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Effect of Gentamicin on kidney in developing chicks

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SUMMARY

Gentamicin is one of the most extensively used aminoglycoside antibiotic. The study was planned to study any teratogenic effect of Gentamicin as a result of single dose injection given therapeutically or accidentally. Sixty fertilized eggs of white leghorn species were incubated for 20 days and divided into treated (n=30) and control (n=30) groups. Eggs of treated and control groups were injected with 0.2mg of Gentamicin sulphate and 'Sterilized water for injection' respectively on the 4th day of incubation. On the 20th day, the chick embryos were extracted and then dissected to remove the kidneys. No effect of Gentamicin was found on either the mortality of chick embryos or the gross appearance of the newborn chicks and kidney as compared to the control group. The mean weight of both right and left kidneys was found less in treated group, though not statistically significant. On light microscopy, various changes were noticed in both control and treated groups which included glomerular enlargement and hypercellularity, glomerular congestion, mesangial proliferation and cystic dilatation and cloudy swelling of proximal convoluted tubules and infiltration by inflammatory cells and congestion of blood vessels. Statistical analysis revealed that all these changes except glomerular congestion were higher in treated group in comparison to control group, either in both the kidneys or in one-sided kidneys.

Key words: Gentamicin – Aminoglycoside – Teratogenic – Glomerular – Mesangial proliferation – Vacuolar degeneration – Congestion

INTRODUCTION

Gentamicin is a well-known aminoglycoside antibiotic that was obtained from *Micromonospora purpurea* in 1964, as reported by Finland (1969). Gyselynck et al. (1971) reported that it is not metabolized to a measurable extent and is eliminated from the body, principally by renal excretion.

Several side effects have been reported by Regec et al. (1986), such as nephrotoxicity, ototoxicity and neurotoxicity. It is estimated that up to 53% of patients treated with Gentamicin undergo some form of kidney damage.

The use of Gentamicin during pregnancy is increasing owing to the following:

a) Infections by Pseudomonas aeruginosa, most enteric bacteria and Staphylococcus aureus reported by Yoshioka et al. (1972).

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b) Maternal infections like pyelonephritis and pneumonia reported by Weinstein et al. (1976).
c) Chorioamnionitis reported by Gilstrap et al. (1988).

Because Gentamicin rapidly crosses from the placenta to the fetal circulation and amniotic fluid, knowledge about its teratogenic potential is essential [Yoshioka et al. (1972), Kauffman et al. (1975), Weinstein et al. (1976), Creatsas et al. (1980) and Gilstrap et al. (1988)].

Mallie et al. (1988) reported degenerative changes such as swollen cells, a cloudy cytoplasm, PAS-positive inclusions, brush border atrophy, and luminal enlargement in the proximal convoluted tubules of rat neonates born to pregnant Wistar rats after subcutaneous Gentamicin administration at doses of 75, 50, 25 or 7.5 mg /kg/day during the 2nd and 3rd weeks of gestation.

Czeizel et al. (2000) observed the teratogenicity of aminoglycoside antibiotics, including parenteral Gentamicin during pregnancy, and reported congenital malformations of 0.08% in 22,865 women who received Gentamicin during pregnancy.

Material and Methods

The present study was conducted in the Department of Anatomy, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, in 2008-2010 on sixty successfully fertilized eggs of white leghorn species. The eggs were brought in 7 series. In each series, the fertilized eggs were divided into two groups:

Group I: The control group (total no.=30)

Group II: The treated group (total no.=30)

Prior permission was obtained from the Institutional Animal Ethics Committee (IAEC).

Incubation of eggs

The eggs were incubated in a digital incubator manufactured by "Universal LABAID equipment", maintaining a temperature ranging between 38ºC to 39ºC and humidity around 85%. The eggs were kept with their broad ends up and were rotated twice daily along their longitudinal and vertical axes as recommended by Olsen and Byerly (1936). After 48 hours of incubation, the candling of eggs was performed. Then, the unfertilized eggs were removed from incubation. In each series, the unfertilized eggs ranged from 10-20% (Table 1).

