



Cytotec Induced Embryotoxicity in Developing *Mus musculus*

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ABSTRACT : The study was carried out to assess the developmental abnormalities induced by Cytotec in mice during intrauterine life. Pregnant mice were exposed to a single dose of 0, 0.02, 0.04, 0.06, 0.08 and 0.1 $\mu\text{g/g}$ BW on day 8 of gestation. Fetuses were recovered on day 18 of gestation. These fetuses were subjected to morphological and morphometric studies. Morphological studies showed abnormalities like anophthalmia, microphthalmia, micromelia and syndactyly. In addition to these, resorptions were also encountered in the higher dose groups. Morphometric analysis showed an overall reduction in body weight, crown rump length, brain and eye circumference, pinna and snout size, length of fore and hind limb and tail size with a significant difference ($p < 0.001$) compared to controls. The outcomes of histological studies revealed some brain defects like hydrocephaly, enlarged third ventricle and undifferentiated ectoneural cells and abnormalities of the heart included right auricle thrombosis and degeneration of trabecular zone. (**Key Words :** Cytotec, Embryotoxicity, Developmental Anomalies, Mice)

INTRODUCTION

Cytotec[®] oral tablets contain Misoprostol which is similar to group of substance known as prostaglandins (Product Information, 2005, 2009). Robert et al. (1967) discovered that naturally occurring prostaglandin E series inhibit the gastric acid secretions. Cytotec[®] is stable at room temperature (Ginath and Zakut, 2001). It has a long shelf life, easy to administer, significantly cheaper and does not require refrigeration (Collins, 1990; Rozenberg et al., 2001).

Upon absorption, misoprostol is converted to its active metabolite, misoprostol acid. Misoprostol acid is then metabolized by the liver into inactive metabolites which are excreted in the urine (Foote et al., 1995). Plasma concentration of misoprostol peaks in approximately 30 minutes and decline rapidly thereafter (Zieman et al., 1997; Tang et al., 2002; Khan et al., 2004; Meckstroth et al., 2006; Andolina et al., 2003) while it has been successfully used by obstetricians and gynecologists for a number of years for the induction of labor for medical termination of pregnancy, cervical ripening and prevention of postpartum hemorrhage (Goldberg et al., 2001). It is administered both orally and vaginally (Adair et al., 1998).

Vaginal as well as oral administration was found effective in medical abortion (El-Rafaey et al., 1995; Tang

et al., 2002; Ho et al., 2006). Some other routes have been found to be effective like buccal (Carlan et al., 2002; Middleton et al., 2005; Castleman et al., 2006) and rectal route (Khan and El-Rafaey, 2003). Misoprostol has been found effective for second-trimester abortions using different doses and administration routes (Carbonell et al., 2004) and induction of labor (Carlan et al., 2002; Shetty et al., 2002; Shetty et al., 2003; Nopdonrattakoon, 2003; Paungmora et al., 2004).

Use of combined oral contraceptives doubles the risk of breast cancer in young women, especially when users are compared with carefully confirmed never-users of hormones (Hemminki et al., 2002). A case report has identifies a woman who died of multi-organ failure following an over dose of misoprostol (Henriques et al., 2007). Misoprostol can also cause uterine rupture (Al-Hussein, 2001) or dehiscence of a prior cesarean scar (Berghahn et al., 2001) during second trimester of pregnancy. The most common side effects on the mother associated with misoprostol include chill, fever, nausea and vomiting (Derman et al., 2006).

Keeping in view the above mentioned wide usage of Cytotec, present study is designed to observe the teratogenic effects of this drug in *Mus musculus*.

