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Comparison of the effects of selected aminoglycoside antibiotics on motor behaviors in mice

Seyed Ali Ayati Najafabadi, Ali Rassouli*, Goudarz Sadeghi-Hashjin

Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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Abstract

Aminoglycoside antibiotics (AGs) can cause neuromuscular blockade and paralysis of skeletal muscles. To compare the paralytic effects of selected AGs on some motor behaviors in mice, 24 male mice were divided into four groups. Each group was given one of AGs (gentamicin, dihydrostreptomycin, apramycin and amikacin) at incremental doses that increased half-logarithmically compared to the therapeutic dose (16.00 mg kg^{-1}). Motor behavioral tests included open field test, inclined plane, horizontal bars, static rods, parallel bars and rotarod. Finally, the data were analyzed using descriptive and analytical statistics. Gentamicin and dihydrostreptomycin at 32.00 times of the therapeutic dose produced complete paralysis of the limbs, respiratory arrest, and even death in some animals. However, apramycin and amikacin did not show significant effects on skeletal muscle and motor behaviors at 32.00 times of the therapeutic dose. After administration of apramycin at 100 times of the therapeutic dose, four out of six mice (66.67%) died from respiratory depression. Amikacin at this dose did not cause animal death, although it caused some changes in motor behaviors with a significant difference in comparison with control values. Gentamicin demonstrated significantly more potent effects on motor behaviors compared to the other AGs. Overall, the order of potency was gentamicin > dihydrostreptomycin > apramycin > amikacin. High doses of AGs could impair the skeletal muscle function and disrupt motor behaviors in mice. Furthermore, the paralytic potency of selected AGs on skeletal muscle was significantly different.

Introduction

Aminoglycosides (AGs) are bactericidal antibiotics mainly used to treat serious infections caused by aerobic Gram-negative bacteria and staphylococci.[1] The commonly used AGs in veterinary and human medicine include streptomycin, dihydrostreptomycin, gentamicin, amikacin, neomycin, kanamycin, tobramycin and apramycin.[2,3] These drugs work by inhibiting protein synthesis in bacteria.[4] They are poorly absorbed by gastrointestinal tract,[5] and when administered through injection they are eliminated in their intact form mainly by renal glomerular filtration.[3]

The great value of AGs lies in their broad anti-bacterial spectrum and fast bactericidal properties.[6] In medicine, these drugs are used to treat diseases such as nosocomial respiratory tract infection, complicated urinary tract infection, peritonitis, endometritis, mastitis and septicemia.[7,8]

However, the relative toxicity of AGs has limited their use for treating severe infections.[1] Nephrotoxicity (acute tubular necrosis) and ototoxicity (vestibular and auditory dysfunctions) are the main side effects of AGs[9,10] and have been investigated in numerous studies.[11-13] Along with these commonly reported adverse effects, AGs can also cause neuromuscular blockade and paralysis of skeletal muscles[14] which may lead to apnea.[10] The AGs inhibit acetylcholine release at the pre-synaptic site[15] by blocking calcium entry in the nerve terminal *via* N-type calcium channels.[2] Neuromuscular blockade is uncommon, but may occur after intraperitoneal (IP) or intravenous (IV) administrations of large doses of these drugs.[2,10] Also, this side effect occurs mainly in association with anesthesia or administration of other neuromuscular blocking agents.[2] Neuromuscular blockade may be reversed by IV administration of calcium salts such as calcium chloride or calcium gluconate.[1,10] Neostigmine may also reverse this side effect.[1]

So far, only a few studies have been documented regarding the neuromuscular blockade caused by AGs in *in vivo* conditions and their effects on motor behaviors in

***Correspondence:**

Ali Rassouli. DVM, PhD
Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
E-mail: arasooli@ut.ac.ir

intact animals. Most previous studies on this side effect have been done in *in vitro* conditions and on isolated organs.[16-20] Based on the available data; it was hypothesized that AGs at high doses can change motor behaviors in mice even before inducing complete skeletal muscle paralysis or death. Another hypothesis suggests that various AGs have different effects and potencies on motor behaviors.

In the present study, the muscle paralytic effects and potencies of four AGs including gentamicin, amikacin, dihydrostreptomycin and apramycin, were investigated through evaluation of a number of motor behaviors in mice.

