 group (Streptomycin injection + high dose oral black cumin). The lowest SNR mean value on the pre-experimental OAE examination for 1500 Hz frequency was 9.7 ± 3.1 in the P4 group (Streptomycin injection + high-dose oral black cumin) and the highest mean value of 19 ± 12.7 in the K0 group (Control). The lowest SNR mean value on the pre-experimental OAE examination for 2000 Hz frequency was 10 ± 0 in the K0 group (Control) and the highest mean value was 13.5 ± 5.5 in the P4 group (Streptomycin injection + high dose oral black cumin).

The lowest SNR mean value on the pre-experimental OAE examination for 3000 Hz frequency was 14 ± 2.8 in the K0 group (Control) and P1 group (Streptomycin injection only) and the highest mean value of 16.7 ± 6.3 in the P4 group (Streptomycin injection + oral black cumin dose dose) high). The lowest SNR mean value on the pre-experimental OAE examination for 4000 Hz frequency was 17 ± 1.4 in the K0 group (Control) and the highest mean value of 24.9 ± 7.5 in the P3 group (Streptomycin injection + medium-dose oral black cumin). The lowest SNR mean value on the pre-experimental OAE examination for 5000 Hz frequency was 19 ± 12.7 in the K0 group (Control) and the highest mean value was 27.9 ± 10.7 in the P3 group (Streptomycin injection + medium dose black cumin) (Table 3).

The lowest SNR mean value on the post-experimental OAE examination for 1000 Hz frequency was 3.6 ± 14.7 in the P3 group (Streptomycin injection + medium dose oral black cumin) and the highest mean value was 50.5 ± 13.5 in the P4 group (Streptomycin injection + high dose oral black cumin). The lowest SNR mean value on the post-experimental OAE examination for 1500 Hz frequency was 7.5 ± 2.1 in the K0 group (Control) and the highest mean value was 14.5 ± 10.8 in the P3 group (Streptomycin injection + medium dose black cumin). The lowest SNR mean value in the post-experimental OAE examination for 2000 Hz frequency was 8.5 ± 1.7 in the P1 group (Streptomycin injection) and the highest mean value was 16.6 ± 2.6 in the P2 group (Streptomycin injection + low dose oral cumin). The lowest SNR mean value in post-experimental OAE examination for 3000 Hz frequency was 13.5 ± 1.6 in the P1 group (Streptomycin injection) and the highest mean value of 19.2 ± 1.7 in the P4 group (Streptomycin injection + high dose oral black cumin). The lowest SNR mean value on the post-experimental OAE examination for the 4000 Hz frequency was 17.5 ± 0.7 in the K0 group (Control) and the highest mean value of 23 ± 4.5 in the P2 group (Streptomycin injection + low dose oral black cumin). The lowest SNR mean value on the post-experimental OAE examination for the frequency of 5000 Hz was 22.1 ± 8.9 in the P3 group (Streptomycin injection + medium dose oral black cumin) and the highest mean value of 32.9 ± 5.9 in the P2 group (Streptomycin injection + low dose oral cumin) (Table 3).

Data analysis of the mean difference in SNR values of the treatment group on post-experimental otoacoustic emissions (OAE) tests was carried out using a nonparametric Kruskal-Wallis test because the data were characterized as not normal distributed and not homogeneous. Based on table 4, the value of ρ: 0.000 was found. The p value proved that there was a significant difference in the mean SNR value $\rho < 0.05$ on the OAE examination between each treatment (K0 to P4) (Table 4).

