Pediatrics & Therapeutics
ISSN: 2161-0665

Kale et al., Pediatr Therapeut 2012, 2:3
DOI: 10.4172/2161-0665.1000116

Research Article Open Access

Evaluation of Anti-teratogenic Effects of Beta cyclodextrin against Tolbutamide Induced Teratogenecity in Wistar Rats

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Abstract

Background: The efforts are done to assess the properties of drug excipient to act as agent to bring about reduced teratogenic effects of drugs that can be used for treatment of metabolic disorders.

Objective: To evaluate anti-teratogenic effects of β cyclodextrin against tolbutamide induced teratogenecity in Wistar rats.

Methods: 3 groups comprising 10 mated female Wistar rats were exposed with β cyclodextrin (750 mg/kg), tolbutamide (400 mg/kg), β cyclodextrin + tolbutamide (750 + 400 mg/kg) during gestation day 6 to 18 and concurrent vehicle control group dosed with 0.5% CMC. β cyclodextrin was given 1 hr prior to tolbutamide treatment. The females were sacrificed on presumed gestation day 20 to assess the uterine parameters and fetal observations including visceral and skeletal examination.

Results: No deaths and clinical signs occurred in this study. No test substance related changes in body weight, food consumption and gross pathological findings noticed. The uterine parameters such as Mild increase in no. of early resorptions was noticed in females treated with tolbutamide at 400 mg/kg B. wt and dam receiving combination of β cyclodextrin and tolbutamide. The post implantation loss was slightly higher in groups treated with in combination of β cyclodextrin and tolbutamide (750 + 500 mg/kg) . In the present study, the mean body weights of fetuses (male and female) were significantly decreased in tolbutamide treated group. Fetus of dam treated with tolbutamide showed decrease in CRL than the control group.

Conclusion: The result of above study can be used to prevent teratogenic action of various useful drugs used in human and veterinary medicine. This experiment can be used as initial steps to study anti-teratogenic effect of various natural, artificial compound and their derivates to maintain therapeutic value of various popular drugs used in human and veterinary medicine (Table 1).

Keywords: Implantation; organogenesis; Teratogen; Tolbutamide; β cyclodextrin

Introduction

Tolbutamide (1-butyl3-*p*-tolylsulfonylurea) is a first-generation sulfonylurea oral hypoglycemic agent used to treat non insulin-dependent diabetes mellitus (NIDDM) [1]. Gestational diabetes usually develops after the 24thweek of pregnancy because of the counter-insulin effects of placental hormones such as human placental lactogen, oestrogen and cortisol. It can be well controlled in most cases by diet alone. However, when diet does not achieve good glycaemic control, insulin treatment is recommended to manage diabetes until delivery after which the gestational diabetes disappears

Tolbutamide produces hypoglycemia in adults by blocking ATP-dependent potassium (KATP) channels in pancreatic β cells, resulting in insulin release [2]. It was demonstrated that transfer of tolbutamide to the organogenesis-stage conceptus after maternal dosing in the mouse and concentration of the drug in the embryonic heart [3]. Thus, tolbutamide has direct access to the embryo during the period of greatest teratogenic sensitivity. The embryonic pancreas is not yet present at this stage, so any direct effect of tolbutamide on the embryo must be due to extra-pancreatic targets [4,5] which may, therefore, serve as an important target for the dysmorphogenic action of tolbutamide. However, it is not possible to discriminate between potential teratogenic effects of TOLB and malformations produced by either drug-induced hypoglycemia or the diabetic state itself. Till today teratogenic data on tolbutamide by using *in vivo* approach is very scanty. The goal of this work is to better define teratogenic risks in order to provide information critical to the therapeutic management of pregnant NIDDM patients.

Technological advances of the 19th century laid the foundation of carbohydrate chemistry and by the middle of the century a number of relatively pure carbohydrates such as sucrose, cellulose from cotton, starch, glucose, fructose, mannose and cyclodextrin derivates were known to chemists in world. Now a days β cyclodextrin have been used as drugs excipient in order to increase the drugs bioavailability. In the US Patent (no5100878) Geber [6] claimed that teratogenic effect of various physical, biological, chemical teratogen can be blocked by using β cyclodextrin as anti teratogen. According to this patent anti-

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Received February 01, 2012; **Accepted** March 05, 2012; **Published** March 06, 2012

Citation: Kale S, Mody SK, Atul C, Patel HB, Modi CM, et al. (2012) Evaluation of Anti-teratogenic Effects of Beta cyclodextrin against Tolbutamide Induced Teratogenecity in Wistar Rats. Pediatr Therapeut 2:116. doi:10.4172/2161-0665.1000116

teratogen β cyclodextrin can be administered either before or onset of organogenesis and either before or after exposure of teratogen. There is little information available whether β cyclodextrin has anti-teratogenic properties against teratogenecity of various drugs indicated for metabolic diseases. Hence, with this background of information present investigations have been under taken to evaluate anti-teratogenic effect of β cyclodextrin against tolbutamide induced teratogenic effects in pregnant Wistar rats.