The fertilized eggs were then divided into control and treated groups. Both the groups were incubated further, and, on the 4th day of incubation, the eggs were injected. Gentamicin was taken in the form of Gentamicin sulphate, marketed as Zygenta, 80 mg in 2 ml ampule, by cadilaheal thcare-limited. The fertilized eggs of study group were injected in a single dose of 0.2mg of Gentamicin sulphate by a tuberculin syringe on 4th day of incubation. The dose was calculated by recommended human dose 3-5mg/kgbw/day by Tripathi (2008), and taking weight of new born chick as 40 g (normal weight 38-42 g). The eggs of control group were injected with corresponding volume of 'sterilized water for injection' on the same day.

Injection Technique

The eggs were cleaned with the cotton soaked in rectified spirit and then, with the help of a needle, two holes were made in the eggshell. One was made at the broad end of the egg for the displacement of air, while a second hole was made at the pointed end of the egg for the purpose of injection. The injection was given through a sterilized no. 24 hypodermic needle fitted to a tuberculin syringe with 100 divisions of 1 ml. The holes were then sealed with molten paraffin wax. The fertilized eggs of both groups were then labeled using a black marker pen and were marked G for the Gentamicin (treated) group and C for the Control group.

The eggs of both groups were again kept for incubation after injection up to the 20th day of incubation. Sufficient measures were taken to maintain a continuous electricity supply. On the 20th day of incubation, the eggs were taken out and their shells were broken to remove the chicks. A variable degree of mortality was noted, both in the control and treated groups of all the seven series, as shown in Table 1.

Kidney Analysis

Any gross malformation was recorded. Each chick was dissected after anaesthetizing it with chloroform. Photographs of the dissected chicks were taken with a digital camera (Fig. 1). The kidneys were located and any gross malformation in them was noted. The kidneys of both the right and left side were carefully dissected out intact. However, some

pairs of kidneys were damaged during dissection (Table 2). The weight of the right and left kidneys of both the control (n=30) and treated (Gentamicin) group (n=30) was recorded separately with an analytical balance manufactured by MeOpta Praha, Czechoslovakia. Photographs of the kidneys of both the groups were taken with a digital camera (Figs. 2 and 3).

Both the right and left kidneys of each chick were kept in ten percent formalin solution in separate labeled containers for 48 hours. The kidney was taken out of the fixative and washed thoroughly in running tap water. The kidney was then dehydrated in an ascending series of alcohol (50, 70, 80, 90, 95%, absolute), cleared in benzene for 3 hours, and embedded in paraffin. The blocks from the two groups were labeled as follows:

sGnRK or sGnLK and sCnRK or sCnLK, where:

s = series, in roman numerals, to which the kidney belonged

G = Gentamicin (treated) group

C = Control group

n = number of newly hatched chicks in the respective series to which the kidney belonged

RK = Right kidney

LK = Left kidney

Sections of each tissue were cut using a Rotary Microtome at six micron thickness. Sections from each block were then stained with haematoxylin and eosin.

Slides from both the control and treated groups were then observed under light microscopy and changes due to Gentamicin administration in different parts of kidney were noted down and microphotographs were taken.

Then, statistical analysis was carried out by using chi-square test to find significant differences in the histological changes between control and treated groups.

Table 1. Number and distribution of eggs at various stages of the experiments.

	Series I	SeriesII	Series III	Series IV	Series V	Series VI	Series VII	Total
Eggs kept for incubation	10	10	10	20	20	35	15	120
Number of unfertilized eggs	2	2	1	3	4	7	1	20
Number of eggs for experimental use	8	8	9	17	16	28	14	100
Grouping of eggs								
Treated	4	4	5	10	8	20	0	51
Control	4	4	4	7	8	8	14	49
Number of dead embryos								
Treated	1	2	1	3	6	1	0	14
Control	4	2	1	2	2	3	1	15
Number of living embryos								
Treated	3	2	4	7	2	19	0	37
Control	0	2	3	5	6	5	13	34

Table 2. Number of damaged pairs of kidneys in each series during dissection.