A post hoc test was then performed to assess differences between treatment groups. Post hoc test results can be seen in table 4 above. The mean difference in the SNR value of the K0 group (control) and the P1 group (Streptomycin injection) was a significant difference $\rho < 0.05$; group K0 (control) with P2 (Streptomycin injection + low dose oral black cumin) was a significant difference $\rho < 0.05$; group K0 (control) with P3 (Streptomycin injection + medium dose oral cumin) was a significant difference $\rho < 0.05$; group K0 (control) with P4 (Streptomycin injection + high dose oral black cumin) was a significant difference $\rho < 0.05$; group P1 (Streptomycin injection) with P2 (Streptomycin injection + low dose oral cumin) there was no significant difference $\rho > 0.05$; group P1 (Streptomycin injection) and P3 (Streptomycin injection + medium dose oral cumin) there was no significant difference $\rho > 0.05$; group P1 (Streptomycin injection) with P4 (Streptomycin injection + high dose oral black cumin) there was no significant difference $\rho > 0.05$; group P2 (Streptomycin injection + low dose oral cumin) and P3 (Streptomycin injection + medium dose oral cumin) there was a significant difference $\rho < 0.05$; P2 group (Streptomycin injection + low dose oral cumin) and P4 (Streptomycin injection + high dose oral cumin) there was a significant difference $\rho < 0.05$; group P3 (injection of Streptomycin + medium dose of black cumin) and P4 (injection of streptomycin + high dose of oral cumin) were not significant differences $\rho > 0.05$ (Table 4).

Table 4. Distribution of SNR Mean Value Analysis

GROUP	p- value
K0 vs other group	0.000
K0 vs P1	0.000
K0 vs P2	0.000
K0 vs P3	0.014
K0 vs P4	0.000
P1 vs P2	1.000
P1 vs P3	0.05
P1 vs P4	0.212
P2 vs P3	0.001
P2 vs P4	0.038
P3 vs P4	1.000

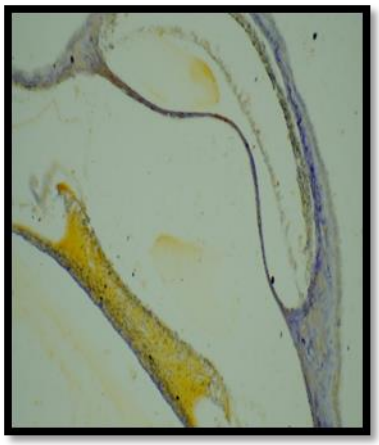
Histopathological Examination

Histopathological examination results were measured using criteria for the degree of damage of Organ corti as in table 5.

Table 5. Criteria The Degree of Organ Corti Damage[14]

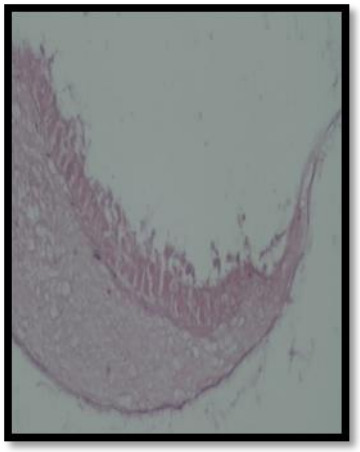
Criteria	Description	Score
Normal	No histological damage to tissue	0
Mild Damage	Several types of histological abnormalities with less than one third of damage in total field of view (≤30%)	1
Moderate Damage	Several types of histological abnormalities with more than one third of damage in total field of view (≥30% - 60%)	2
Severe damage	Several types of histological abnormalities with more than two third of damage in total field of view (≥60%)	3

Histopathological examination showed in the K0 group that there was no damage to the cochlear Organ corti structure. In the P1 group there was greater structural damage especially in the outer hair cells according to the damage scores 2 and 3 (moderate and severe damage). In group P2 there was less damage to the Organ corti structure than the P1 group seen in the outer hair cells and the cortical organ supporting cells with a score of 1 (mild damage). In the P3 group there was Organ corti damage equal to the P1 group with a score of 2 and 3 (moderate and severe damage). In group P4 there was less Organ Corti damage than groups P1 and P3 in outer hair cells and cortical organ supporting cells (Fig. 1).