Materials and Methods

Experimental animals. Twenty-four male albino mice weighing 20.00 - 25.00 g were used in this study. The mice were kept under standard laboratory conditions with a temperature of 22.00 ± 2.00 ˚C and a 12:12 hr light-dark schedule starting at 7:00 am. They had free access to food and water. The mice were divided into four treatment groups with six mice in each one. The protocol of this study was approved by the institutional Ethics Committee at the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran (No. 7506006/6/12).

Drugs and treatment protocol. The AG preparations used in the present study were gentamicin, amikacin (Caspian Tamin Pharmaceutical Co., Rasht, Iran), dihydrostreptomycin (Erfan Darou Pharmaceutical Co., Tehran, Iran) and apramycin (Rooyan Darou Pharmaceutical Co., Tehran, Iran). Each group was given one of these AGs at incremental doses that increased half-logarithmically compared to their therapeutic dose (16.00 mg kg^{-1})[21,22] equal to the therapeutic dose, and 3.20, 10.00 and 32.00 times of the therapeutic dose. Before administrating AGs, a single IP injection of sterile 0.90% saline solution was given to the animals in each group and behavioral tests were performed. The results of these tests were recorded as the control values for comparison. Twenty-four hr after administrating the saline solution, the therapeutic dose of each drug was administered to the mice and then, incremental doses were administered at 72-hr intervals. Injectable sterile water was used to prepare different dilutions of the drugs just before each dose was injected, ensuring that the injection volume ranged from 0.20 to 0.50 mL for each animal. If the injected doses did not affect the motor behavior of the animal, the dose was increased until it caused complete paralysis of the limbs, respiratory arrest, or death in one-third of the animals in the group. In animals where the higher doses of a drug caused complete paralysis of the limbs or respiratory arrest, resulting in loss of limb strength, calcium gluconate (Nasr Pharmaceutical Co., Mashhad, Iran) was administered at a dose of 100 mg kg^{-1},[22] in an attempt to reverse the paralytic effect of the AG drug.

Behavioral tests. The motor behavioral tests in this study included open field test, inclined plane, horizontal bars, static rods, parallel bars and rotarod. Twenty to 30 min after the administration of different doses of AGs, the evaluation of these tests was started. All analyses were done during the light phase. Animals were allowed at least one week to adapt to the laboratory conditions before the experiments. One day before the administration of saline solution and recording of the control values, these tests, except the open field, were performed several times; so that, the animals were familiar with the testing method.

Open field test. To perform this test, a circular white cardboard surface with a diameter of 50.00 cm was used. The surface was divided into four equal parts by two intersecting perpendicular lines and surrounded by a 15.00 cm high wall. The animals were gently placed in the center of the field and then, the number of times they completely crossed the lines during 5 min was recorded as their locomotor activity data.

Inclined plane test. A wooden surface measuring 25.00 × 30.00 cm was used for this experiment. The angle of this wooden surface could be adjusted from 30.00° to 85.00°. This apparatus was set at an angle of 55.00° and the mouse was placed in the middle of the inclined plane. The time that the animal could remain in that position was recorded. This process was repeated three times for each mouse and the average time that the animal remained on the inclined plane was reported as a final result.

Horizontal bars. The bars were made of iron with different diameters (2.00 and 4.00 mm) and the same length of 38.00 cm. They were held 49.00 cm above the bench surface by a wooden support column at each end. The mouse was placed on the bar with its forepaw. The maximum test time was 30 sec. During this period, mice could either hold on for 30 sec, fall off, or traverse the bar and touch one of the support columns. Scoring was done in a similar manner as described by Deacon.[23] If the mouse held on for the full 30 sec or touched the column with its forepaw; it received a score of 5. If the animal fell off between 1 - 5, 6 - 10, 11 - 20 or 21 - 30 sec, it received a score of 1 to 4, respectively. The scores of each mouse on each bar were recorded separately.

Static rods. According to the method presented by Deacon,[23] five wooden rods with different diameters (35.00, 28.00, 22.00, 15.00 and 9.00 mm), each 60.00 cm in length, were used being fixed to a bench as parallel lines with their height being 60.00 cm above the floor. At first, the mouse was placed at the far end of the widest rod (35.00 mm) and allowed to do anything as its desire; while, two items were measured and recorded: Orientation time (the time taken to orientate 180° from the starting position towards the bench) and transit time (the time taken the nose of mouse to reach the 10.00 cm mark from the bench end of the rod). After testing on one rod, the mouse was given a short rest, then placed on the

next smaller-size rod and tested in the same way again. The maximum time considered for this test was 120 sec. If the mouse spent the maximum test time to reach on one rod, 120 sec was considered for it, but the animal was not tested on remaining smaller rods any more.