Materials and Methods

In-house bred healthy adult nulliparous and non-pregnant female Wistar rats of 8-10 wks age were used. The animals were housed in IVC cages with rice husk as bedding material in an AAALAC accredited facility with 12 h light / dark cycle at 25 ± 3°C and relative humidity between 30-70%. The experimental animals were provided with, UV treated rodent diet as per study schedule, supplied by Pranav Agro Industries Ltd, Sangli – 416 436, India and filtered drinking water was provided *ad libitum*.

The treatment groups include independent tolbutamide and β cyclodextrin treated at dose of 500 mg/kg and 750 mg/kg respectively and in combination of β cyclodextrin + tolbutamide at dose of 750+500 mg/kg. Independent vehicle control group was maintained in this study.

The mated females from treatment groups and vehicle control group were administered with test substance from gestation day (GD) 6 to 18 by oral gavage at dose volume 10 mL/kg body weight. The day on which vaginal plug or sperms observed in vaginal smear, was considered as day '0'of gestation

The following observations were made during experimental and terminal phase of the study. Mortality and clinical signs of toxicity, body weight and feed consumption were recorded on presumed gestation day 0, 6, 9, 12, 15, 18 and 20. Mated females were sacrificed on presumed gestation day 20 by CO_2 asphyxiation and subjected to gross pathological examination (Table 2).

Immediately after gross pathological examination, uterus from each dam along with ovaries was removed to ascertain the pregnancy status. Once the uterine observations recorded, each fetus was separated by cutting the umbilical cord of each fetus as close to the body as possible and the placenta was placed in sequential order in a compartmentalized tray filled with normal saline (0.9% w/v NaCl) . The fetuses were humanely euthanized by inducing hypothermia by placing them in sequential order on a wet towel placed over cooling plate. The fetal evaluation include, identification of fetal sex, individual fetal body weight, placental weight, crown rump length, gross external observation, visceral (including head razor) examination and skeletal examination [7].

Statistical tests were performed using Graph Pad Prism version 4.0. Two way ANOVA was used for feed consumption, one way ANOVA for continuous data and non-parametric ANOVA test such as [8] Kurskal-Wallis test was used for the comparison of different dosage groups for discontinuous data. Malformations in fetuses and number of dams with major malformed fetuses were analyzed using Chi square test followed by Fischer-exact test. Post-hoc test included Dunnett's and Dunn's test for parametric and non parametric ANOVA respectively, (if significance was noted), while Bonferroni's test was used for two way ANOVA.

Results and Discussion

The present investigation was undertaken to determine the anti-teratogenic action of β cyclodextrin against tolbutamide induced teratogenecity in Wistar rat. Furthermore it was planned secondary to know teratogenic effect of tolbutamide in Wistar pregnant rat. The

Gestation Day	Group	I	II	III	IV
	Dose	0 mg/kg	750 mg/kg	400 mg/kg	750 + 400 mg/kg
0	Mean	182.48	180.73	185.83	183.69
	SD	13.92	11.17	7.88	13.98
	n	9	9	8	9
6	Mean	200.79	199.68	206.61	203.26
	SD	16.12	9.73	7.62	16.41
	n	9	9	8	9
9	Mean	204.57	207.63	207.35	206.07
	SD	17.35	11.55	5.58	18.88
	n	9	9	8	9
12	Mean	211.81	216.63	217.70	219.63
	SD	16.79	10.96	6.39	22.69
	n	9	9	8	9
15	Mean	220.94	229.37	230.59	228.79
	SD	23.02	11.09	5.11	23.29
	n	9	9	8	9
18	Mean	240.67	248.73	251.69	253.01
	SD	34.86	15.07	10.80	28.99
	n	9	9	8	9
20	Mean	279.62	268.00	275.73	271.90
	SD	28.32	19.97	13.57	32.82
	n	9	9	8	9

Group I : Control, **II**: β Cyclodextrin, **III**: Tolbutamide, **IV**: β Cyclodextrin + tolbutamide

Table 1: Summray of group mean body weight – gestation.

previous teratological data and U. S. E. P. A. guideline of bench mark dose for developmental toxicology were taken into consideration. No signs of toxicity or mortality were observed in group of dam treated with β cyclodextrin. The same result was reported by [9]. Dam treated with tolbutamide does not show any signs of toxicity except depression in early days of treatment which was due to stress. Also there were no signs of toxicity or mortality in dam treated with combined treatment of tolbutamide and β cyclodextrin. In group of dam treated with tolbutamide showed reduction in feed consumption at gestation day 9-12. The result reported by previous workers shows reduction body weight which was not statistically significant [3].