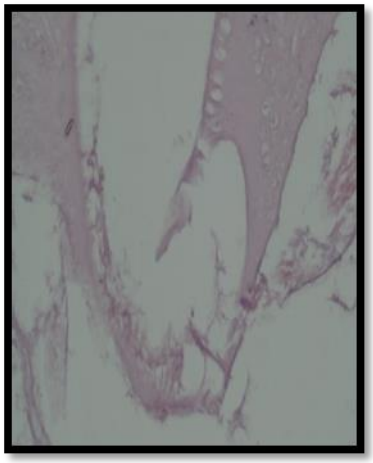


P0

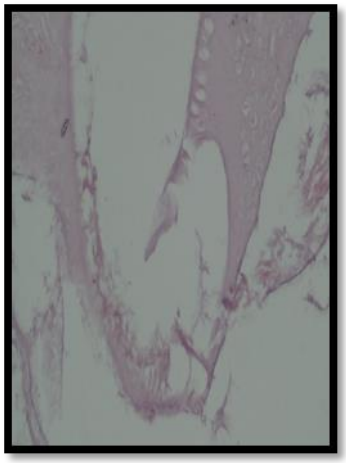
P1



P2



P3



P4

Data analysis of the mean difference in Organ corti damage scores in the treatment group was found to be ρ: 0.001. The p value proved that there was a significant difference in mean Organ corti damage score ρ <0.05 in the treatment group (K0 to P4) (Table 6).

Table 6. Distribution of Mean and Standard Deviation of Degree of Organ Corti Damage in Post-experimental Histopathological Examination

Group	Means
K0	0±0
P1	2.4±0.55
P2	1.4±0.55
P3	2.6±0.55
P4	1.6±0.55

Table 7. Analysis of Differences in Mean Values of Organ Corti Damage Scores

Group	p- value
K0 vs Other group	0.000
K0 vs P1	0.000
K0 vs P2	0.002
K0 vs P3	0.000
K0 vs P4	0.000
P1 vs P2	0.042
P1 vs P3	1.000
P1 vs P4	0.178
P2 vs P3	0.009
P2 vs P4	1.000
P3 vs P4	0.042

A post hoc test was then performed to assess differences between treatment groups. The difference in the mean score of cortical organ damage score in K0 group (control) and P1 (Streptomycin injection) group is a significant difference ρ <0.05; group K0 (control) with P2 (Streptomycin injection + low dose oral black cumin) was a significant difference ρ <0.05; group K0 (control) with P3 (Streptomycin injection + medium dose oral cumin) was a significant difference ρ <0.05; group K0 (control) with P4 (Streptomycin injection + high dose oral black cumin) was a significant difference ρ <0.05; group P1 (Streptomycin injection) with P2 (Streptomycin injection + low dose oral black cumin) there was a significant difference ρ <0.05; group P1 (Streptomycin injection) with P3 Streptomycin injection + medium dose black cumin) there was no significant difference ρ> 0.05; group P1 (Streptomycin injection) with P4 (Streptomycin injection + high dose oral black cumin) there was no significant difference ρ> 0.05; group P2 (Streptomycin injection + low dose oral cumin) and P3 (Streptomycin injection + medium dose oral cumin) there was a significant difference ρ <0.05; P2 group (Streptomycin injection + low dose oral black cumin) and P4 (Streptomycin injection + high dose oral black cumin) there was no significant difference ρ> 0.05; group P3 (injection of Streptomycin + medium dose of black cumin) and P4 (injection of streptomycin + oral high dose of cumin) have a significant difference ρ <0.05 (table 7).

Analysis of Relationship to Study Variables

Relationship Between Body Weight Examination Post Examination and Post Examination OAE Examination

Table 8. Relationship Between Body Weight Examination Post Examination and Post Examination OAE Examination

Parameter			OAE		Total	p -value
			Refer	Pass		
Body Weight	Underweight	Frequency (n)	0	0	0	0.465
		Percentage (%)	0	0	0	
	Normal	Frequency (n)	9	15	24	
		Percentage (%)	36	60	96	
	Overweight	Frequency (n)	0	1	1	
		Percentage (%)	0	4	4	
Total		Frequency (n)	9	16	25	
		Percentage (%)	36	64	100	

From 24 rats (96%) with normal weight, 15 rats (60%) obtained pass in SNR results and 9 rats (36%) obtained refer in SNR results in OAE examination. 1 rat (4%) who overweight gained pass in SNR result in OAE examination. The nonparametric Spearman correlation test obtained from SPSS analysis are ρ: 0.465. It was concluded that there was no significant relationship (ρ> 0.05) between experimental post weight and post-OAE examination (Table 8).