Parallel bars. Two parallel iron bars, each measuring 1.00 m in length and 4.00 mm in diameter, were fixed 30.00 mm apart by wooden supporting columns at their ends. The height of the bars above the floor was 60.00 cm. The method used was the same as one described by Deacon.[23] For each mouse, the orientation time (the time taken to orientate 90.00° from the starting position) and the transit time (the time taken to reach one of the end supports) were measured. The maximum time allowed for this test was 120 sec.

Rotarod test. The apparatus used for this test consisted of a base platform and a rotating rod, positioned 22.00 cm above the base. The 30.00 cm-long rotating rod was divided into four equal sections with separating partitions. The surface of the rod featured parallel ridges along the longitudinal axis, enabling the mice to grip it effectively. Five different speeds (15, 20, 30, 35 and 40 RPM) were specified on this apparatus, which could be adjusted and changed manually. The test method was the same as one described by Deacon.[23] Firstly, the device was set to a speed of 15 RPM and then, the mouse was placed on the rotating rod, facing away from the direction of rotation. The apparatus speed was increased by 10 sec after placing the mouse on the rod. This process continued until the mouse fell off and the RPM at which the mouse fell was recorded.

Overall, the timeline of the behavioral tests for each mouse was as follows: Open field test took 5 min and then, there was a resting time in solitary cage for 2 min. Inclined plane test took 2 min at maximum and there was a resting time for 2 min afterwards. Horizontal bar test also took 2 min at maximum (30 sec + 1 min rest + 30 sec) and then, there was a resting time for 2 min, too. Static rod test took 14 min at maximum (120 sec + 1 min rest + 120 sec + 1 min rest + 120 sec + 1 min rest + 120 sec+ 1 min rest + 120 sec) and then, there was a resting time for 2 min, again. Parallel bar test also took 2 min at maximum and there was a resting time for 2 min afterwards. At the end, rotarod test took 50 sec at maximum for each animal.

Statistical analysis. The statistical analysis was performed using SigmaPlot Software (version 14.0.0.124; Systat Software Inc., USA). Data were analyzed using a one-way ANOVA *post-hoc* Tukey test. The results were expressed as mean ± standard deviation. A value of $p < 0.05$ was considered significant. To compare the potencies of four selected AGs to induce alterations in mice motor behaviors, the responses of animals at 32.00 times of therapeutic dose of each drug were analyzed statistically.

Results

Maximum testable/ tolerable dose of each AG. The administration of gentamicin at 32.00 times of the therapeutic dose caused hind limb muscle paralysis in all six mice and both hind limb and forelimb muscles paralysis in three out of six mice (50.00%). By administrating calcium gluconate (100 mg kg^{-1}) through IP injection, muscle strength returned to normal in two mice after a few min, but the third mouse died due to the paralysis of respiratory muscles. Dihydrostreptomycin at 32.00 times of the therapeutic dose led to muscle paralysis in both limbs in two out of six mice (33.33%). Fortunately, both mice recovered after using calcium gluconate. The animals that received calcium gluconate were excluded from the study. Amikacin and apramycin at 32.00 times of the therapeutic dose made no changes in the motor behaviors of the tested animals; therefore, these drugs were administered at 100 times of the therapeutic dose. Apramycin at this higher dose caused respiratory arrest and death in four out of six mice (66.67%); hence, the maximum tolerable dose for apramycin in this study was considered to be 32.00 times of the therapeutic dose. In general, gentamicin demonstrated significantly more potent effects on motor behaviors compared to the other selected AGs and the order of potency was as follows: Gentamicin > dihydrostreptomycin > apramycin > amikacin (Table 1, Figs. 1 - 4).

Open field test. Increasing the dose of drugs in all treatment groups caused a decrease in locomotor activity (the average number of lines crossed during 5 min). The locomotor activity of the mice after the administration of each drug, at 10.00 times of the therapeutic dose and higher, decreased significantly compared to the control values ($p < 0.05$; Table 1).

Inclined plane test. Gentamicin and dihydrostreptomycin, at 32.00 times of the therapeutic dose, caused a significant decrease in the time the animal remained on the surface compared to the control values ($p < 0.05$). But, amikacin at 100 times of the therapeutic dose demonstrated a significant decrease in the time the animal remained on the inclined plane compared to the control values ($p < 0.05$). On the other hand, administration of apramycin at the highest dose (32.00 times of the therapeutic dose) did not lead to any significant difference in the time the animal remained on the surface compared to the control group (Fig. 1).

