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Original Research Article

TERATOLOGICAL EFFECTS OF CARBOPLATIN: A MORPHOLOGICAL STUDY IN MICE

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ABSTRACT

Background: Carboplatin (*cis*-diammine 1,1cyclobutanedicarboxylateplatinumII), is an antitumor platinum complex derived from cisplatin. Preclinical studies suggest that it may have greater antitumor activity and lower toxicity than cisplatin. The potential of Carboplatin to induce embryo toxicity was investigated in the albino mice.

Materials and Methods: Fourty pregnant mice were distributed among treated [n=30] and a control [n=10] group. Carboplatin was administered intraperitoneally to pregnant mice of treated group on day 7th of gestation at dose level of 6 mg/kg. All dams were subjected to caesarean section on Day 19 of gestation under deep anesthesia according to the animal ethical guidelines.

Result: At given dose an increase in the resorption rate and a reduction in the fetal weight and height were found. Gross malformations obsereved were intraperitoneal hemorrhage, increased neck bending and limb hemorrhage. There were no signs of maternal toxicity.

Conclusion: The results show that carboplatin is embryotoxic at minimally maternal toxic dose in mice.

KEY WORDS: Teratology, Carboplatin, Abortion, Haemorrhage, Mice, Anomaly.

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INTRODUCTION

Teratology, the study of abnormal prenatal development and congenital malformations induced by exogenous chemical or physical agents, continues to be a burgeoning area of medical research in the quest for the

eradication of preventable birth defects. Identification of agents with teratogenic potential from the plethora of drugs and chemicals that human beings come into contact with in their everyday environment is crucial; although only some 10% of congenital anomalies are thought to be caused by teratogens [1] representing roughly one in every thousand live births, they compromise the quality of life for millions of individuals worldwide and cost billions of dollars in health care every year.

Carboplatin (*cis*-diammine-1,1 cyclobutanedi-carboxylateplatinum II), a second-generation platinum-containing anti-cancer drug, which was developed by Bristol-Myers Company, USA is currently being used in the clinic against lung, ovarian, head, and neck cancers. Cisplatin, a platinum coordinated complex like Carboplatin, has been reported to be embryotoxic in rats [2, 3] and mice [4, 2, 5] and chicks [6].

This study was performed to evaluate the potential of Carboplatin to induce fetal dysmorphogenesis, prenatal mortality, and intrauterine growth retardation at a dose level of 6 mg/kg body weight in mice fetuses which correspond to the teratogenic dose in the previously described Bristol-Myres's report for rat fetuses.

MATERIALS AND METHODS

Forty female albino mice of an average weight of 20 +/- 3 gm and an average age of 80-100 days were used in the study from the Institutional Central Animal Research Facility. Animals were kept individually in plastic cages in noise free, air conditioned animal house with temperature maintained at 75°F and on a light dark cycle of 12: 12 hours. Humidity was maintained with a minimum of 50%. Mice were fed on diet pellets (Hindustan Lever Bombay, India), tap water and libitum and treated with utmost humane care. All experiments were designed in strict adherence to the guidelines of the Institutional Animal Ethics Committee. Female albino mice were kept overnight in the same cage with male mice of same stock in the ratio of 3:1 (female:male = 3:1). At 8:00 A.M. in next morning vaginal smear was examined. If vaginal smear was positive, then smear was made on glass slide and examined under light microscope. If sperms were found, pregnancy was confirmed, and it was considered as day ' ZERO' of pregnancy. The pregnant mice were weighted on alternate days and kept individually in separate cages.

Drugs and Chemical: Commercially available carboplatin BP (trade name Carbowel) was procured from Getwell Oncology Pvt. Ltd.

The animals were randomly assigned to two groups control and treated containing 10 mice and 30 mice respectively and received the following treatments: Mice of control group received disstiled water intraperitoneally on 7th day of gestation. Mice of treated group received carboplatin 0.012ml (6 mg/kg body weight) intraperitoneally by tuberculin syringe on 7th day of gestation.