Relationship between Treatment Group and Body Weight Examination Post-experiment

Table 9. Relationship between Treatment Group and Body Weight Examination Post-experiment

Parameter			Body Weight			Total	ρ -value
			Underweight	Normal	Overweight		
Group	K0	Frequency (n)	0	5	0	5	0.491
		Percentage (%)	0	20	0	20	
	P1	Frequency (n)	0	5	0	5	
		Percentage (%)	0	20	0	20	
	P2	Frequency (n)	0	5	0	5	
		Percentage (%)	0	20	0	20	
	P3	Frequency (n)	0	4	1	5	
		Percentage (%)	0	16	4	20	
	P4	Frequency (n)	0	5	0	5	

	Percentage (%)	0	20	0	20
Total	Frequency (n)	0	24	1	25
	Percentage (%)	0	96	4	100

From 24 rats (96%) who had normal weight, 5 rats (20%) from the K0 group, 5 rats (20%) from the P1 group, 5 rats (20%) from the P2 group, 1 rat (4%) from the P3 group and 5 rats (20%) from the P4 group. Of 1 rat (4%) who had overweight, all of them (4%) were from the P3 group. The results of the nonparametric Spearman correlation test obtained from the SPSS analysis, were ρ: 0.491. It was concluded that there was no significant relationship (ρ> 0.05) between the treatment group and post-experimental examination body weight (Table 9).

Relationship between Treatment Group and OAE Examination Post-experiment

Table 10. Relationship between Treatment Group and OAE Examination Post-experiment

Parameter			OAE		Total	ρ -value
			Refer	Pass		
Group	K0	Frequency (n)	0	5	5	
		Percentage (%)	0	20	20	
	P1	Frequency (n)	5	0	5	
		Percentage (%)	20	0	20	
	P2	Frequency (n)	0	5	5	
		Percentage (%)	0	20	20	0.780
	P3	Frequency (n)	2	3	5	
		Percentage (%)	8	12	20	
	P4	Frequency (n)	2	3	5	
		Percentage (%)	8	12	20	
Total		Frequency (n)	9	16	25	
		Percentage (%)	36	64	100	

From 16 rats (64%) who obtained pass in SNR results on the post-experimental OAE examination, 5 rats (20%) from the K0 group, 5 rats (20%) from the P2 group, 3 rats (12%) from the P3 group and 3 rats (12%) from the P4 group. From 9 rats (36%) who got refer in SNR results on the post-experimental OAE examination, 5 rats (20%) from the P1 group, 2 rats (8%) from the P3 group, 2 rats (8%) from the group P4. The results of the nonparametric Spearman correlation test obtained from the SPSS analysis are ρ-values: 0.780. It was concluded that there was no significant relationship (ρ> 0.05) between the treatment group and post-experimental examination body weight (Table 10).

Relationship between Body Weight Post-experiment and Degree of Organ Corti Damage

From 5 rats (20%) that got normal results, 5 rats (20%) had normal weight. From 5 rats (20%) that got mild damage, 5 rats (20%) had normal weight. From 10 rats (40%) that got moderate damage, 9 rats (36%) had normal weight and 1 rats (4%) had overweight. From 5 rats (20%)

that got severe damage, 5 rats (20%) had normal weight. The results of the nonparametric Spearman correlation test obtained from the SPSS analysis, are ρ: 0.725. It was concluded that there was no significant relationship (ρ> 0.05) between body weight examination and the degree of organ corti damage (Table 11).