Horizontal bars. The average score obtained by gentamicin at 32.00 times of the therapeutic dose on the 2.00 mm horizontal bar, showed a significant decrease ($p < 0.05$) compared to the control value. However, the administration of the highest doses of other drugs in the treatment groups did not show significant changes (Fig. 2A). In addition, the administration of gentamicin and amikacin at the highest dose led to a significant decrease in

the scores on the 4.00 mm bar compared to the control values ($p < 0.05$); while, the highest doses of dihydrostreptomycin and apramycin did not significantly change the scores on this bar (Fig. 2B).

Static rods. Gentamicin and amikacin at the highest dose (32.00 and 100 times of the therapeutic dose, respectively) significantly increased the orientation and transit times on all wooden rods compared to the control values ($p < 0.05$). However, the highest dose of two other drugs including dihydrostreptomycin and apramycin, did not create any significant difference in the orientation and transit times of the animals on the wooden rods. To summarize the data and for clarity, only the orientation and transit times on three specific rods (35.00, 22.00 and 15.00 mm) were presented in Table 2.

Table 1. The effects of incremental doses of selected aminoglycosides on locomotor activity in mice. The therapeutic dose (TD) was 16.00 mg kg^{-1}.

Antibiotic	No.	Doses	No. of lines crossed
Gentamicin	6	Control	41.50 ± 6.02
	6	TD	37.50 ± 4.68
	6	TD × 3.20	35.83 ± 4.95
	6	TD × 10.00	32.83 ± 3.08^{a}
	3	TD × 32.00	9.00 ± 1.63^{c*†}
Apramycin	6	Control	45.50 ± 3.91
	6	TD	41.50 ± 4.99
	6	TD × 3.20	40.00 ± 3.83
	6	TD × 10.00	35.83 ± 3.72^{b}
	6	TD × 32.00	32.00 ± 3.51^{c}
Dihydrostreptomycin	6	Control	40.33 ± 4.78
	6	TD	36.83 ± 2.34
	6	TD × 3.20	35.33 ± 5.85
	6	TD × 10.00	31.00 ± 4.43^{a}
	4	TD × 32.00	20.25 ± 3.56^{c‡}
Amikacin	6	Control	45.50 ± 4.31
	6	TD	41.83 ± 4.06
	6	TD × 3.20	39.50 ± 5.22
	6	TD × 10.00	36.17 ± 3.18^{a}
	6	TD × 32.00	32.50 ± 4.92^{c}
	6	TD × 100	10.33 ± 4.31^{c}

^{abc} indicate a significant difference compared to the control values ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively).
* indicates a significant difference compared to the values of groups received apramycin and amikacin at 32.00 times of the therapeutic dose ($p < 0.001$). † indicates a significant difference compared to the values of groups received dihydrostreptomycin at 32.00 times of the therapeutic dose ($p < 0.01$). ‡ indicates a significant difference compared to the values of groups received apramycin and amikacin at 32.00 times of the therapeutic dose ($p < 0.01$).

Parallel bars. The orientation and transit times on parallel bars significantly increased after the administration of the highest doses of gentamicin and amikacin compared to the control values ($p < 0.05$). However, dihydrostreptomycin and apramycin at the highest doses did not cause any significant difference in the orientation and transit times of the animals on parallel bars (Fig. 3).

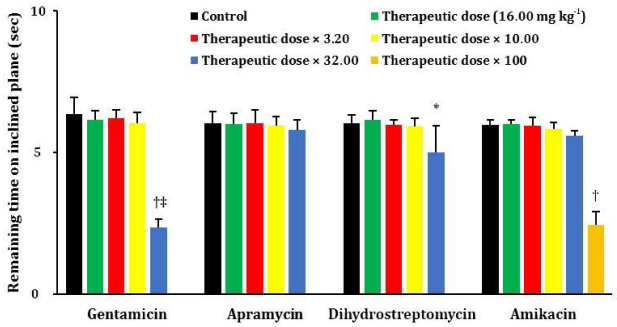

Fig. 1. The effect of incremental doses of selected aminoglycosides on the time the mice remained on the 55.00° inclined plane; the data show the performance of six animals, except for the columns of 32.00 times of the therapeutic dose of gentamicin and dihydrostreptomycin, where the results were obtained from three and four mice, respectively.
* and † indicate significant differences compared to the control values ($p < 0.01$ and $p < 0.001$, respectively). ‡ indicates a significant difference compared to the values of groups received apramycin and amikacin at 32.00 times of the therapeutic dose ($p < 0.05$).

