

Rifampicin Induced Embryotoxicity in *Mus musculus*

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Abstract. During this study, an anti-tuberculosis drug, rifampicin was tested for its embryotoxicity in mice. For this purpose, different concentrations of the drug 0.00, 3.00, 6.00 and 12.00 µg/g B.W. were given orally to pregnant mothers on days 6-12 of the gestation repeatedly and fetuses were recovered on day 18 of gestation. Morphological studies of the fetuses showed abnormalities such as hemorrhage, hydrocephaly, microphthalmia, limb deformities (forelimb micromelia and hyperextension, drooping wrist, low set arm, hindlimb dysplasia), scoliosis, sacral hygroma, open eyelids and curly tail. Dose dependent intrauterine growth retardation was also observed. Morphometric studies indicated fetal body weight and CR length, head circumference, eye circumference, forelimb and hindlimb size and tail length had a significant ($P < 0.001$) dose dependent reduction as compared to control fetuses. Histological defects included neural tube defects, pericardial hemorrhage and hepatic necrosis. By this study it is concluded that rifampicin is potential toxic to the developing mice.

Key words: Rifampicin, embryotoxicity, hydrocephaly microphthalmia.

INTRODUCTION

Rifampicin or rifampin I is a semisynthetic compound derived from *Amycolatopsis rifamycinica* (Sensi *et al.*, 1959). It is a bactericidal antibiotic drug of the rifamycin group (Masters *et al.*, 2005). It is one of the oldest and most effective chemotherapeutic agents available for the treatment of tuberculosis (Agrawal and Panchagnula, 2005). It is quickly absorbed from the gastrointestinal tract, with peak serum concentrations (6 to 7 g/mL) occurring 1.5 to 2 h after ingestion. Therapeutic concentrations are achieved in the saliva, reaching 20% of serum concentrations. These concentrations cross the placenta, with fetal serum concentrations at birth found to be approximately 33% of the maternal serum concentration (Venkatesan, 1989). It penetrates into aqueous humor and is distributed into the breast milk (Outman, 1992).

Its action is based on its ability to bind to and inactivate the bacterial DNA-dependent RNA polymerase, a mode not shared by any other anti-infective agent in use today. This action usually produces a bactericidal effect at very low concentrations against most Gram-positive and many Gram-negative organisms. It also appears to

be one of few antimicrobial agents that are capable of killing bacteria within the protected environment of phagocytic cells (Sande, 1983).

Rifampicin is well distributed, although to a different degrees, in the various tissues of the human body. Probably in the hepatocyte, rifampicin undergoes a process of desacetylation (Acocella, 1978). Rifampicin is also used in combination with other agents in the treatment of certain atypical (nontuberculous) mycobacterial infections, such as those caused by *Mycobacterium avium* Complex (MAC) (Peloquin, 1993). The most common adverse reaction to rifampin is gastrointestinal upset. Other reactions include skin eruptions, hepatitis, and rarely thrombocytopenia and cholestatic jaundice (Baciewicz *et al.*, 1987). Rifampicin-induced hepatic injury and increased lipid peroxidation in growing rats (Sodhi *et al.*, 1997).

During a comparative study on histopathological hepatotoxic effects of antitubercular drugs (rifampicin and isoniazid) in *Mus musculus*, different groups of mice were exposed to different doses of rifampicin and isoniazid (15 mg/kg and 30 mg/kg of body weight). Major histopathological changes in liver involved disintegration and disorganization of epithelial cells, cirrhosis, nuclear pyknosis and necrosis. These pathological effects were drug and dose dependent. These effects were more severe in case of rifampicin (Bashir *et al.*, 2004).

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Rifampicin is excreted into human milk. In one report, the concentrations were 13 mg/ml with about 0.05% of the daily dose appearing in the milk (Vorherr, 1974). The evolution of rifampicin as a broad-spectrum antibiotic has been exciting and certainly unconventional by current standards. It is a drug that to a large extent has sold itself but desperately needs a careful evaluation by clinical investigators and researchers for definition of its proper role.