Table 11. Relationship between Body Weight Examination and Degree of Organ Corti Damage

Parameter			Degree of Damage				Total	P-value
			Normal	Mild	Moderate	Severe		
Body weight	Underweight	Frequency (n)	0	0	0	0	0	0.725
		Percentage (%)	0	0	0	0	0	
	Normal	Frequency (n)	5	5	9	5	24	
		Percentage (%)	20	20	36	20	96	
	Overweight	Frequency (n)	0	0	1	0	1	
		Percentage (%)	0	0	4	0	4	
	Total	Frequency (n)	5	5	10	5	25	
		Percentage (%)	20	20	40	20	100	

Relationship between OAE Examination Post-experiment and Degree of Organ Corti Damage

Table 12. Relationship between OAE Examination and Degree of Organ Corti Damage

Parameter			Degree of Damage				Total	p-value
			Normal	Mild	Moderate	Severe		
OAE	Refer	Frequency (N)	0	0	5	4	9	0.001; r: 0.635
		Percentage (%)	0	0	20	16	36	
	Pass	Frequency (N)	5	5	5	1	16	
		Percentage (%)	20	20	20	4	64	
Total		Frequency (N)	5	5	10	5	25	
		Percentage (%)	20	20	40	20	100	

From 5 rats (20%) that got normal results, 5 rats (20%) pass in OAE examination results. From 5 rats (20%) that got mild damage, 5 rats (20%) pass in OAE examination results. From 10 rats

(40%) that got moderate damage, 5 rats (20%) pass in OAE examination results and 5 rats (20%) refer in OAE examination results. From 5 rats (20%) that got heavy damage, 4 rats (16%) refer in OAE examination and 1 rat (4%) pass in OAE examination. The results of the nonparametric Spearman correlation test obtained from the SPSS analysis are ρ-values: 0.001 values with r-values: 0.635. It was concluded that there was a strong relationship ($\rho < 0.05$) with $r > 0.59$ between OAE examination and the degree of cortical organ damage (Table 12).

Relationship between Treatment Group and Degree of Organ Corti Damage

Table 13. Relationship between Treatment Group and Degree of Organ Corti Damage

Parameter			Degree of Damage				Total	P-value
			Normal	Mild	Moderate	Severe		
Group	K0	Frequency (n)	5	0	0	0	5	
		Percentage (%)	20	0	0	0	20	
	P1	Frequency (n)	0	0	3	2	5	
		Percentage (%)	0	0	12	8	20	
	P2	Frequency (n)	0	3	2	0	5	
		Percentage (%)	0	12	8	0	20	
	P3	Frequency (n)	0	0	2	3	5	0.036,
		Percentage (%)	0	0	8	12	20	r: 0.421
	P4	Frequency (n)	0	2	3	0	5	
		Percentage (%)	0	8	12	0	20	
Total		Frequency (n)	5	5	10	5	25	
		Percentage (%)	20	20	44	20	100	

5 rats (20%) that got normal results, 5 rats (20%) from the K0 group. 5 rats (20%) that got mild damage, 3 rats (12%) from the P2 group and 2 rats (8%) from the P4 group. From 10 rats (40%) that got moderate damage, 3 rats (12%) from group P1 and group P4; 2 rats (8%) from the P2 group and from P3 group. 5 rats (20%) that got severe damage, 3 rats (12%) from the P3 group and 2 rats (8%) from the P1 group. The results of the nonparametric Spearman correlation test obtained from the SPSS analysis are ρ-values: 0.036 with r-values: 0.421. It was concluded that there was a moderate relationship ($\rho < 0.05$) with a value of $r > 0.40$ between the treatment group and the degree of organ corti damage (Table 13).

4 Discussion

Body Weight Examination

The weight of all subjects must be measured at the pre-experimental examination (day 0) to determine whether the subjects are eligible to be included in the study or not. A total of 25 Wistar rats were measured for weight and all of them were eligible to be grouped randomly into five treatment groups. The body weight included in the study was 100-200 grams.