	Series I	Series II	Series III	Series IV	Series V	Series VI	Series VII	Total
Number of Living Embryos								
Treated	3	2	4	7	2	19	0	37
Control	0	2	3	5	6	5	13	34
Number of pairs of kidneys damaged during dissection								
Treated	3	1	2	1	0	0	0	7
Control	0	2	1	1	0	0	0	4
Number of pairs of kidneys available for processing								
Treated	0	1	2	6	2	19	0	30
Control	0	0	2	4	6	5	13	30

Table 3. Mean and weight range (in mgs) of right and left kidneys of control and treated (Gentamicin) group.

(mg)	Range (mg)	Mean ±SD
Right kidney of control group	132-198	164.43±17.97
Left kidney of control group	130-197	163.37±18.61
Right kidney of treated (Gentamicin) group	127-192	155.2±18.44
Left kidney of treated (Gentamicin) group	130-190	155.87±17.3

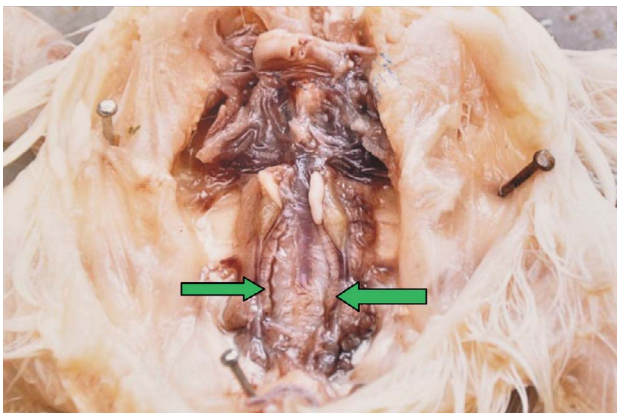

Figure 1. Dissected chick (Magnified view). Arrows point towards right and left kidney.

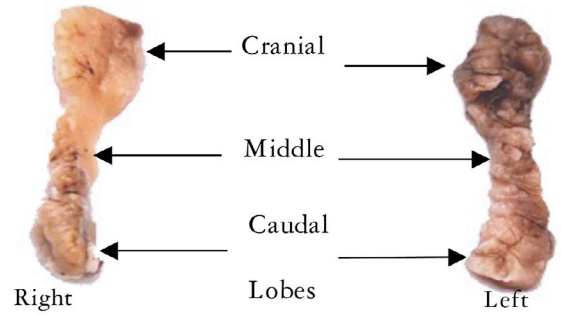

Figure 2. Right and left kidneys of the control group. The kidney is an elongated organ consisting of three successive lobes (cranial, middle & caudal) connected with each other by parenchymatous bridges.

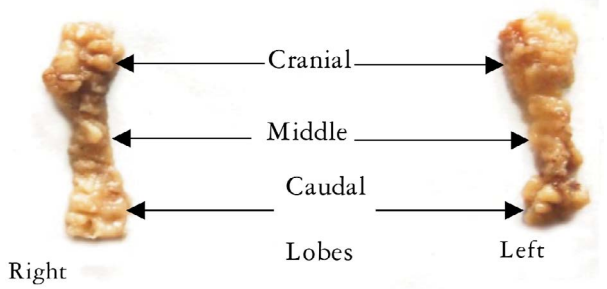

Figure 3. Right and left kidneys of the Gentamicin group. No gross malformation is observed.

Results

The present study was based on observations made in 60 newborn chicks from two groups comprising 30 chicks (control group) and 30 chicks (treated group).

In the control group, 15 chick embryos were dead out of total 49 eggs i.e. 30.6% whereas in the treated (Gentamicin) group, 14 chick embryos were dead out of total 51 eggs i.e. 27.4% (Table 1).

No gross malformations were observed in chicks or kidneys of either the control or treated (Gentamicin) groups (Figs. 1, 2, 3).