MATERIAL AND METHODS

Sexually mature Swiss Webster strain of *Mus musculus*

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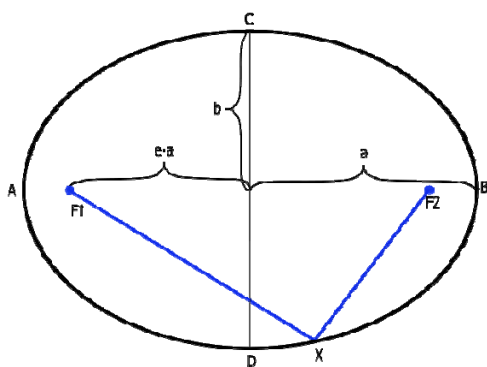
was used during this experiment. Females in proestrus stage were caged overnight with males of the same stock. Presence of vaginal plug was taken as day 0 of gestation. A total of 60 pregnant females divided into a group of 10 for control and 10 for each of the five dose groups.

An antiulcer drug Cytotec (Misoprostol) Searle Pharmaceuticals Inc. was used. The doses were prepared by diluting a tablet of 200 μ g in distilled water in such a way that each 0.1 ml of the solution contained desired dose. Five doses used during this experiment were 0.02, 0.04, 0.06, 0.08 and 0.1 μ g/g BW. The doses were applied orally on day 8 of gestation. A vehicle treated control was also maintained alongside which was given 0.1 ml of distilled water.

On day 18 of gestation, the treated dams were weighed and anaesthetized with anesthetic Ether. The dams were given midline incision in the abdomen and the uteri were exposed. The number of implantations and resorptions were recorded. The fetuses were removed from the uteri. These fetuses were dried on tissue paper and weighed then were fixed in Bouin's fluid for 48 h.

After 48 h, fetuses were shifted to 70% ethanol. The morphological studies were done to record anomalies of craniofacial region, trunk, limbs, tail and axis. The fetuses were macrophotographed.

The morphometric studies involved recording of fetal weight, crown rump length, head circumference, eye circumference, snout length, pinna length, length of fore and hind limbs and tail length. The head circumference was calculated by the measurement of fetal head-occipital-frontal (AB) and width-bi-parietal distances (CD) were obtained as shown below:



The head circumference values ($p = \text{mm}^2$) were calculated for each fetus with the help of a computer based program the "Ellipse Circumference Calculator" downloaded from CSG Network (CSGN, 2006). This program involves the following standard mathematical formula for the calculation of circumference of ellipsoid.

$$P = 2\pi \frac{\sqrt{a^2 + b^2}}{2}$$

Where, $a = (A-B)/2$ and $b = (C-D)/2$

Similar measurements and calculations were made for each fetal eye separately. Measurements of other organs were done by using the digital Vernier Calipers. Statistical analysis was performed on the morphometric data through analysis of variance (ANOVA) on SPSS version 12.0 software. Moreover, percentages of malformed and resorbed fetuses were also subjected to multiple comparisons through ANOVA on SPSS.

For histological preparation selected fetuses from all groups were processed for paraffin sections which were stained with hematoxylin and eosin. Abnormalities of brain, eyes, heart and lungs were considered.

RESULTS

The fetuses recovered from vehicle control group were well formed. They had well developed brain, eyes, snout, pinnae, limbs and tail (Figure 1A). The fetuses studied from the dose group 0.02 μ g/g BW showed some morphological anomalies including underdeveloped eyes (Figure 1B), micromelia and degenerated claws (Table 1). Whereas in 0.04 μ g/g BW dose group the deformities observed included forelimb micromelia (Figure 1D) and hemorrhagic spots (Table 1). The 0.06 μ g/g BW dose group exhibited sacral spina bifida, anophthalmia (Figure 1E), imbalanced axis, underdeveloped eyes and hemorrhagic spots (Table 1). The dose group 0.08 μ g/g BW had imbalanced axis, microcephaly, anophthalmia and forelimb micromelia (Figure 1F) (Table 1). The abnormalities observed in 0.1 μ g/g BW dose group were hydrocephaly (Figure 1G), underdeveloped eyes and meromelia (Table 1).

A significant decrease ($p < 0.001$) was observed in the weight, crown rump length, eye circumference, brain circumference, forelimb and hindlimb lengths as compared to control (Figure 2, 3, 4 and 5).