The present study aims at determining toxicity of rifampicin on developing *Mus musculus*.

MATERIALS AND METHODS

A colony of the Swiss Webster variety of *Mus musculus* was established to fulfill the demands of experiment. The animals were kept under conditions, which were according to the standard protocols of the approved animal treatment condition of medical ethics committee of Punjab University, Lahore, Pakistan. These animals were kept in an air-conditioned animal room to maintain temperature. Steel racks and cages (14" x 10" x 7") were used for this purpose. Two females were caged with one male in five different cages. Breeding stock was kept in controlled environmental conditions in the form of 12 hour light/dark cycles, temperature of $27\pm 2^{\circ}\text{C}$ and relative humidity of 40-55% (Janet *et al.*, 2011). Animals were feed with the National Chick Feed#12 made in Pakistan and have a free access to water. Mice were divided into 5 groups. Each group contains 5 females. The females in the estrus phase were grouped with the experienced males overnight. Presence of a vaginal plug indicated day 0 of gestation.

Rifampicin was available in capsule form with trade name "Lederrif" (Wyeth Pakistan Limited). Different dose concentrations 0.00, 3.00, 6.00 and 12.00 $\mu\text{g/g}$ B.W were prepared by dissolving Rifampicin in distilled water in such a way that 0.1ml of solution contains the desired concentration. These doses were given to pregnant mice with the help of 1 ml plastic syringe. At the end of syringe, a stainless steel tube with blunt end covered by a rubber tube was attached which was specially prepared for oral feeding. Mice readily accepted the rubber tube and these doses (0.1ml of

each dose group) were then pumped into the gullet, which was readily engulfed by the mice. By this technique, there was minimum escape of dose. These doses were given at days 6-12 of gestation daily, once a day.

On 18th day of gestation, the pregnant mothers were weighed and then anaesthetized with Ether. After giving a cesarean section, the two horns of the uterus were taken out of the body and weighed. The implantation sites were counted and fetuses were dissected out of the uterus and then fixed in Bouin's fixative for 48 hours. After 48 hours fetuses were preserved in 70% alcohol.

The preserved fetuses were subjected to morphological, morphometric and histological studies. Morphological defects of axis, craniofacial region, limbs and trunk of fetuses were noted and selected fetuses from all dose groups were macrophotographed by using microscope Labomed, CZM6, of Japan and camera, Panasonic TZ15.

Morphometric studies involved the fetal body weight, crown rump length, head and eye circumferences, length of forelimb and hindlimb. All the measurements were made by analytical balance and digital vernier caliper. While the circumferences of head and eye were calculated with the help of computer based programmed the "Ellipse Circumference Calculator", downloaded from CSG network (CSGN, 2006).

The morphometric data were analyzed by a computer based programme SPSS. ANOVA was used for analysis of data obtained in this study the values were further subjected to Duncan's Multiple Range Test (DMRT) for multiple comparisons for each dose group.

For histological observations selected fetuses from all groups were preserved in Bouin's fixative for 48hrs and then transferred in 70% alcohol at room temperature. They were processed for paraffin sections. The processed tissues were embedded in wax and then 5μ thick transverse sections were cut on Microtome. After staining with hematoxylin and eosin sections were studied under light microscope (Spencer and Bancroft, 2008).

RESULTS

During the present study, a significant ($p < 0.001$) increase in percentage of malformed and

resorbed fetuses was observed in all dose groups as compared to control (Table I).

Table I.- Toxic effects on development of 18-day-old mouse fetuses by Rifampicin, administered orally to pregnant mice on days 6-12 of gestation.