There were no gross pathological findings observed during necropsy examination in any of the treatment and control group animals.

The group mean uterine data revealed no significant changes in parameters such as uterus weight, corpora lutea count, total implants, live fetus, dead fetus, early and late resorptions among treatment groups when compared with control group. However, mild increase in no. of early resorptions was noticed in females treated with tolbutamide at 400 mg/kg and the same was found to be comparable in females of combination treatment of β cyclodextrin with tolbutamide. The similar work done by Leroy et al. [10] for β cyclodextrin showed the mean

Gestation Day	Group	I	II	III	IV
	Dose	0 mg/kg	750 mg/kg	400 mg/kg	750 + 400 mg/kg
0-6	Mean	**96.12**	**101.41**	**108.63**	**97.80**
	SD	8.59	30.97	26.14	7.07
	n	9	9	8	9
6-9	Mean	**47.48**	**52.73**	**45.71**	**45.97**
	SD	17.72	26.81	6.96	5.47
	n	9	9	8	9
9-12	Mean	**33.39**	**36.36**	**49.61**	**53.84**
	SD	19.35	15.86	8.39	6.03
	n	9	9	8	9
12-15	Mean	**45.82**	**47.68**	**46.21**	**46.32**
	SD	11.67	16.18	16.46	16.74
	n	9	9	8	9
15-18	Mean	**49.32**	**53.33**	**52.06**	**54.86**
	SD	10.78	12.26	7.05	6.10
	n	9	9	8	9
18-20	Mean	**31.31**	**38.37**	**40.54**	**35.79**
	SD	10.98	10.57	9.72	9.67
	n	9	9	8	9

Group I : Control, **II**: β Cyclodextrin, **III**: Tolbutamide, **IV**: β Cyclodextrin + tolbutamide.

Table 2: Summray of group mean feed consumption during gestation.

	Group	I	II	III	IV
	Dose	0 mg/kg	750 mg/kg	400 mg/kg	750 + 400 mg/kg
Uterus Weight (g)	Mean	**55.06**	**47.36**	**52.70**	**54.62**
	SD	17.12	16.61	7.51	17.26
	n	9	9	8	9
CL	Mean	**10.22**	**8.56**	**11.75**	**10.33**
	SD	2.64	2.88	1.16	2.6
	n	9	9	8	9
Implants	Mean	**10.00**	**8.44**	**11.63**	**10.11**
	SD	2.83	2.79	1.19	2.42
	n	9	9	8	9
Live Fetuses	Mean	**9.89**	**8.11**	**10.38**	**9.78**
	SD	2.8	2.85	1.6	3.27
	n	9	9	8	9
Dead Fetuses	Mean	**0.00**	**0.00**	**0.00**	**0.00**
	SD	0.0	0.0	0.0	0.0
	n	9	9	8	9
Early Resorptions	Mean	**0.11**	**0.00**	**1.00**	**0.33**
	SD	0.33	0.00	2.07	1.00
	n	9	9	8	9
Late resorption	Mean	**0.11**	**0.33**	**0.38**	**0.00**
	SD	0.33	0.5	0.52	0.0
	n	9	9	8	9

Group I: Control **Group II:** β Cyclodextrin, **III:** Tolbutamide **IV:** β Cyclodextrin + tolbutamide

Table 3: Summary of uterine data.

number of implants (12.7 -14.1), resorptions (1.3-7.3%) fetal viability (93-99%) (Table 3, 4).

The derived uterine derived parameters such as corrected body weight, relative weight of uterus, pre implantation loss, percent live and dead fetus in treated animals were found to be comparable with control group. The post implantation loss was higher in tolbutamide (400 mg/kg) and combination of β cyclodextrin and tolbutamide (750 + 400 mg/kg) treated group.

The litter data such as mean number of live fetuses, male fetuses and female fetuses and the sex ratio expressed as percent of male fetuses was found slightly higher in tolbutamide (400 mg / kg) treated group (Table 5).