The histological studies from 0.02 μ g/g BW dose group showed malformations including the thickening of meninges around the fourth ventricle (Figure 6E) degeneration of jaw muscles and atrophy of the nasal septum and the inferior concha. Moreover in the cardiac region, degeneration of the trabecular zone was observed at the histological level (Figure 7C). Tissue necrosis of the liver was also observed in the serial section of the fetuses in this dose group.

In the selected sections of the fetus from 0.04 μ g/g BW dose group multiple histological defects were observed. The cranial studies revealed enlargement of third ventricle as

Table 1. Morphological abnormalities in 18 days old fetuses recovered from pregnant mice treated with different doses of Cytotec on day 8 of gestation

Parameters Dose groups	Resorptions (% age)	Malformations (% age)	Axis (% age)	Brain (% age)	Eyes (% age)	Limbs (% age)	Claws (% age)	Hemorrhagic spots (% age)
Vehicle control (n = 100)	-	-	-	-	-	-	-	-
0.02 µg/g BW (n = 90)	-	3.33	-	-	Underdeveloped (2.2)	Micromelia (2.2)	Degenerated Claws (2.94)	-
0.04 µg/g BW (n = 80)	-	19.84***	-	-	-	Forelimb Micromelia (2.5)	Degenerated Claws (4.3)	Hemorrhagic Spots (26.1)
0.06 µg/g BW (n = 68)	-	30.09***	Axis Tortion (11.5)	-	Anophthalmia (2.9) Underdeveloped (3.8)	-	-	Hemorrhagic Spots (15.4)
0.08 µg/g BW (n = 68)	4.76**	32.5***	Axis Tortion (7.1)	Microcephaly (2.9)	Anophthalmia (2.9)	Forelimb Micromelia (2.9)	-	-
0.1 µg/g BW (n = 66)	15.07***	35.46***	-	Hydrocephaly (3.03)	Underdeveloped (3.6)	Meromelia (3.03)	Syndactyly (1.5)	-

n = Number of fetuses recovered. Asterisks indicate significant difference against control p<0.01, p<0.001.