Dose groups (µg/g B.W)	No. of implantations recovered (N)	Malformed fetuses (%)	Resorbed fetuses (%)
Control	30	0.00	0.00
0.00	25	0.00	0.00
3.00	28***	64.29***	0.00
6.00	26***	80.76***	0.00
12.00	20***	59.38***	40.63***

***P<0.001 The asterisks indicate significant differences compared to control mice.

Morphological analysis showed that the fetuses recovered from control and vehicle control group had normal size and well developed body organs (Fig. 1A). The fetuses from dose group 3.00 µg/g B.W showed abnormalities such as distorted axis (10.71%), scoliosis (3.17%), microphthalmia, dysplasia and micromelia (3.57%), hyper extension, flexed wrist and lower set arm (7.41%), curly tail (10.74%). The dose group 6.00 µg/g B.W has congenital anomalies like hydrocephaly and microcephaly (5.77%), hyperextension (7.69%), flexed wrist (3.85%), dysplasia (15.38%), hygroma (3.85%), runt (underdeveloped small fetus) and dead fetuses (7.69%). Developmental abnormalities such as scoliosis (8.15%), open eyelids (10.23%), Hemorrhagic spots (9.09%) and runt (18.18%) were observed in the dose group 12.00 µg/g B.W (Table II).

The morphometric observations during this study showed a significant ($p < 0.001$) reduction in mean body weight of the fetuses, head and eye circumferences, fore and hind limbs lengths, tail length as compared to control mice in all dose groups 3.00, 6.00, 12.00 µg/g B.W (Table III).

Histological studies through cardiac, cranial and visceral regions were carried out to determine the anatomical defects. The selected sections of control from cardiac region showed well developed spinal cord, heart, lungs and brain. In the treated groups brain anomalies included poorly formed

spinal cord leaving upside a cavity and meningocele, Heart anomalies included hemorrhage of pericardium and liver anomalies included necrosis of liver.

DISCUSSION

Rifampicin is one of the oldest and most effective chemotherapeutic agents available for the treatment of tuberculosis (Agrawal and Panchagnula, 2005). Rifampicin is an effective liver enzyme-inducer, promoting the upregulation of hepatic cytochrome P450 enzymes, increasing the rate of metabolism of many other drugs that are cleared by the liver through these enzymes. As a consequence, rifampicin can cause a range of adverse reactions when taken concurrently with other drugs (Stockley and Ivan, 1994).

The data available on this drug from the stand point of view of teratology are controversial and not conclusive. Many reports are available about safety of this drug. Bothamley (2001) described that first line treatment of tuberculosis with isoniazid, rifampicin and ethambutol is widely recognised to be safe in pregnancy. Reports show rifampicin crosses the placenta to the fetus (Kenny and Strates, 1981). Other reports explaining its teratogenicity are also available like that of Wise (1987) stated that rifampicin teratogenicity has been suggested but not confirmed.

In the present study morphological defects like hydrocephaly and limb defects were observed in the mouse fetuses. These observations are supported by Bothamley (2001). He reported a study in which malformation rate of 4.4% in 204 pregnancies was noted in which mothers took rifampicin during first trimester. These malformations included hydrocephaly, anencephaly and limb defects.

The current morphometric analysis showed a decrease in fetal body weight, crown rump length and other structural parameters with increasing the dose concentration. These observations are in accordance with the findings of Anufrieva *et al.* (1980), in which teratogenic effects of rifampicin were studied on mice exposed to the antibiotic orally during the whole period of gestation. It was found that at higher concentrations there was a