The litter data such as mean number of live fetuses, male fetuses and female fetuses and the sex ratio expressed as percent of male fetuses was found to be normal in the tolbutamide treated groups alone and in combination with β cyclodextrin in comparison to control group. The group means fetal absolute body weight and group mean fetal average total, male and female body weight data was found to be normal when compared with control group. The reduction in placental weight was found in fetus exposed with tolbutamide (400 mg/kg). In all other treatment group placental weight found comparable with control group. The crown-rump length of fetuses was found to be normal when compared with control group fetuses. Slight decrease in crown rum length was noticed in fetuses exposed to tolbutamide (GIII, 400 mg/kg).

Fetal gross examination was carried by method described by [7] Taylor and Barrow. The gross external examination of fetuses' revealed treatment related effects in GIII (400 mg/kg). General abnormality like small size of fetus was found to be significant in tolbutamide (Gr. III) treated groups. Major abnormalities observed was absence of digits (ectodactly) in forelimb specifically in right side in the fetus exposed to tolbutamide treatment at dose of 400 mg/kg.

Visceral examination was carried out by as per staples technique [11]. No treatment related incidences of visceral anomalies was

Uterine Paramaters	Group	I	II	III	IV
	Dose	0 mg/kg	750 mg/kg	400 mg/kg	750 + 400 mg/kg
20th day Corrected B. wt	Mean	279.62	19.53	275.73	271.9
	SD	28.32	5.43	13.57	32.82
	n	9	9	8	9
Relative Uterus Wt. (%)	Mean	19.53	17.37	19.08	19.66
	SD	5.43	5.19	2.3	5.33
	n	9	9	8	9
(%) Pre Implantation Loss	Mean	2.47	1.01	1.04	1.78
	SD	7.41	3.03	2.95	3.54
	n	9	9	8	9
(%) Post Implantation Loss	Mean	1.01	4.2	9.56	6.67
	SD	3.03	6.55	16.92	1.01
	n	9	9	8	3.03
% Live fetuses	Mean	98.99	95.8	90.44	93.33
	SD	3.03	6.55	16.92	20.00
	n	9	9	8	9
% Dead Fetuses	Mean	0.00	0.00	0.00	0.00
	SD	0.0	0.0	0.0	0.0
	n	9	9	8	9

Group I : Control **Group II**: β Cyclodextrin, **III**: Tolbutamide **IV**: β Cyclodextrin + tolbutamide.

Table 4: Summary of derived uterine data.

Parameters	Group	I	II	III	IV
	Dose	0 mg/kg	750 mg/kg	400 mg/kg	400 + 750 mg/kg
Mean No. of Live Fetuses	Mean	9.89	8.11	10.13	9.67
	SD	2.80	2.85	1.64	3.24
	n	9	9	8	9
Mean No. of Male Fetuses	Mean	6.00	4.38	4.88	4.78
	SD	3.54	1.85	1.64	2.39
	n	9	8	8	9
Mean No. of Female Fetuses	Mean	4.25	4.22	5.50	4.89
	SD	2.12	2.44	1.41	2.03
	n	8	9	8	9
% Male Fetuses	Mean	59.65	51.58	46.57	48.76
	SD	22.12	22.09	12.58	14.64
	n	9	8	8	9

Group I : Control **Group II**: β Cyclodextrin, **III**: Tolbutamide **IV**: β Cyclodextrin + tolbutamide.

Table 5: Summary of litterdata.

Parameters	Group	I	II	III	IV
	Dose	0 mg/kg	750 mg/kg	400 mg/kg	400+750 mg/kg
Absolute Total Fetal B. wt (g)	Mean	**35.21**	**29.00**	**36.38**	**35.21**
	SD	12.72	11.69	5.92	12.32
	n	9	9	8	9
Placenta wt. (g)	Mean	**0.43**	**0.43**	**0.38**	**0.45**
	SD	0.06	0.04	0.03	0.07
	n	9	9	8	9
Crown-rump length (cm)	Mean	**3.22**	**3.20**	**2.99**	**3.27**
	SD	0.10	0.28	0.24	0.15
	n	9	9	8	9
Average Total Fetal B. wt (g)	Mean	**3.51**	**3.56**	**3.50**	**3.60**
	SD	0.32	0.51	0.16	0.17
	n	9	9	8	9
Average Male Fetal B. wt (g)	Mean	**3.58**	**3.57**	**3.53**	**3.64**
	SD	0.39	0.56	0.18	0.20
	n	9	8	8	9
Average Female Fetal B. wt (g)	Mean	**3.38**	**3.57**	**3.46**	**3.55**
	SD	0.51	0.48	0.15	0.15
	n	8	9	8	9

Group I : Control **Group II**: β Cyclodextrin, **III**: Tolbutamide **IV**: β Cyclodextrin + tolbutamide.