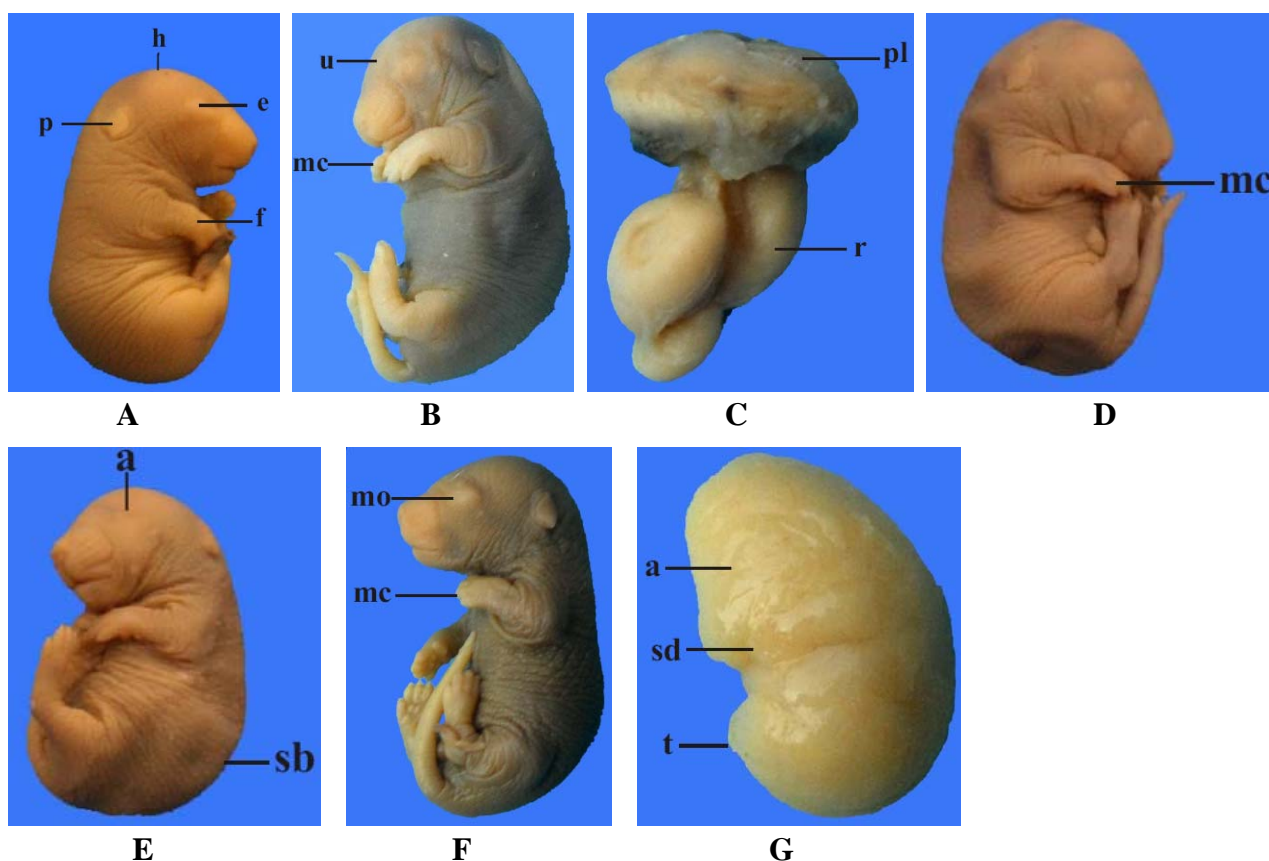


Figure 1. Macrophotographs of 18 days old fetuses exposed to different concentrations of Cytotec on day 8 of gestation. A: Vehicle Control B: Dose 1 (0.02 µg/g BW) C and D: Dose 2 (0.04 µg/g BW) E: Dose 3 (0.06 µg/g BW) F: Dose 4 (0.08 µg/g BW) and G: Dose 5 (0.1 µg/g BW). NOTE: p; well developed pinna, h; fully developed head, e; well formed eye with closed eye lid, f; well developed forelimb, u; underdeveloped eye, mc; forelimb micromelia, pl; placenta, r; resorbed fetus, a; anophthalmia, sb, sacral spina bifida, mo; microphthalmia, sd; syndactyly, t; degenerate tail.

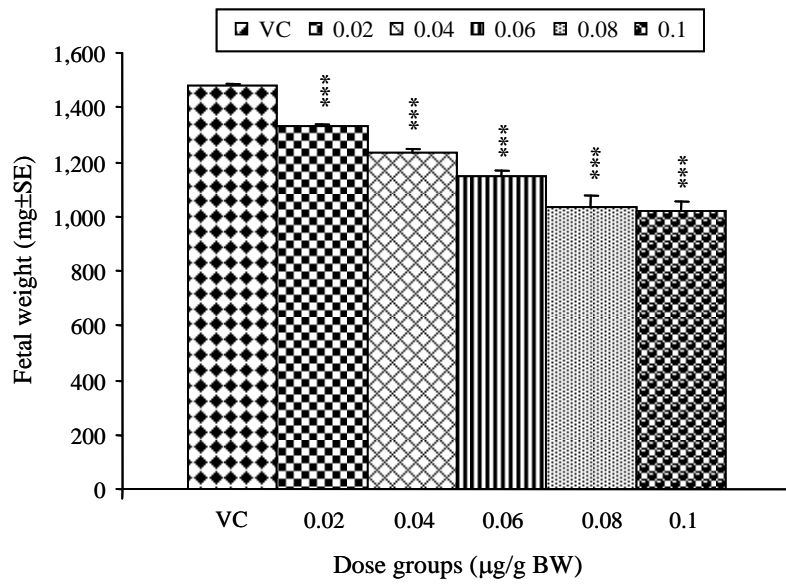


Figure 2. Histogram showing effects of different doses of Cytotec on body weight of 18 days old mice fetuses. Asterisks indicate significant difference against control *** $p < 0.001$.