Effect of Gentamicin on the kidney weight of chick embryos

The mean weight of both the right and left kidneys of the control group was greater than that of the treated (Gentamicin) group, which means that there is a reduction in weight, although this difference was found statistically non-significant on applying unpaired t-test ($p>0.05$). In addition, no significant difference was found between the weights of the right and left kidneys of both control and treated (Gentamicin) groups on applying the paired t-test ($p>0.05$) (Table 3).

Effect of Gentamicin on the kidney histology of chick embryos

1. Glomerular changes (Tables 4, 5).
   a) Glomerulus enlargement and hypercellularity (Fig. 4)
   b) Glomerulus congestion (Fig. 5)
   c) Mesangial proliferation (Fig. 5)
2. Proximal convoluted tubules (Tables 6, 7)
   a) Cystic dilatation (Fig. 6)

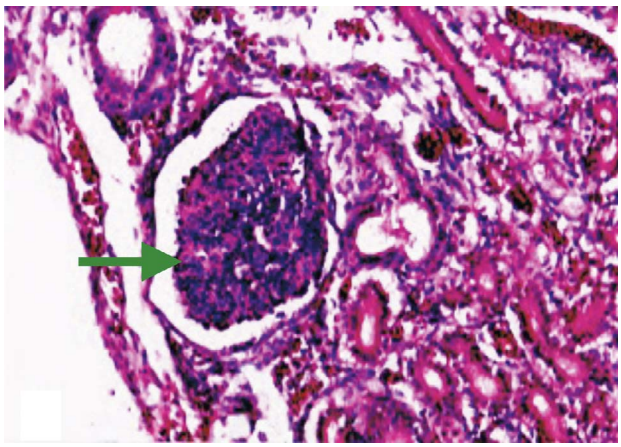

Figure 4. Microphotograph of kidney of treated (Gentamicin) group (H&E, x10) with arrow pointing towards glomerular hypercellularity obliterating the capillary lumina.

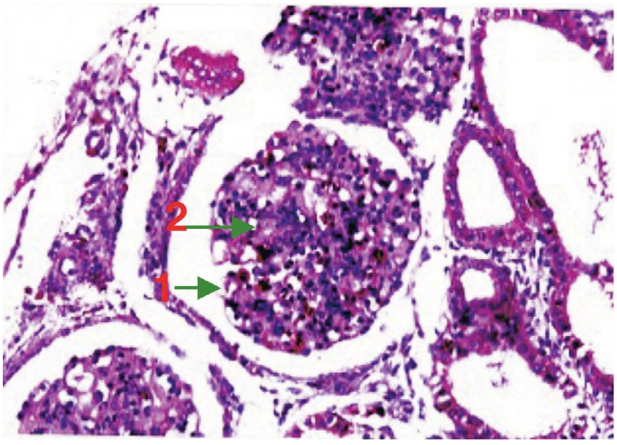

Figure 5. Kidney of treated (Gentamicin) group (H&E, x10) with arrow 1 pointing towards congested glomeruli and arrow 2 pointing towards mesangial proliferation.

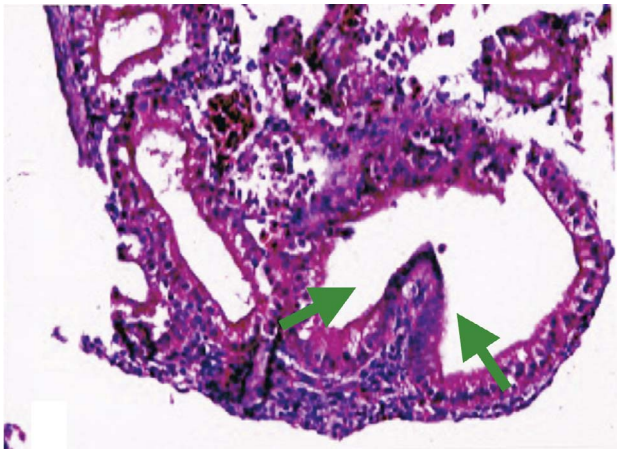

Figure 6. Kidney of treated (Gentamicin) group (H&E, x10) with arrows pointing to cystic dilatation of the proximal convoluted tubules.