Table 6: Summary of fetus data.

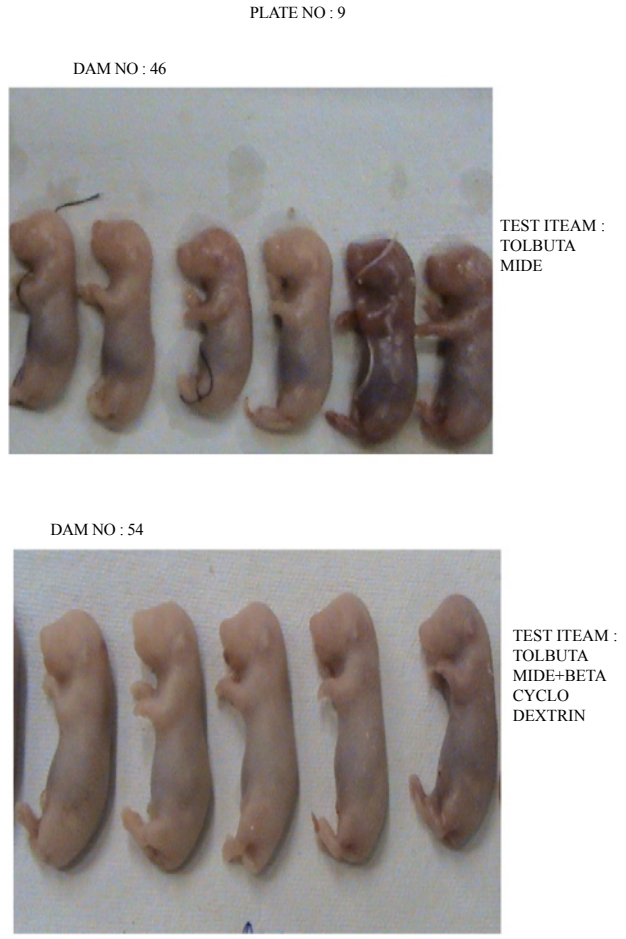

Figure 1: Images of Gross and skeletal abnormalities during Tolbutamide teratogenesis.

observed in this study. But in contrast to our result various other workers reported visceral malformations in tolbutamide teratogenesis. In contrast to our result *in vivo* exposure to tolbutamide in a variety of animal species has also produced embryonic malformations. Pregnant rabbits treated with 200 mg/kg tolbutamide orally on GD 8–16 produced a high incidence of fetal cardiac defects and mortality [12]. Rats treated orally with 200 mg tolbutamide per day during the first 12 days of gestation produced offspring with a low level (2–4%) of CNS and eye defects [13] whereas 500 mg tolbutamide produced a 41% malformation rate [14]. Medaka fish embryos exposed to high concentrations of tolbutamide demonstrated cardiovascular, ocular and body curvature defects [15].

Rat embryos exposed to tolbutamide *in vitro* also demonstrated abnormal development of heart, neural tube, eye, ear and limb buds [16] although at higher concentrations than in mice (Figure 1).

Skeletal examination was carried by using method described by McLeod MJ [17]. For fetal skeletal abnormalities, a total 47, 39, 45, 46 fetuses were examined from 0, 750, 400, 750 + 400 mg/kg dose groups, respectively. Variety of alterations noticed during skeletal examination of fetuses in degree of ossification, shape and in number of skeletal structures were non-dose dependent. Skeletal anomalies like incomplete ossification of interparietal, absence of fronto-parietal suture, poor ossification of sternebra and short supernumerary ribs were found to be statistically significant in treated groups when compared to that of control group of animals. Due to lack of dose dependency in the type of incidences, these anomalies could not be considered as a treatment related effect. All other variations such as incomplete or delayed ossification of skull bones, sternebrae, thoracic centrum, lumbar centrum, metacarpals, phalanges and presence of supernumerary ribs were comparable between control and treated animals. The pattern of internal and external developmental defects recorded in the treated group fetuses on GD 6 and 7 were almost similar in pattern and type as reported earlier in pregnant rats [18]. A few skeletal anomalies such as discontinuous ribs, supernumerary rib (unilateral) and