Collection of Fetuses: The pregnant mice were anesthetized with an overdose of ether on day 19th of gestation (full term pregnancy). The uterine horns were exteriorized after opening the abdomen by midline caesarean incision. The sacs were inspected for sites of resorptions and viable fetuses. The fetuses were removed from the uterus and were dried on a bloating paper. The weight of fetuses were recorded by electronic weighing balance. Fetuses were examined for external abnormalities and crown to rump length (CRL) was recorded.

OBSERVATIONS

Out of 198 treated fetuses, 144 were live and 54 were found to be dead. And out of 144 live treated fetuses, 115 fetuses showed congenital malformations [Table 1].

Fetal Mortality: In treated groups, fetal mortality was much more [27.2%] as compared to control where it was zero (Table 1).

Intrauterine Growth Retardation (IUGR): Marked growth retardation was observed in Carboplatin treated fetuses as compared to control fetuses (45.1%) (Table 2);[Figure 5].The average weight of control fetuses was 1.414 gm and that of treated group fetuses was 1.071 gm. p-value was found to be < 0.001, which is highly significant (Table 3 & 4);[Figure 2]. Significant decrease in Crown to Rump length (CRL) was observed. The average CRL of control fetuses

was 21.89 mm and that of treated fetuses was 18.87 mm. p-value was found to be < 0.001, which is highly significant (Table 5 & 6); [Figure 3].

Brain Morphology: Weight and size of brain of control and treated fetuses were taken after dissection. The average weight of control fetuses brain was 0.048 gm and that of treated fetuses brain was 0.038 gm. p-value was found to be < 0.001, which is highly significant (Table 7 & 8);[Figure 4].The average anterior-posterior and transverse diameter of control fetuses brain was 8.06 mm and 4.78 mm respectively and that of treated fetuses brain was 6.42 mm and 3.9 mm respectively. p-value was found to be < 0.001,which is highly significant (Table 9);[Figure 9].

Gross Malformations: Various gross malformations were observed in fetuses of treated group [In 115 out of 144 fetuses, 79.8%] (Table 1). The gross malformations observed were IUGR (45.1%), Intraperitoneal hemorrhage (31.2%), Limb anomalies(19.4%) and Neck bending (14.6%) (Table 2);[Figure 1].

Out of 115 abnormal fetuses, 65 fetuses showed Intrauterine growth retardation [IUGR] [Figure 5], 45 fetuses showed intraperitoneal hemorrhage [Figure 6]. On examination of limbs in treated group, 28 fetuses showed angular and torsional deformities [Figure 7]. Forward neck bending was also observed in 21fetuses of treated group [Figure 8].

Fig. 1: External Malformations in carboplatin treated living mice embryos (total number = 144).

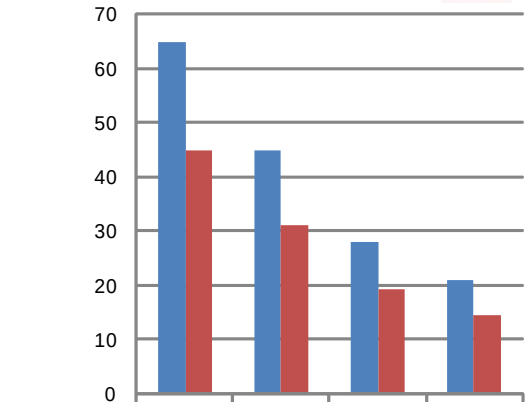


Table 1: Teratologic effects of Carboplatin [0.12 mg/mice] in developing mice

	Total number of experimental mice	Total number of embryos	Number of embryos dead / resorptions	Number of embryos living	Number of abnormal out of living embryos
Control	10	76	0	76	0
Treated	30	198	54 [27.2%]	144 [72.8%]	115 [79.8%]

Table 2: Observed external malformations in Carboplatin treated living mice embryos [Total number = 144].