Table 4. Glomerular changes in the control group as seen by light microscopy.

		Glomerular Changes		
		Glomerulus enlargement & hypercellularity	Glomerulus congestion	Mesangial proliferation
Number of chick embryos showing positive changes	Bilateral	1	1	1
	Unilateral in Right kidney	5	1	3
	Unilateral in Left kidney	1	0	0
	Total	7(23.33%)	2(6.67%)	4(13.33%)

Table 5. Glomerular changes in the treated (Gentamicin) group as seen by light microscopy.

		Glomerular Changes		
		Glomerulus enlargement & hypercellularity	Glomerulus congestion	Mesangial proliferation
Number of chick embryos showing positive changes	Bilateral	5	1	3
	Unilateral in Right kidney	6	5	6
	Unilateral in Left kidney	7	1	5
	Total	18(60%)	7(23.33%)	14(46.67%)

Table 6. Changes in proximal convoluted tubules in the control group as seen by light microscopy.

		Changes in Proximal Convoluted Tubules	
		Cystic dilatation	Cloudy swelling
Number of chick embryos showing positive changes	Bilateral	2	1
	Unilateral in Right kidney	3	1
	Unilateral in Left kidney	3	5
	Total	8(26.67%)	7(23.33%)

Table 7. Changes in proximal convoluted tubules in the treated (Gentamicin) group as seen by light microscopy.

		Changes in Proximal Convoluted Tubules		
		Cystic dilatation	Cloudy swelling	Vacuolar degeneration
Number of chick embryos showing positive changes	Bilateral	8	12	3
	Unilateral in Right kidney	7	6	2
	Unilateral in Left kidney	6	4	1
	Total	21(70%)	22(73.33%)	6(20%)

Table 8. Other changes in the control group as seen by light microscopy.

		Infiltration by inflammatory cells	Congestion in blood vessels
Number of	Bilateral	1	9
chick embryos	Unilateral in Right kidney	2	2
showing	Unilateral in Left kidney	1	0
positive changes	Total	4(13.33%)	11(36.67%)

Table 9. Other changes in the treated (Gentamicin) group as seen by light microscopy.

		Infiltration by inflammatory cells	Congestion in blood vessels
Number of	Bilateral	5	20
chick embryos	Unilateral in Right kidney	5	0
showing	Unilateral in Left kidney	2	0
positive changes	Total	12(40%)	20(66.67%)

Table 10. p-values related to the comparison of histological changes in the right and left kidneys of the control and treated (Gentamicin) groups.

		Right Kidney of Control vs Treated (Gentamicin) group	Left Kidney of Control vs Treated (Gentamicin) group
Glomerular changes	Glomerulus enlargement & hypercellularity	0.152	0.002*
	Glomerulus congestion	0.129	0.554
	Mesangial proliferation	0.117	0.010*
Changes in proximal	Cystic dilatation	0.006*	0.012*
convoluted tubules	Cloudy swelling	0.000*	0.007*
Infiltration by inflammatory cells		0.028*	0.071
Congestion in blood vessels		0.020*	0.004*

*= statistically significant ($p<0.05$)

b) Cloudy Swelling or Hydropic transformation (Fig. 7)

No change was observed in the distal convoluted tubules, the thin segments of the loop of Henle and collecting ducts in either the right or left kidneys of the treated (Gentamicin) group.

3. Infiltration by inflammatory cells (Tables 8, 9) (Fig. 9)
4. Congestion in blood vessels (Tables 8, 9) (Fig. 10)

Statistical Analyses (Chi-Square Test) (Table 10)

Changes in the proximal convoluted tubules and congestion in blood vessels were found to be significantly higher in both the right and left kidneys of the treated group in comparison to the control group. Histological changes such as glomerular enlargement and mesangial proliferation were significantly higher in the left kidney of the treated group than in the control group. There was a significant increase in infiltration by inflammatory cells in the right kidney of the treated group in comparison with the control group.