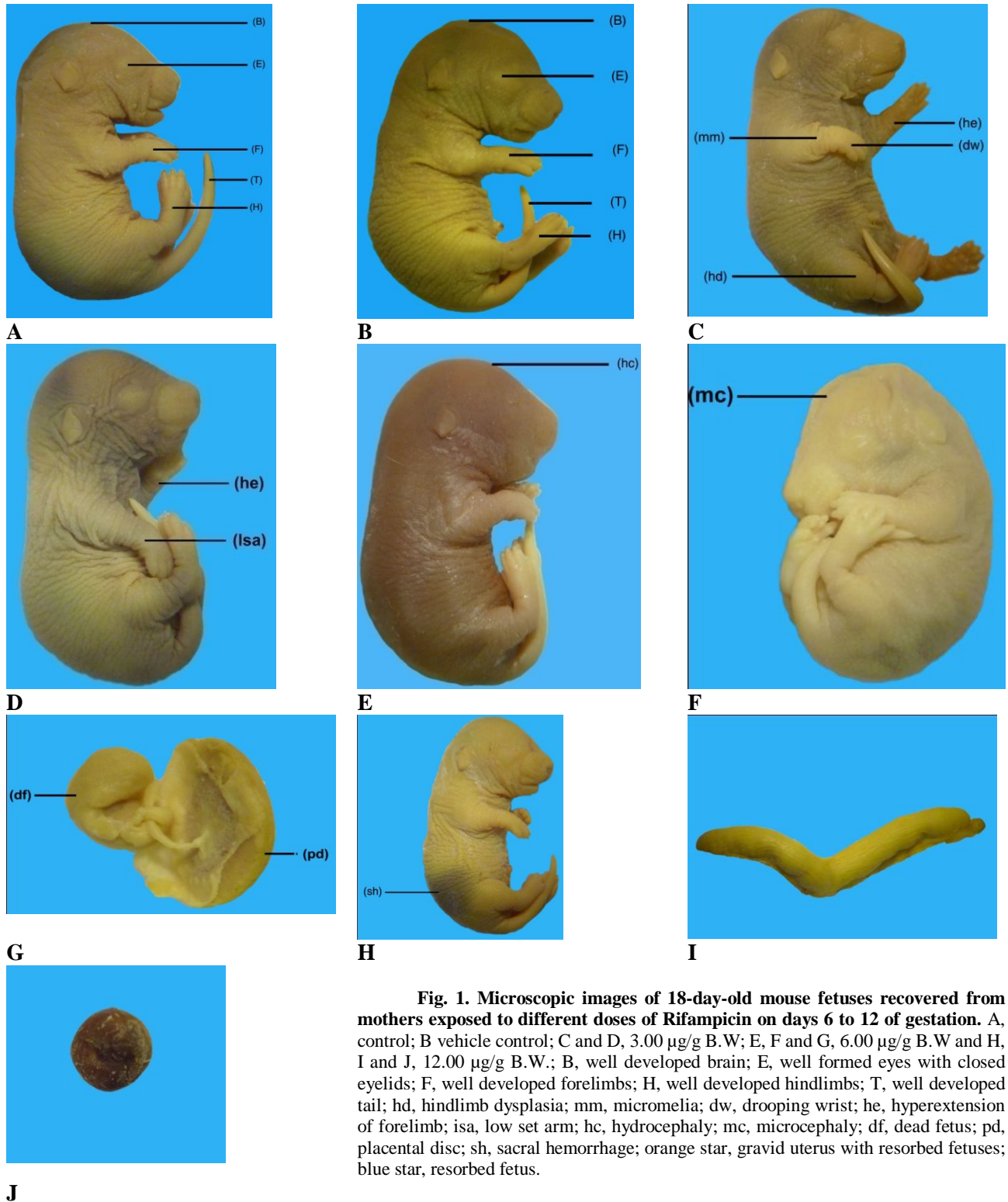


Table II.- Developmental anomalies induced by Rifampicin in 18-day-old fetuses recovered from pregnant mice, administered orally with different concentrations on days 6-12 of gestation.

Parameters	Dose groups ($\mu\text{g}/\text{gB.W}$)				
	Control	0.00	3.00	6.00	12.00
Axis (%)	0.00	0.00	Distorted axis (10.71) Scoliosis (3.17)	0.00	Scoliosis (8.15)
Brain (%)	0.00	0.00	0.00	Hydrocephaly (5.77) Microcephaly (5.77)	
Eye (%)	0.00	0.00	Microphthalmia (3.57)	0.00	Open eye (10.23)
Forelimb (%)	0.00	0.00	Micromelia (3.57) Hyperextension (7.14) Flexed wrist (7.14) Low set arm (7.14)	Hyperextension (7.69) Flexed wrist (3.85)	0.00
Hindlimb (%)	0.00	0.00	Dysplasia (3.57)	Dysplasia (15.38)	0.00
Tail (%)					
Hemorrhagic spots (%)	0.00	0.00	0.00	0.00	Abdomen (9.09) Foot (9.09)
Intrauterine growth retardation (%)	0.00	0.00	0.00	Runt (7.69) Dead fetus (7.69)	Runt 18.18
Hygroma (%)	0.00	0.00	0.00	Abdomen (3.85)	0.00