The results of pre-experimental body weight examination (day 0) and post-experimental examination (day 30) made the average weight value of each group to see the comparison of the average weight value between groups. From the results on both days, weight gain was found for each group on the post-experimental examination compared to the pre-experiment examination. There is a few of factors that increase body weight such as the increasing age of the subject, the frequency of eating a day, the type of food given and the side effects from the administration of Streptomycin and black cumin. Kruskall-Wallis analysis test was conducted to test the differences in the mean weight value of each group. From the results of the analysis test group K0 (control) with the treatment groups P1, P2, P3 and P4, found no significant difference $\rho > 0.05$ with the value ρ-value (ρ: 0.549). This proves that the difference in oral cumin dose did not cause a significant difference in weight gain for the treatment group. From research before, prove that black cumin is able to reduce levels of fat in the blood and is able to remove antidiabetic effects, however, the study did not explain the effectiveness of black cumin in losing weight. [15]

In addition, the type of food provided also plays a role in weight gain. In this study given food in the form of a mixture of 552 pellets (pork food) and ground corn. The food provided is a high-fat diet. From the research of Thatit Nurmawati, there is a correlation between body weight with a high-fat diet. [16] This proves that a high-fat diet has an effect on increasing the subject's weight.

Intramuscular administration of Streptomycin also plays a role in weight gain. This was explained by Fernando in his study, which stated that the accumulation of fat was due to the administration of Streptomycin.[17] Giving a high-fat diet and giving Streptomycin cause weight gain without neglecting the factors that increase the subject's age which may be a side cause of the subject's weight gain.

In this study there was no relationship between the treatment group with body weight with a value of $\rho > 0.05$; ρ-value (ρ: 0.491) and there is no relationship between body weight with the results of OAE examination with a value of $\rho > 0.05$; ρ-value (ρ: 0.465).

OAE Examination

Otoacoustic emission examination were performed on all groups (K0 to P4) on the pre-experiment (day 0) and on the post-experiment (day 30) before surgical procedures to take tissue. From the results of the DPOAE pre-experimental examination conducted, it was found that all the rats taken fulfilled the inclusion criteria and none of them followed the exclusion criteria. From the results of the post-experimental DPOAE examination conducted, it was found that the average SNR value was decreased in each group. From the above data, in the K0 (control) group there was a decrease in SNR values at a frequency of 1500 Hz and an increase in SNR values at frequencies of 3000 Hz, 4000 Hz and 5000 Hz. In group P1 (Streptomycin

injection) SNR values were found at frequencies of 1000 Hz, 1500 Hz, 2000 Hz and 4000 Hz and an increase in SNR values at frequencies of 3000 Hz and 5000 Hz. In the P2 group (Streptomycin injection + low dose oral black cumin) there was a decrease in SNR values at a frequency of 1500 Hz and an increase in SNR values at frequencies of 3000 Hz, 4000 Hz and 5000 Hz. In group P3 (injection of Streptomycin + oral black cumin medium dose) SNR values were found at frequencies of 1000 Hz, 3000 Hz, 4000 Hz and 5000 Hz; an increase in SNR values at a frequency of 4000 Hz was found. In group P4 (injection of Streptomycin + high-dose oral black cumin) there was a decrease in SNR values at frequencies of 2000 Hz, 4000 Hz and 5000 Hz; an increase in SNR values at frequencies of 1000 Hz, 1500 Hz and 3000 Hz was found.

DPOAE examination is one of OAE examinations, other than TEOAE (Transient evoked OAE). This OAE examination is to measure the stimulus captured by outer hair cells in the cochlea. The stimulus released by the OAE device through a probe placed in the subject's right and left ears will produce readings on the OAE device in the form of amplitude, noise floor and SNR value; which is the measurement of this research. **[18]** SNR values can be obtained from the Distortion Product (DP) value reduced by Noise Floor (NF), assessed the results of several frequencies starting from 1000 Hz, 1500 Hz, 2000 Hz, 3000 Hz, 4000 Hz and 5000 Hz. [14] This OAE examination is associated with cochlear and inner ear function. This OAE examination can measure the sensitivity of the ototoxic properties of Streptomycin and the protective properties of black cumin against cochlea and ears in the subject through decreasing SNR values in the treatment group. **[19]**