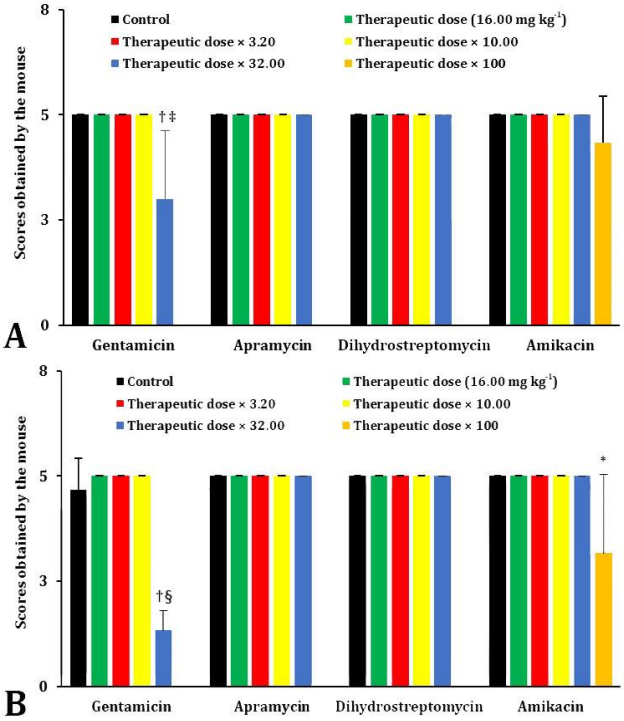

Fig. 2. The effect of incremental doses of selected aminoglycosides on the scores obtained by mice on horizontal bars. **A)** Scores on the 2.00 mm horizontal bar; **B)** Scores on the 4.00 mm horizontal bar. The results of all columns were obtained from six animals, except for the columns of 32.00 times of the therapeutic dose of gentamicin and dihydrostreptomycin, where the results were obtained from three and four mice, respectively.
* and † indicate significant differences compared to the control values ($p < 0.01$ and $p < 0.001$, respectively). ‡ and § indicate a significant difference compared to the values of groups received other aminoglycosides at 32.00 times of the therapeutic dose ($p < 0.01$ and $p < 0.001$, respectively).

Table 2. The performance of mice on different static rods following administration of incremental doses of selected aminoglycosides. The therapeutic dose (TD) was 16.00 mg kg^{-1}.