Fig. 2. Histological structure of cross sections through cranial region of 18-day-old mouse fetuses recovered from mothers exposed to different doses of Rifampicin on days 6 to 12 of gestation. A, fetus from control group 'C' showing well formed spinal cord (Sc), pharynx (P) and tongue (T); B, fetus from treated group $3\mu\text{g}/\text{g B.W.}$; C, Resorbed fetus from treated group $6\mu\text{g}/\text{g B.W.}$ Note poorly formed spinal cord leaving upside a cavity (green arrow); meningocele (yellow arrow).

decrease in the length of the limbs of fetus, higher incidence of hemoperitoneum and disorders in liver and renal functions.

Hemorrhages at different body parts of the

fetuses were also seen during this study which follows the findings as reported by Eggermont *et al.* (1976) that rifampin has been implicated as one of the agents responsible for hemorrhagic disease of

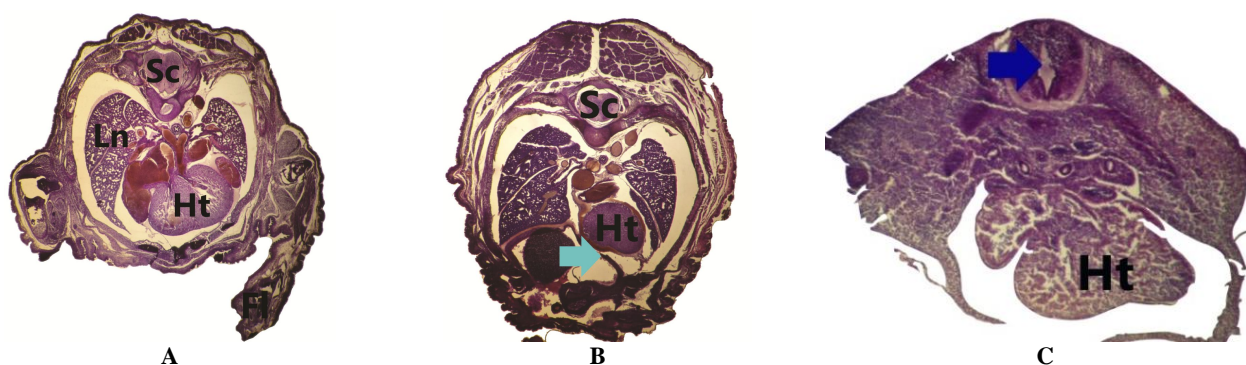


Fig. 3. Histological structure of cross sections through cardiac region of 18-days-old mouse fetuses recovered from mothers exposed to different doses of Rifampicin on days 6 to 12 of gestation. A, fetus from control group showing well formed spinal cord (Sc), lungs (Ln), heart (Ht) and forelimb (Fl); B, fetus from treated group 6µg/g B.W.; C, resorbed fetus from treated group 12µg/g B.W. Note pericardial hemorrhage (light-blue arrow); meningocele (dark-blue arrow).