From the results of the OAE above, a decrease in the value of SNR at several frequencies in several groups showed the existence of ototoxic effects of Streptomycin and protective effects released by black cumin. *World Health Organization* (WHO) has proven that Streptomycin is ototoxic.[2] Black cumin is also capable to give protective effect from the ototoxic effect of Streptomycin. [10,20,21] Group P1 (Streptomycin injection) found a significant difference in mean SNR value $\rho < 0.05$ compared to group K0 (control). This was also proven in the research of several researchers. [4,5,22] In that study, the researchers proved that Streptomycin and aminoglycoside drugs are ototoxic. Ototoxic streptomycin causes hearing loss and damage to outer hair cells in the cochlea which causes a decrease in the value of the SNR in OAE examination. P2 group (Streptomycin injection + low dose oral black cumin) showed significant difference in mean SNR value $\rho < 0.05$ with group K0 (control). This happens because the protective effect of Black Cumin is still minimal. The P3 group (Streptomycin injection + medium-dose oral cumin) showed no significant difference $\rho > 0.05$ with the K0 group (control). This proves the protective effect of black cumin; group P4 (Streptomycin injection + high dose oral black cumin) showed a significant difference in mean SNR value $\rho < 0.05$ with group K0 (control) which showed the protective effect of black cumin was still minimal.

This proves that the ototoxicity effect of Streptomycin can be inhibited by the protective effect released from black cumin. Black cumin has an active compound such as thymoquinine.

Thymoquinine will produce antioxidant effects that will inhibit the formation of ROS while inhibiting the toxicity effect of Streptomycin. [10] Decreasing and increasing in SNR values on OAE examination clearly indicate differences in the effects of Streptomycin toxicity and the protective effect of each dose of black cumin.

In this study there was no relationship between the treatment group with the results of OAE examination with a value of ρ> 0.05; ρ-value (ρ: 0.473) and there is no relationship between body weight with the results of OAE examination with a value of ρ> 0.05; ρ-value (ρ: 0.684).

Histopathological Examination

Histopathological examination was performed on all groups (K0 to P4) after surgical procedures to take tissue. The above results show that in the K0 group there was no damage to the cochlear organ corti structure with a damage score of 0 (normal). In group P1 there were heavier structural damage especially in the outer hair cells according to damage scores 2 and 3 (moderate and severe damage). In P2 group there was minimal structural damage to organ corti seen in outer hair cells and organ corti support cells with a score of 1 (mild damage). In group P3 there was organ corti damage that was as severe as group P1 with damage scores 2 and 3 (moderate and severe damage). In the P4 group there was less organ corti damage than the P1 and P3 groups in the outer hair cells and organ corti support cells with damage scores of 1 and 2 (mild and moderate damage). There is a moderate relationship between the treatment group and the degree of organ corti damage with a value of r: 0.421 with a value of p: 0.036. Furthermore, there is a strong relationship between OAE post experimental examination and the degree of organ corti damage with a value of r: 0.635 with a value of p: 0.001. The degree of cortical organ damage was assessed from histopathological examination by Hematoxylin-Eosin (HE) staining. This examination aims to assess the structure of microanatomies from each treatment that has been done. The microanatomy structure was assessed based on the criteria for the degree of damage to organ corti above. Histopathological examination also reinforces the results taken in OAE examination because OAE examination only assesses the value of the SNR and measures the function of the ear from stimulus generation. The results of this study are consistent with the study. [23,24]

In this study, each treatment group was induced by Streptomycin intramuscularly every day which would cause damage to stereocilia and finally ended with apoptotic cell death inducing ROS formation. P2, P3 and P4 groups were also given orally different black cumin doses for each group. The protective effect of black cumin is still not known with certainty for ototoxicity, but there are some studies mentioning the ototoxic properties of streptomycin can be inhibited from the antioxidant properties of black cumin. [25] From this study, the antioxidant properties of black cumin released by thymoquinine (TQ) can inhibit from ototoxicity. In this study there was no relationship between body weight and the degree of organ corti damage with a value of ρ> 0.05; ρ-value (ρ: 0.72)

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