Antibiotics	Doses	No.	35.00 mm static rod		22.00 mm static rod		9.00 mm static rod	
			Orientation time (sec)	Transit time (sec)	Orientation time (sec)	Transit time (sec)	Orientation time (sec)	Transit time (sec)
Gentamicin	Control	6	5.25 ± 1.15	5.10 ± 1.19	8.41 ± 0.86	6.36 ± 0.57	13.50 ± 1.58	7.61 ± 0.85
	TD	6	5.49 ± 1.22	5.46 ± 1.24	8.58 ± 0.85	6.22 ± 0.97	13.80 ± 0.77	7.53 ± 1.21
	TD × 3.20	6	5.83 ± 1.53	4.94 ± 1.11	8.40 ± 1.25	6.76 ± 0.53	13.90 ± 1.87	7.36 ± 1.11
	TD × 10.00	6	5.53 ± 1.16	6.07 ± 0.44	8.48 ± 1.11	6.87 ± 0.68	13.10 ± 1.58	7.48 ± 1.11
	TD × 32.00	3	92.00 ± 39.50^{c*}	82.70 ± 52.80^{c*}	120 ± 0.00^{c*}	120 ± 0.00^{c*}	120 ± 0.00^{c*}	120 ± 0.00^{c*}
Apramycin	Control	6	5.59 ± 1.27	5.34 ± 0.69	8.76 ± 0.95	6.15 ± 1.15	13.30 ± 1.94	7.51 ± 1.20
	TD	6	5.87 ± 1.01	5.43 ± 0.81	8.53 ± 0.97	6.22 ± 1.12	13.60 ± 2.04	7.54 ± 1.28
	TD × 3.20	6	5.71 ± 0.92	6.12 ± 0.47	8.68 ± 1.09	6.35 ± 0.33	13.50 ± 1.13	6.57 ± 1.05
	TD × 10.00	6	5.85 ± 0.74	5.71 ± 0.53	9.04 ± 1.12	6.05 ± 0.29	13.90 ± 0.71	7.26 ± 0.58
	TD × 32.00	6	6.29 ± 1.17	6.10 ± 0.94	9.40 ± 1.22	6.23 ± 1.15	13.10 ± 1.95	7.00 ± 1.05
Dihydrostreptomycin	Control	6	5.83 ± 0.97	5.52 ± 0.85	8.06 ± 0.74	6.02 ± 0.70	12.70 ± 1.19	7.51 ± 1.37
	TD	6	5.08 ± 0.99	5.86 ± 1.11	8.23 ± 1.51	6.26 ± 0.59	13.30 ± 1.02	7.17 ± 1.72
	TD × 3.20	6	5.97 ± 0.89	5.80 ± 1.06	8.16 ± 1.31	6.37 ± 1.27	12.50 ± 1.24	6.75 ± 0.83
	TD × 10.00	6	6.12 ± 0.94	5.32 ± 1.01	8.28 ± 0.97	6.18 ± 1.38	13.10 ± 1.95	6.70 ± 1.36
	TD × 32.00	4	7.31 ± 3.84	6.34 ± 1.54	11.50 ± 4.28	6.84 ± 1.65	15.70 ± 7.40	7.11 ± 1.13
Amikacin	Control	6	5.96 ± 1.09	5.00 ± 0.11	9.25 ± 1.57	6.22 ± 1.23	13.80 ± 0.84	7.20 ± 1.39
	TD	6	5.60 ± 0.83	5.87 ± 1.27	9.09 ± 0.75	5.75 ± 0.67	13.80 ± 1.31	6.99 ± 1.13
	TD × 3.20	6	5.85 ± 1.10	5.39 ± 1.02	9.26 ± 1.29	5.98 ± 0.83	13.50 ± 1.59	7.64 ± 1.42
	TD × 10.00	6	6.06 ± 0.63	5.89 ± 1.47	9.47 ± 1.00	5.57 ± 0.96	13.80 ± 0.92	6.77 ± 1.11
	TD × 32.00	6	5.90 ± 0.86	5.13 ± 0.98	9.71 ± 1.07	5.67 ± 0.44	14.20 ± 0.75	7.57 ± 1.27
	TD × 100	6	53.20 ± 47.60^{b}	45.70 ± 25.70^{a}	92.20 ± 39.60^{c}	83.90 ± 51.10^{c}	108 ± 27.00^{c}	101 ± 42.10^{c}

^{abc} indicate a significant difference compared to the control values ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). * indicates a significant difference compared to the groups received other aminoglycosides at 32.00 times of the therapeutic dose ($p < 0.001$).

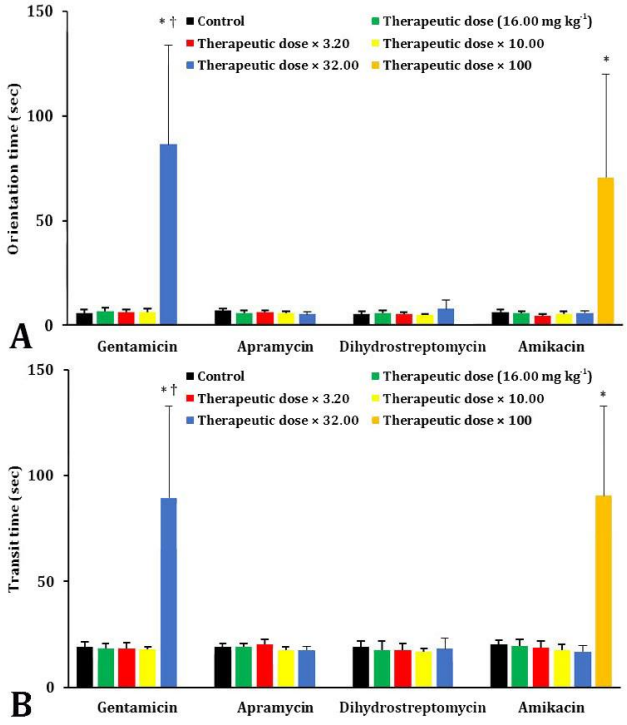

Fig. 3. The effect of incremental doses of selected aminoglycosides on the performance of mice on parallel bars. **A)** Orientation time; **B)** Transit time.
* indicates a significant difference compared to the control value ($p < 0.001$). † indicates a significant difference compared to the values of groups received other aminoglycosides at 32 times of the therapeutic dose ($p < 0.001$).