Discussion

The study was planned to know the teratogenic potential of this drug, if administered even in a single dose, to pregnant females unaware of their pregnancies especially in the first few months of pregnancy. The study was performed in chick embryos since the embryogenesis in chick is similar to human beings. Particular attention was paid to the effect of Gentamicin on mortality of chick embryos, gross appearance of newborn chick, gross appearance of kidney and its weight and histological structure of the kidney.

Teratogenic effect of Gentamicin on chick embryo mortality

In the present study, no effect of Gentamicin on the mortality of the chick embryos was found in comparison with the control group after a single therapeutic dose.

Lelievre-Pegorier et al. (1987) found similar mean numbers of guinea pig pups per litter in both saline- and Gentamicin-treated groups after daily injection of 4 mg/kg of Gentamicin to pregnant animals from days 48 to 54 of gestation.

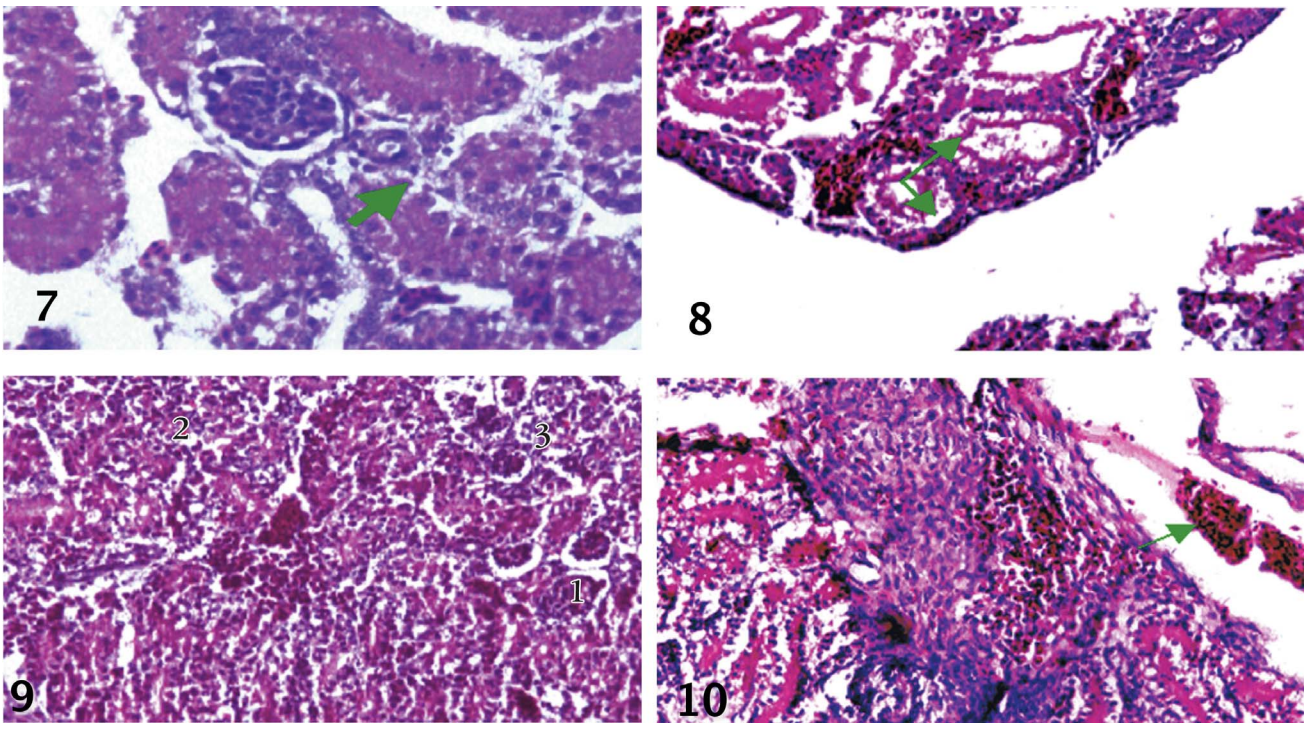

Figure 7. Kidney of the treated (Gentamicin) group (H&E, x40) with the arrow pointing to cloudy swelling of the proximal convoluted tubules.