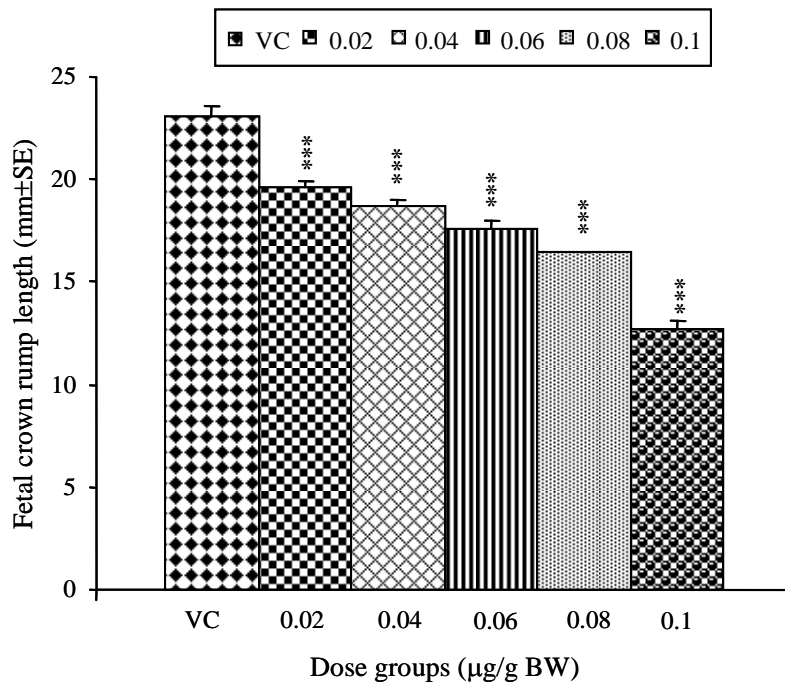


Figure 3. Histogram showing effects of different doses of Cytotec on crown rump length of 18 days old mice fetuses. Asterisks indicate significant difference against control *** $p < 0.001$.

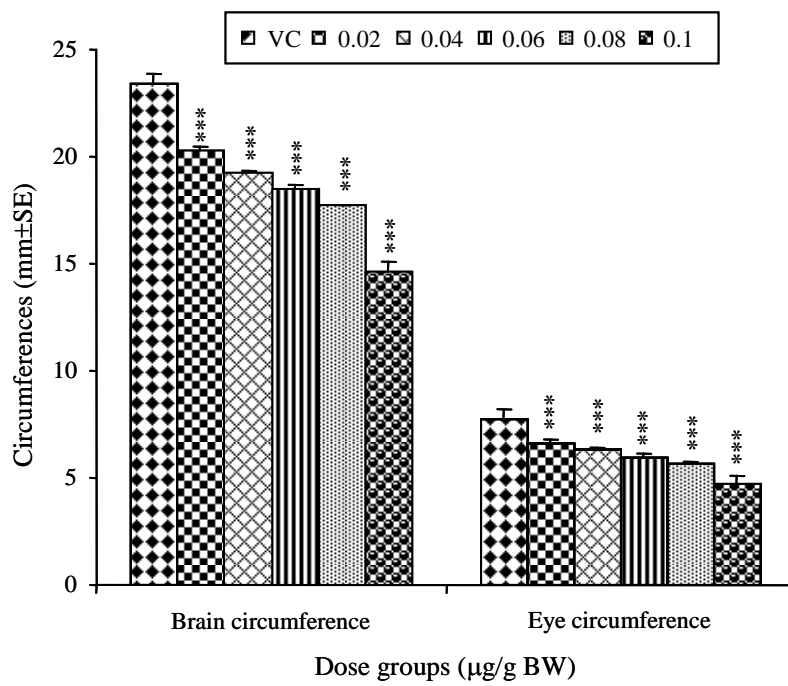


Figure 4. Histogram showing effects of different doses of Cytotec on Brain and Eye Circumference of 18 days old mice fetuses. Asterisks indicate significant difference against control *** p<0.001.