Rotarod test. The highest doses of gentamicin and amikacin caused the mice to fall down at lower speeds of the apparatus, being significantly different compared to the control values ($p < 0.05$). While, the dihydrostreptomycin and apramycin at the highest dose did not lead to a significant difference in the average speed at which the mice fell off compared to the control values (Fig. 4).

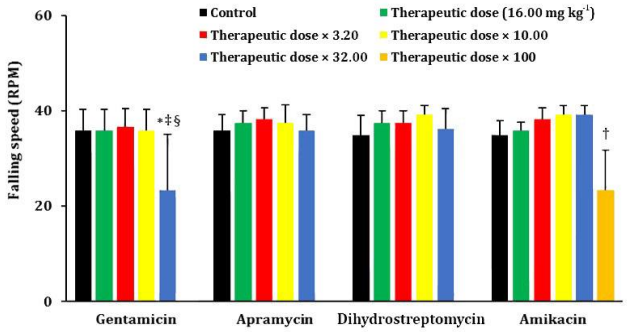

Fig. 4. The effect of incremental doses of selected aminoglycosides on the performance of mice in the rotarod test. The results of all columns were obtained from six animals, except for the columns of 32.00 times of the therapeutic dose of gentamicin and dihydrostreptomycin, where the results were obtained from three and four animals, respectively.
* and † indicate significant differences compared to the control values ($p < 0.05$ and $p < 0.001$, respectively). ‡ indicates a significant difference compared to the values of groups received apramycin and dihydrostreptomycin at 32.00 times of the therapeutic dose ($p < 0.05$). § indicates a significant difference compared to the values of groups received amikacin at 32.00 times of the therapeutic dose ($p < 0.01$).

Discussion

This study showed that the effects of four selected AGs on skeletal muscle paralysis and motor behaviors varied. Overall, gentamicin demonstrated significantly more potent effects on motor behaviors compared to the other AGs. These results are consistent with previous studies comparing the effects of different AGs on neuromuscular transmission and paralysis in skeletal muscles.[16-20] Reportedly, kanamycin produces a more potent neuromuscular blockade than gentamicin.[17]

In the present study, gentamicin demonstrated the highest potency to induce flaccid paralysis in skeletal muscles. After administrating at 32.00 times of the therapeutic dose, paralysis in limb muscles occurred in all six mice of this group and one of them suffered from respiratory arrest and eventually died. The administration of dihydrostreptomycin at this dose led to paralysis in limb muscles and motor behavioral changes in three out of six mice (50.00%). However, the administration of amikacin and apramycin at this dose had no effect on the limb muscles in mice. Singh *et al.* also showed that gentamicin was more potent than dihydro-streptomycin in causing muscle paralysis in an isolated organ study (phrenic nerve-hemidiaphragm preparation).[19] The present study also found that amikacin had the lowest potency in inducing muscular paralysis since after its administration at 100 times of the therapeutic dose; only flaccid paralysis in the limb muscles and behavioral changes were observed in mice, and respiratory arrest did not occur. In contrast, after administration of apramycin at this dose (100 times of the therapeutic dose), four out of six mice died due to skeletal muscle paralysis and respiratory failure.

In the present study, the order of occurrence of the muscular paralysis (in the limb and respiratory muscles) of AGs was also investigated. In all dead animals (five mice), the paralytic effects first appeared on the hind limb; so that, the animal became unable to move and then, the muscle strength of its forelimb was lost. Finally, the animal died due to the respiratory arrest and apnea. However, Liu *et al.* found that the median effective dose ratios for four AGs (Arbekacin, Astromicin, Isepamicin and Netilmicin) were greater for limb muscle paralysis than those for the diaphragm in rabbits.[24] The discrepancy may be due to the differences in both materials and methods. The present study was done in *in vivo* conditions and in intact animals, but the study mentioned above was done on isolated organs and the animals were anesthetized with halothane. In addition, the tested drugs in the present study and the cited study are different.