	IUGR	Intraperitoneal hemorrhage	Limb Anamolies	Neck bending
Number	65	45	28	21
Percentage	45.1	31.2	19.4	14.6

[IUGR = Intrauterine growth retardation]

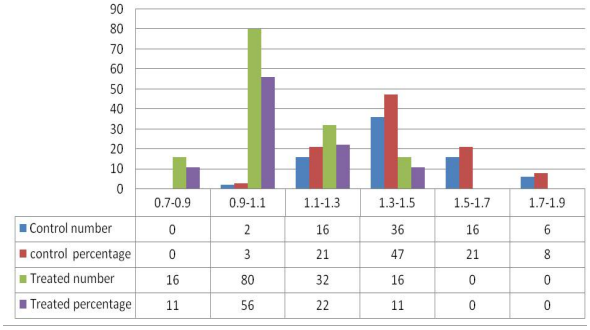
Table 3: Drug response effect in weight of mice embryos (gm) among control and treated groups.

	Control (n=76)	Treated (n=144)
Range	1.886-1.126	1.412-0.748
Mean	1.421	1.067
Standard deviation (S.D.)	0.1835	0.1639
t-test	14.626	14.123
p-value	< 0.001	

Table 4: Drug response effect in weight of mice embryos (gm) among control and treated groups

Group Variable (gm)	Control (n=76)		Treated (n=144)	
	Number	Percentage	Number	Percentage
0.7 - 0.9	0	0	16	11
0.9 -1.1	2	3	80	56
1.1 – 1.3	16	21	32	22
1.3 – 1.5	36	47	16	11
1.5 – 1.7	16	21	0	0
1.7 -1.9	6	8	0	0

Fig. 2: Drug response effect in weight of mice embryos (gm) of control and treated groups.



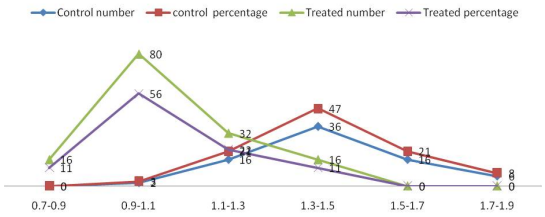


Table 5: Drug response effect in crown to rump length [CRL] of mice embryos (mm) among control and treated groups.

	Control (n=76)	Treated (n=144)
Range	18-27	15-24
Mean	21.78	19.14
Standard deviation (S.D.)	2.398	1.708
t-test	9.429	8.516
p-value	< 0.001	

Table 6: Drug response effect in crown to rump length [CRL] of mice embryos (mm) among control and treated groups

Group Variable (mm)	Control (n=76)		Treated (n=144)	
	Number	Percentage	Number	Percentage
15-17	0	0	16	11
17-19	8	11	72	50
19-21	24	32	48	33
21-23	30	39	8	6
23-25	10	13	0	0
25-27	4	5	0	0

Fig. 3: Drug response effect in crown to rump length [CRL] of mice embryos (mm) of control and treated groups.

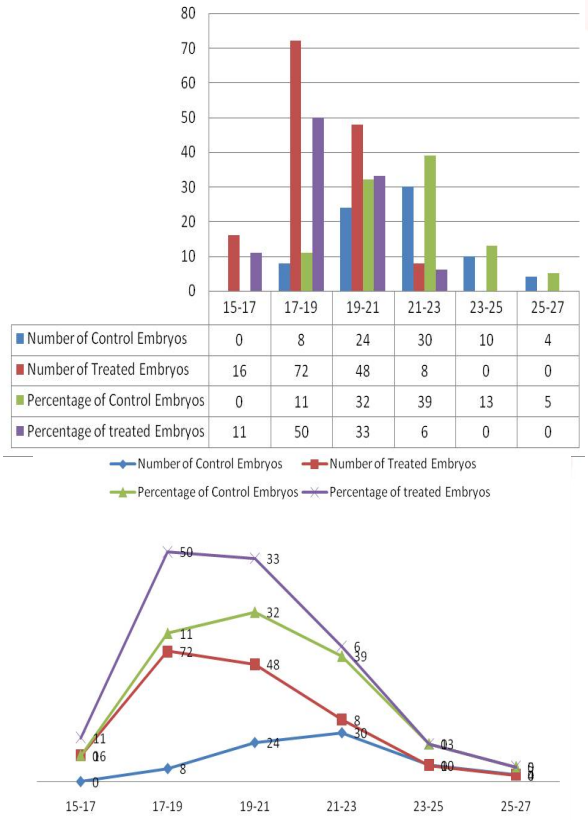


Table 7: Drug response effect in brain weight of mice embryos (gm) among control and treated groups