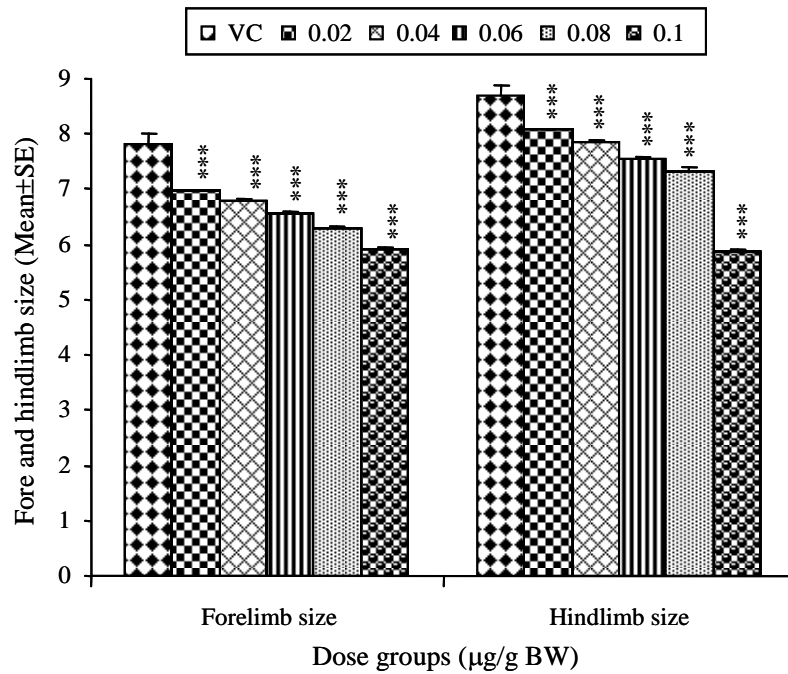


Figure 5. Histogram showing effect of different doses of Cytotec on Fore and hindlimb size of 18 days old mice fetuses. Asterisks indicate significant difference against control *** p<0.001.