In the present study, by increasing the dose of all AGs, the locomotor activity of mice in the open field decreased in a dose-dependent manner. Following the administration of each drug at 10.00 times of the therapeutic dose and higher, locomotor activity decreased significantly compared to the control values. In contrast, the administration of drugs at 10.00 times of the therapeutic dose did not affect the limb muscles in the animal performance in other tests. Several factors can influence the animal behavior in the open field, such as the intensity of light and sound during the test, the size of the open field and the habituation of animals to the test conditions.[25] In our study, the light and sound remained constant during the test periods. In addition, the field size did not change during the experiments and the same field was used for all animals. An important consideration is the habituation of animals to the test area, which may have led to a decrease in the locomotor activity of animals in the open field and may interfere with the results of the test. In this regard, the results of the previous studies may explain why we should be cautious about the interpretation of the findings of present study.[26-28] Contet *et al.* showed that the number of squares crossed by animals significantly decreased from day 1 to day 2.[26] This finding indicates the habituation of animals to the open field. Naggy and Forrest found that the mean number of squares crossed by animals decreased over the four days of the test period.[27] Since the open field tests were done several times on the same animals in the present study, the habituation of the animals to the test condition could have some interfering effects on the obtained results. So, the protocol used for open field test in the present study could not be suitable enough for evaluating the side effects of AGs on locomotor activity and applying proper control group using a separate group of animals during all experimental period along with AG-treated groups is suggested.

The decrease in the time that the animal remains on the inclined plane may be due to flaccid paralysis of the limb muscles, as one of the side effects of AGs is flaccid paralysis of skeletal muscles. Bakre *et al.* showed that unlike the control group, mice given diazepam (which has a muscle relaxant effect) were unable to stay on a 45.00° inclined plane for 2 min.[29]

The horizontal bar test measures the strength of animal, particularly in the forelimbs, but the performance of the mouse can also be influenced by its motor coordination.[30] Incoordination is caused mainly by lesions of the cerebellum, vestibular system, or the general proprioceptive (upper motor neuron).[31] Since AGs have poor penetration of central nervous system (CNS),[2] and the drugs were not continuously or chronically administered in this study, the likelihood of ototoxicity (damage to the vestibular system) or other CNS abnormalities is very low.[6] However, more research is needed to clarify the role and involvement of CNS damage in the results of this test. Therefore, it seems that the effect of AGs on the performance of animal is most likely related to the strength of its forelimbs. An important consideration in this test is that the ability of mouse to grip

the bar is inversely proportional to its diameter, meaning the animal can grab the 2.00 mm bar more easily than the 4.00 mm bar.[23] In this study, the performance of animals on the 2.00 and 4.00 mm bars showed that higher doses of the drugs decreased the strength and scores obtained by the animal compared to the control values especially on the 4.00 mm one. Various studies support these findings.[32,33] Jacquez *et al.* concluded that ethanol-exposed mice held the bars for less time than the control group due to the deficit in their motor strength.[32] In the present study, the administration of gentamicin at 32 times of the therapeutic dose resulted in a significant decrease in the scores obtained by the animal on the 2.00 and 4.00 mm bars compared to the control values. In comparison, amikacin at 100 times of the therapeutic dose significantly decreased the scores only on the 4.00 mm bar compared to the control values, with no change in the performance of animal on the 2.00 mm bar. These findings indicate that the paralytic potency of these drugs differs.

Static rods, parallel bars and rotarod tests are designed to assess motor coordination and strength. Various studies have evaluated motor coordination using these tests.[23,33,34] In the present study, as mentioned above, these drugs rarely affected motor coordination in mice. Instead, they mostly caused flaccid paralysis in the limb muscles leading to motor dysfunction in these tests. Ahmad *et al.* showed that the administration of midazolam and diazepam in mice resulted in the less time spending of animals on the rotarod compared to the control group.[35]

In conclusion, the over-dose of AGs harms skeletal muscle and motor behaviors in mice, disrupting the behaviors. In addition, the potencies of the four selected AGs in altering motor behaviors differ significantly, with gentamicin inducing significantly more potent paralytic effects compared to the other three AGs. These drugs have little effect on skeletal muscles at therapeutic doses and even up to 10.00 times of the therapeutic dose. However, at higher doses, their paralyzing effects increase. Muscle paralysis caused by AGs is clinically important. This study highlights the importance of careful calculation of these drugs dosage in animals to prevent skeletal muscle paralysis and potential death due to the respiratory failure caused by paralysis of the intercostal and diaphragm muscles.

Further research is suggested to investigate the cause of death by high doses of AGs, the possible involvement of CNS damage and abnormalities in respiratory failure and alterations in motor behaviors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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