	Control (n=20)	Treated (n=40)
Range	0.038-0.062	0.029-0.048
Mean	0.0475	0.037
Standard Deviation [S.D.]	0.005	0.004
t-test	8.555	7.844
p-value	< 0.001	

Table 8: Drug response effect in brain weight of mice embryos (gm) among control and treated groups.

Group Variable (gm)	Control (n=20)		Treated (n=40)	
	Number	Percentage	Number	Percentage
0.028-0.033	0	0	4	10
0.033-0.038	0	0	20	50
0.038-0.043	3	15	12	30
0.043-0.048	8	40	4	10
0.048-0.053	6	30	0	0
0.053-0.058	2	10	0	0
0.058-0.063	1	5	0	0

Fig. 4: Drug response effect in brain weight (gm) of mice embryos of control and treated groups.

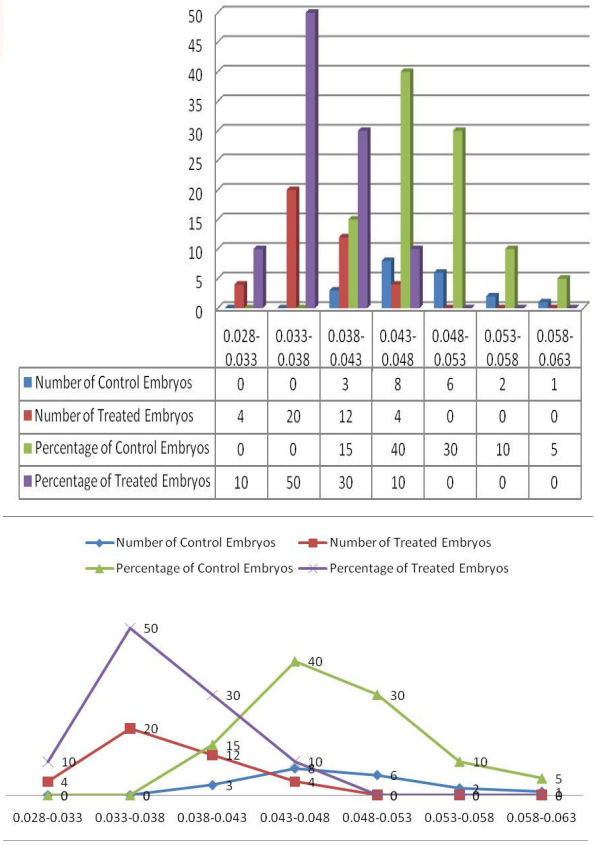


Table 9: Drug response effect in brain dimensions of mice embryos (mm) among control and treated groups.

	Antero-posterior		Transverse	
	Control	Treated	Control	Treated
Range	07-09	5.6-8.8	04-06	2.8-5.2
Mean	8.06	6.42	4.78	3.9
Standard Deviation [S.D.]	0.745	0.642	0.476	0.465
t-test	12.634	12.342	9.154	9.12
p-value	< 0.001		< 0.001	

Fig. 5: Gross Appearance of Control and Treated Embryos - Treated embryos showing intrauterine growth retardation [IUGR] in comparison to control embryos.