Figure 8. Kidney of the treated (Gentamicin) group (H&E, x10) with arrows pointing to vacuolar degeneration of two proximal convoluted tubules.

Figure 9. Kidney of the treated (Gentamicin) group (H&E, x10) showing inflammatory infiltrate in 1. Glomeruli, 2. Proximal convoluted tubule, and 3. Interstitium, respectively.

Figure 10. Kidney of the treated (Gentamicin) group (H&E, x10) with the arrow pointing to congestion in blood vessels.

Mallie et al. (1988) also found similar mean numbers of neonates per litter in both control and Gentamicin groups after subcutaneous Gentamicin administration to pregnant Wistar rats at doses of 7.5, 25, 50 or 75 mg/kg/day during the 2nd and 3rd weeks of gestation.

Similarly, no effect of Gentamicin on the mortality of neonates was observed by Smaoui et al. (1993) after intraperitoneal administration of 75 mg/kg/day of Gentamicin to pregnant Wistar rats on days 7 to 11 and 14 to 18 of gestation.

Thus, all the findings in the available literature regarding the teratogenic effect of Gentamicin in multiple doses on the mortality of neonates matched our findings with a single therapeutic dose.

Teratogenic effect of Gentamicin on the weight of the kidneys of newborn chicks

In the present study, it was found that although the mean weight of both the right and left kidneys of Gentamicin-treated chicks was less than that of the control group, this difference was not statistically significant.

Lelievre-Pegorier et al. (1987) reported that the mean weight of the kidneys of pups born to Gentamicin-treated guinea pigs was less than that of pups born to saline-treated animals, athough this difference was not statistically significant. Hence, their findings matched our own. However, those authors performed this experiment with multiple doses of Gentamicin to pregnant guinea pigs from days 48 to 54 of gestation.

On the other hand, Smaoui et al. (1993) found significantly decreased kidney weights in prenatally Gentamicin-exposed neonates born to pregnant Wistar rats that were administered 75 mg/kg of Gentamicin on days 7 to 11 and 14 to 18 of gestation.

Teratogenic effect of Gentamicin on the histological structure of kidneys of newborn chicks

In the present study, with a single therapeutic dose of Gentamicin to fertilized eggs, a teratogenic effect was observed on the histological structure of kidney in the chick using light microscopy. Out of 30 chicks of the treated group, 18 showed glomerular enlargement and hypercellularity (60%); 7 showed glomerular congestion (23.33%); 14 showed

mesangial proliferation (46.67%); 21 showed cystic dilatation of the proximal convoluted tubules (70%); 22 showed a cloudy swelling of the proximal convoluted tubules (73.33%); 12 showed infiltration by inflammatory cells (40%), and 20 showed congestion of blood vessels (66.67%).

Lelievre-Pegorier et al. (1987) reported diminished glomerular volume of juxtamedullary nephrons of about 40% and of superficial nephrons of about 30% and about 20% decrease in the proximal tubular length of thejuxtamedullary nephrons in young pups born to guinea pigs that were administered 4 mg/kg/day of Gentamicin from days 48 to 54 of gestation.

Smaoui et al. (1993) observed no glomerular necrosis on light microscopy in rat neonates born to Wistar rats after 75 mg/kg/day Gentamicin on days 7 to 11 and 14 to 18 of gestation. On electron microscopy, they noticed altered epithelial cells, myeloid bodies in lysosomes, phospholipid deposits in mitochondria and swollen golgi apparatus' cisternae in glomeruli. Lesions found in the proximal convoluted tubules were same as reported by Mallie et al. (1988).

In the present study, various histological changes in the structure of kidney were noted even with a single dose of Gentamicin. The effect with multiple dose administration during pregnancy in human beings will be obviously much more.

In conclusion, from analysis of the results, it can be definitely concluded that Gentamicin has a teratogenic effect on the kidney even if administered in a single dose during the development of an embryo, and further studies need to be conducted to check its teratogenic potential in human embryos.

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