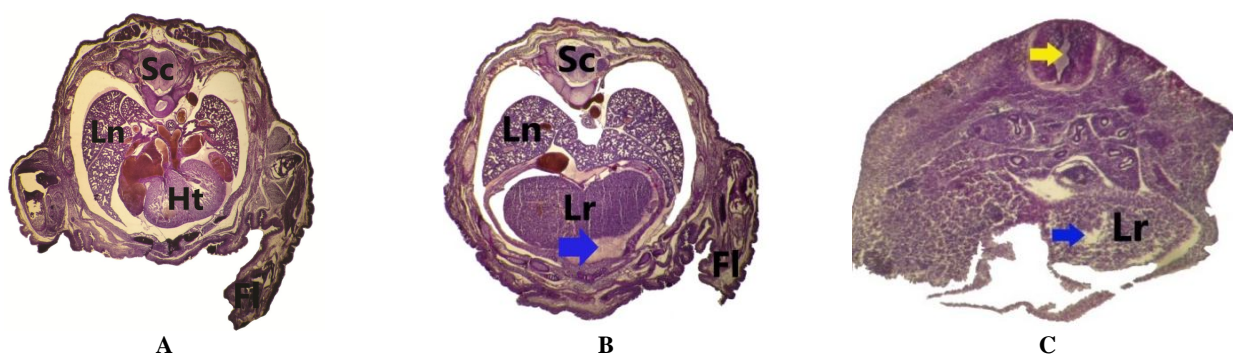


Fig. 4. Histological structure of cross sections through visceral region of 18-days-old mouse fetuses recovered from mothers exposed to different doses of Rifampicin on days 6 to 12 of gestation. A, fetus from control group showing well formed spinal cord (SC), Lungs (Ln), liver (Lr) and forelimb (Fl); B, fetus from treated group 6µg/g B.W; C, resorbed fetus from treated group 12µg/g B.W. Note necrosis of liver (blue arrow); meningocele (yellow arrow).

Table III.- Effects of Rifampicin on development of 18-day-old fetuses recovered from pregnant mice, administered orally with different concentrations on days 6-12 of gestation.

	Dose groups (µg/gB.W)				
	Control	0.00	3.00	6.00	12.00
Body weight (mg±S.E.)	1526.70±191.80	1463.44±82.00	955.03±217.42 ***	758.27±131.39 ***	598±103.85 ***
CR length (mm±S.E.)	23.79±1.43	23.52±0.36	19.72±1.93 ***	17.41±1.83 ***	16.28±1.56 ***
Head circumference (mm ² ±S.E.)	26.9±1.82	26.65±0.41	24.08±1.15 ***	22.71±1.10 ***	21.81±1.48 ***
Eye circumference (mm ² ±S.E.)	7.42±0.40	7.34±0.46	6.33±0.47 ***	5.70±0.40 ***	5.08±0.44 ***
Forelimb length (mm±S.E.)	8.08±0.68	7.97±0.70	6.78±0.52 ***	5.94±0.42 ***	5.27±0.18 ***
Hindlimb length (mm±S.E.)	8.31±0.32	8.30±0.39	7.07±0.49 ***	6.58±0.25 ***	6.22±0.60 ***
Tail length (mm±S.E.)	11.04±0.57	10.96±0.34	9.36±0.55 ***	8.48±0.70 ***	8.00±0.82 ***

Asterisks show significant differences compared to control mice; *** = P < 0.001

the newborn. Eye abnormalities observed during this study ranges from open eyelids to microphthalmia and anophthalmia. These observations may be supported by a human case reported by Roy (1990). A male child was born with defects of eyes to a mother who was under treatment with isoniazid, ethambutol and rifampicin, at 38th week of gestation by caesarian section.

The recent histological study showed hepatotoxicity in mouse fetuses. These toxicities included lipid accumulation and necrosis in liver. These findings are parallel to the previous studies made by Yew and Leung (2007). They described that hepatotoxicity is of increasing concern in the treatment of tuberculosis.

Neural tube defects were also found in the present histological study, which is in agreement with the findings of Tuchmann-Duplessis and Mercier-Parot (1969). They reported that reproduction studies with rifampicin in mice and rats at doses greater than 150 mg/kg produced spina bifida in both species and cleft palates in the mouse fetuses.

As the present study has clearly shown that oral administration 3.00, 6.00 and 12.00µg/g B.W. of rifampicin, used during this study, has potentially embryotoxic properties in mouse embryos particularly if such exposure occurs at beginning of organogenesis. Therefore, it is strongly recommended that rifampicin should be used with utmost care in human beings especially its use should be checked during pregnancy and also during breast-feeding.

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