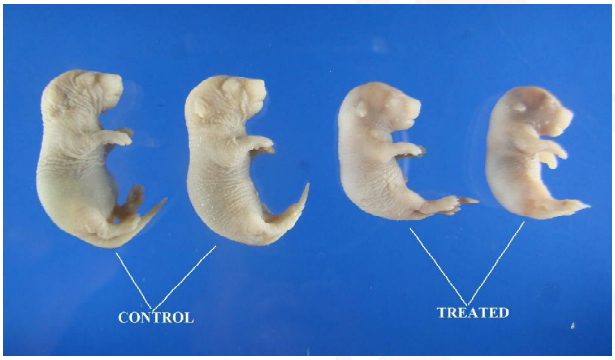


Fig. 6: Gross appearance of control and treated embryos-showing Intraperitoneal hemorrhages in comparison to control embryos.

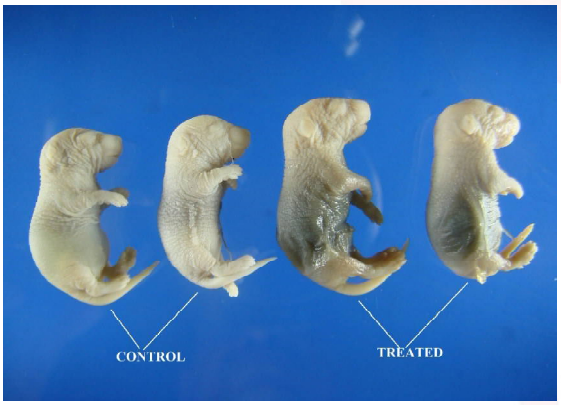


Fig. 7: Gross Appearance of Control and Treated Embryos-Treated embryos showing Torsion and Angular limb deformities in comparison to control embryos.

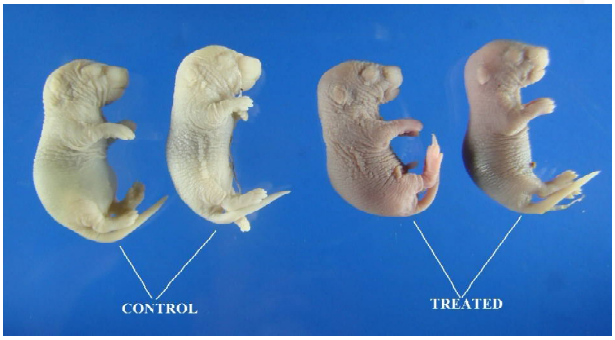


Fig. 8: Gross Appearance of Control and Treated Embryos -Treated embryos showing increased Neck bending in comparison to control embryos.

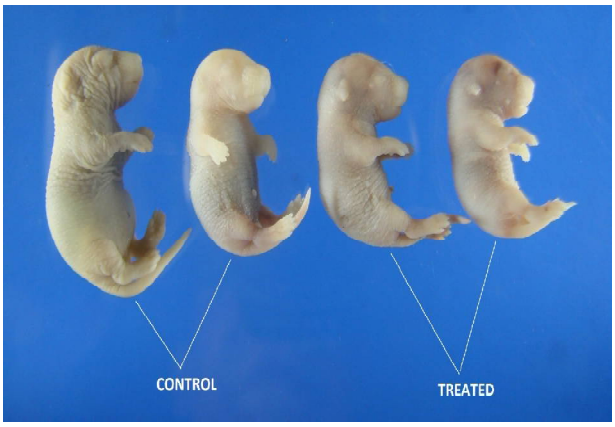
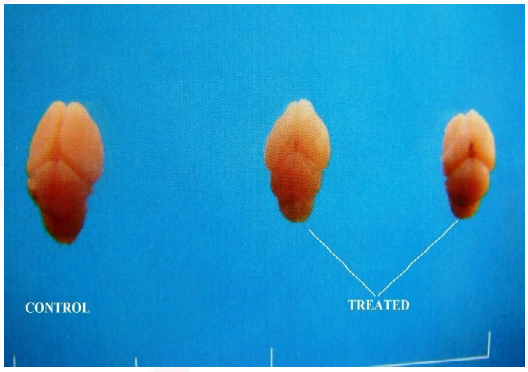


Fig. 9: Gross Appearance of Brain of Control and Treated Embryos -Treated embryos showing reduced size of brain in comparison to control.