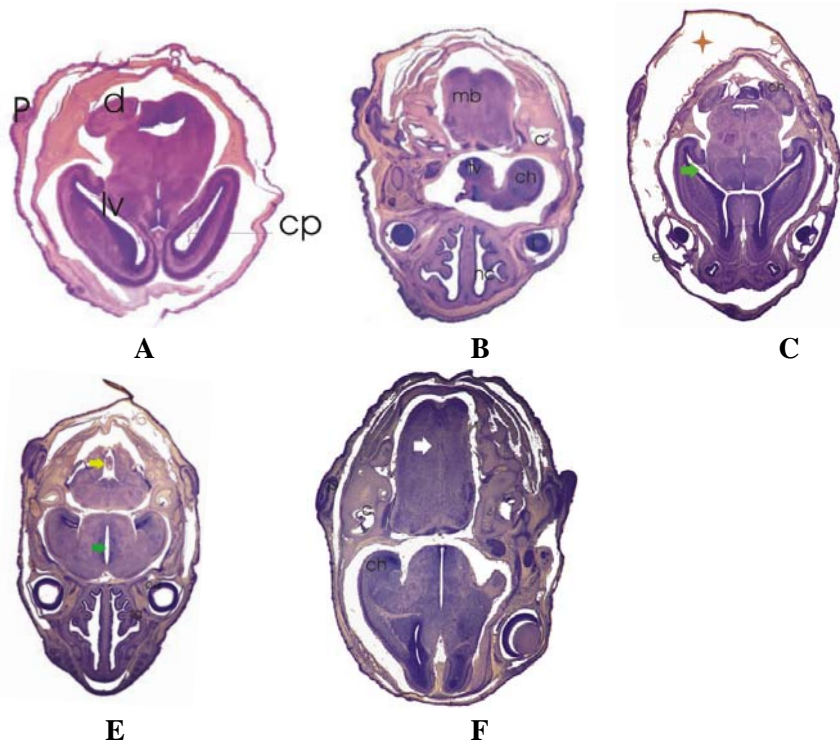


Figure 6. Sections of the 18 days old mouse fetus from cranial and ophthalmic regions. A and B: Sections from control group. C-E: Sections from drug treated groups. NOTE: p; pinna, d; diencephalons, lv; lateral ventricle, cp; choroids plexus within third ventricle, mb; medulla oblongata, tv; third ventricle, ch; cerebral hemisphere, c; cochlea, l; lens, nc; nasal cavity, brown star; hydrocephaly, green arrow; enlarged third ventricle, e; eye lids fused at this stage, yellow arrow; undifferentiated ectoneuronal cells, od; optic disc, sg; serous glands within the lateral wall of the middle meatus.

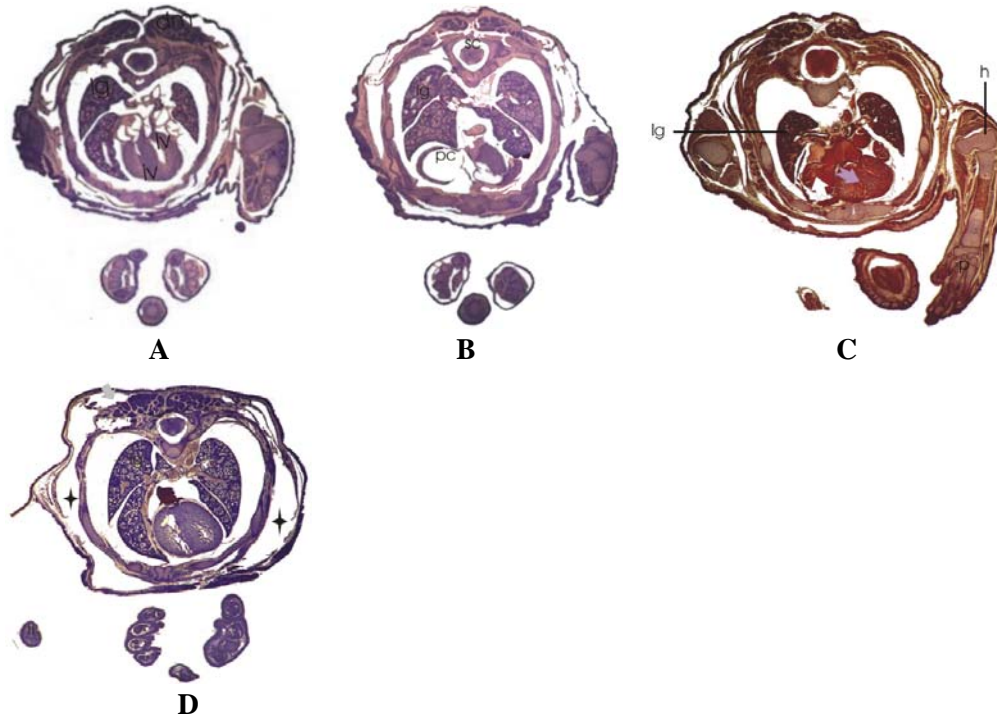


Figure 7. Sections of 18 days old mouse fetus through Cardiac and Pulmonary Regions. A and B: Sections from control group. B and C: Sections from drug treated fetuses. NOTE: sc; spinal cord, lg; lungs, dm; dorsal muscles, lv; lumen of ventricle, iv; interventricular septum, pc; peritoneal cavity, white notched arrow; right auricle thrombosis, purple arrow; degeneration of trabecular zone of heart, h; humerus, p; phalanges.

