

The Ototoxic Potential of Digoxin in Laboratory Animals

Supplementary file

Estimation of hearing by Preyer's reflex test

This test was performed as a modification of the original procedure of **Jero et al., 2001**¹¹. The method depends on animal's reflexive reaction which takes the form of contraction of the post-auricular muscle, resulting in movement of the pinna towards the cranium in case of guinea pigs or the rapid movement of the whole body of the animal in case of rats. The observation of the Preyer's reflex has mainly been used for determining whether an animal is sound responsive or deaf. The room used should be quiet to avoid any sound distraction. Each animal was transferred in a separate cage.

According to the traditional method, a handclap is used; however, we used the sound of air horn, 30 cm away from the cage this was repeated three times for each animal with a 30 seconds interval between each one. Each animal was observed individually and its movement was recorded.

- Scoring

- For guinea pigs, animals with more than one response (contraction of the pinna) in the three trials were classified as having a positive Preyer's reflex, otherwise considered negative.
- For rats, instead of experimental traditional dependence on reaction reflex (response or deaf), a quantitative index for evaluation was conducted as following:

Body reflex	2
Body movement	1
No movement of any kind	0

Final score is the average three trials

Histopathological method

The cochlea specimens from guinea pigs in different groups were extracted and put in formalin 10% for 2 days then placed in trichloroacetic acid for one week till decalcification, with frequent checkup¹² (Bassily, 1989). Then, they were prepared for the histopathological examination as they were rinsed in formalin, dehydrated, cleared, impregnated and embedded in paraffin to facilitate ease of cutting for histological assessment of tissue damage using haematoxylin and eosin^{13, 14}.