DISCUSSION

The present study has demonstrated that Carboplatin (a platinum based chemotherapeutic agent) when administrated on 7th day of gestation at a dose of 6 mg/kg body weight is teratogenic to albino mice which includes fetal resorptions / death, intrauterine growth retardation [IUGR], limb anomalies and other anomalies without significant maternal toxicity.

Shuichi et al (1989) suggested that carboplatin is embryotoxic , inducing intrauterine death and congenital malformations in rats, when injected during the early stages of development i.e. 6th - 8th day of gestation at a dose level of 6 mg/kg body weight [7]. Teratological effects of carboplatin in mice have not been established till date. In the preceding studies, carboplatin achieved the embryolethal effects when administered before the organogenesis period [8] whereas this drug retarded fetal development but revealed no embryolethality when dosed

during the organogenic period [9]. Since cisplatin, one of platinum coordinated complexes, is a bifunctional agent which causes interstrand/intrastrand DNA crosslinking in mammalian cells [10-12], its major mechanism has been proposed to be the inhibition of DNA replication due to these crosslinking effects [13-15]. The mechanism of action of carboplatin seems to be similar to that of cisplatin because of resemblance in the chemical structure between these two compounds. Further, it has been shown that cisplatin-induced embryolethality varies with the stage of gestation [2, 5]. These results suggest that carboplatin administrated to pregnant mice during the pregnancy may induce fetal death and fetal gtowth retardation. Though elevations in the number of dead fetuses during the early gestation period were considered to be predominantly due to the direct effects of carboplatin, secondary effects through dams could not be denied. Carboplatin induced various external malformations when administrated to pregnant mice at 6 mg/kg body weight on 7th day of gestation. Especially, the incidences of intraperitoneal hemorrhages (31.2%), limb anomalies (19.4%) and neck bending (14.6%) were higher in these fetuses than corresponding controls.

Carboplatin reveals the teratogenic action against mice conceptus when dosed at 6 mg/kg body weight on 7th day of gestation. This finding was supported by the fact that a significant elevation in the number of empty implantation sites/fetal death/ resorptions (27.2%), was revealed when carboplatin was injected to pregnant mice at 6 mg/kg body weight on 7th day of gestation. In the present study, microcephaly was observed as evidenced by the reduction in weight and various dimensions of the brain. As stated by Singh and Padmanabhan [16] central nervous system defects can be induced in experimental animals, by a variety of agents like nutritional deficiency, dietary restrictions, ionizing radiations, maternal viral infections chemicals and therapeutic agents [17].

They also noted microcephaly and hydrocephaly along with various brain anomalies induced by such agents. This was associated with fetal intrauterine growth retardation [IUGR]. Fetal IUGR and microcephaly due to ischaemia have also been observed by Wigglesworth [18].The present study also showed IUGR as well as microcephaly. The limb defects and rumplesness observed in the present study can be explained by the fact that these mesodermal elements require neuroectodermal tissues for induction. The damage of neuroectodermal tissues must have failed to induct the mesoderm to proceed for proper development of these skeletal elements. The highest death rate of the embryos (from 25% to 100%) due to exposure to the tested agents was observed in the periods of embryogenesis when embryos are most susceptible to adverse factors (days 4 to 6 of embryodevelopment in the stage of blastogenesis). The teratogenic effects of the tested agent was observed only during the period of organogenesis (day 7, 10 and 14, in the stage of organogenesis).

CONCLUSION

Statistically significant incidence of severe malformations which includes increase fetal mortality in dams and delayed fetal development in surviving conceptuses were observed in this study. Fetal mortality and intrauterine growth retardation (IUGR) was much more in the treated group as compared to control. Various external malformations including deformities of limb, neck bending and intraperitoneal hemorrhage were also observed in carboplatin treated group.These external deformities were mostly due to abnormal development of appendicular skeleton. Thus, the present study establishes the drug to be teratogenic as well as lethal in developing mice embryos.

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Conflicts of Interests: **None**

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