well as the presence of hydrocephaly was also observed (Figure 6C and D). Along with that, undifferentiated ectoneural stem cells were seen escaped in the fourth ventricle cerebrospinal fluid were also observed (Figure 6D). Moreover, degeneration of jaw muscles was also observed in the facial region of the fetuses of this dose group. At the same time, extra vascularization of thymus was also brought into study. In the cardiac region, hypoplasia of thick ventricular walls was observed (Figure 7D).

DISCUSSION

Extremely low cost and stability at room temperature of Cytotec has made it particularly useful in developing countries like Pakistan (Javaid et al., 2004). The risk of adverse effects on fetuses exposed to misoprostol is still not fully explored due to illegal abortions and unsatisfactory technical conditions (Song, 2000; Goldberg et al., 2001). Norman et al. (1991) reported that 80% of pregnancies in which misoprostol was used were not terminated. Experimental data related to the teratogenic potential of Cytotec is extremely sparse.

The LD₅₀ of misoprostol administered orally in rats is 81-100 mg/kg BW and in mice it is 27-138 mg/kg BW (McEvoy, 1996). The subchronic studies conducted in rodents for 30 days and 26 weeks with daily doses up to 200 and 125 mg/kg BW, respectively showed no toxicity but induced some antihormonal effects of the compound (Deraedt et al., 1985; Jost, 1986). The present study was mainly focused at the teratogenic potential of misoprostol available with the trade name Cytotec®.

Different patterns of abnormalities were observed at different dose groups. The head deformities included microcephaly and hydrencephaly at different dose groups (Table 1) were comparable with reports by Qazi (2006) from Pakistan where a woman gave birth to a baby girl after failed attempt to pregnancy termination with misoprostol. The offspring was with multiple anomalies like fronto-nasal bone, scalp defect and protrusion of duramater, microcephaly and equinovarus deformities were also present. Dal Pizzol et al. (2008) reported birth defects associated with misoprostol which included meningomyelocele and microcephaly, musculoskeletal defects, club foot, syndactyly and fingernail defects.

Like wise the eye abnormalities ranged from underdeveloped eyes to microphthalmia and anophthalmia. Furthermore, limb anomalies included micromelia, amelia and meromelia as well as webbed claws were also observed. Gonzalez et al. (1993) reported seven cases with first trimester exposure to misoprostol resulting in limb defects. Another study reported by Gonzalez (1998) described the

presence of arthrogryposis and terminal transverse limb defects with or without Mobius syndrome. Certain other reports also presented the case of misoprostol-induced arthrogryposis and Mobius sequence (Coelho et al, 2000; Dal Pizzol et al., 2006).

Uzumcu et al. (2009) suggested that pathogenesis of Moebius syndrome is due to abnormal development of cranial nerves V through XII and performed microdeletion analyses on 13q12.11-q13 in nine patients, and sequenced three candidate genes in nineteen patients for functional relevance and further resolution of our screening. Jonakait and Ni (2009) demonstrated that misoprostol enhanced the microglial proliferation which promotes cholinergic neuronal differentiation of undifferentiated precursors in the embryonic forebrain *in vitro*.

CONCLUSION

The present study showed the Cytotec is teratogenic in developing mice. The embryotoxic effects were observed on morphological, morphometric and histological levels. The morphological analysis showed microcephaly, hydrocephaly, anophthalmia, microphthalmia, micromelia and syndactyly etc. Morphometric analysis showed significant difference in body weight, crown rump length, brain and eye circumference and forelimb and hindlimb size as compared to control. Similarly, histological findings revealed underdevelopment of various organs of the body. In lieu of above mentioned findings, it is concluded that Cytotec has teratogenic potential. Therefore, this drug should be prescribed with extreme care to women of childbearing age and should be completely avoided in pregnant women.

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