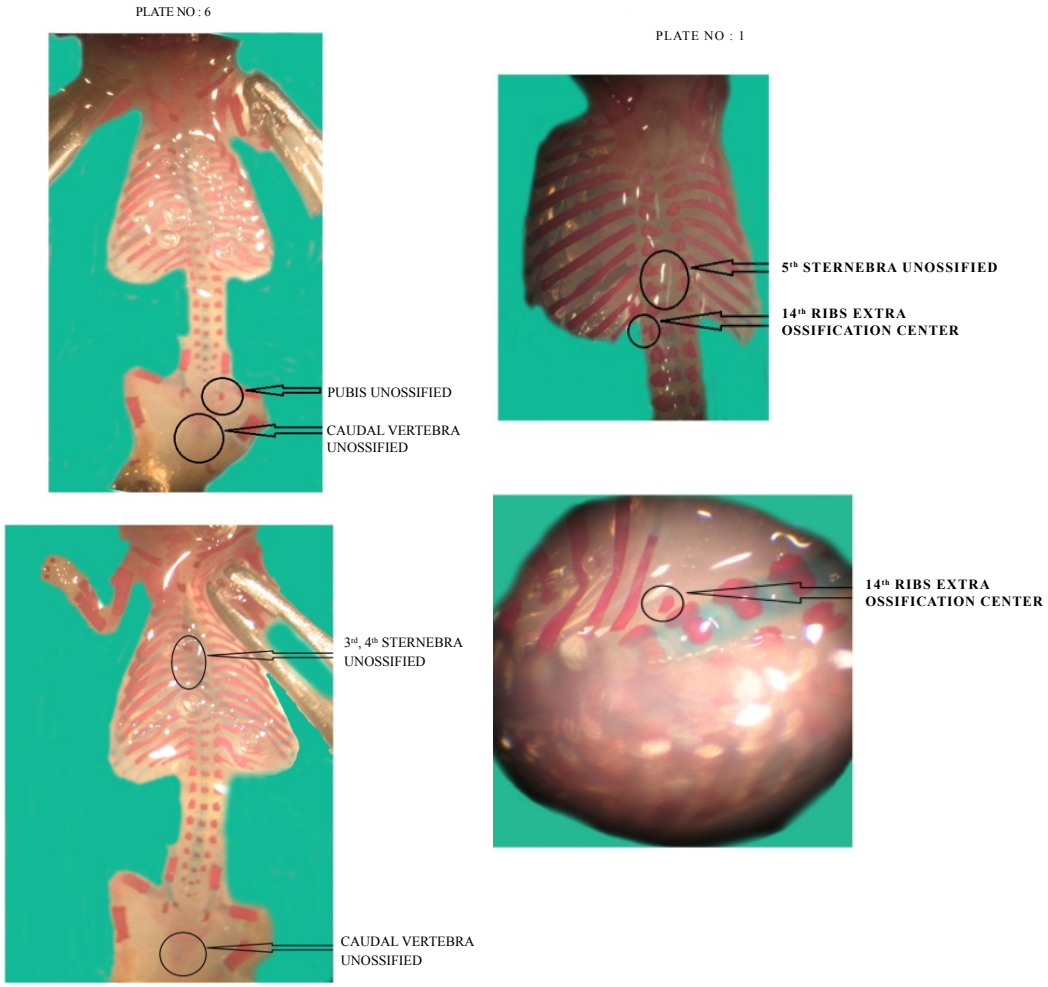

Figure 2:

malformed bones of fore and hind limbs were additionally observed in the present study. The incidence of these anomalies were very low (overall up to 4.0%) which might be due to the individual variation for the drug toxicity. These skeletal abnormalities are discussed as skull, ribs, strenebra, thoracic centrum, lumbar centrum, sacral centrum, limbs abnormalities with the respective group. For skull abnormalities frontal, parietal, inter-partials, super-occipital, hyoid bone were examined (Table 6).

Conclusion

The present study provided a novel approach to explore the anti-teratogenic effects of beta cyclodextrin against teratogenic effects produced by tolbutamide by single oral daily administration during GD 6 to 18. For tolbutamide, the gross defects seen in fetuses were small in size of fetus, hemorrhage on body and skeletal defects such as superaoccipatal split, parietals and interparietals unossification, incomplete ossification of strenebra, biparate ossification of thoracic centrum, unossified sacral and caudal vertebra, unossified ischium and pubis are new findings for such hypoglycemic agent. Co-administration of beta cyclodextrin showed reduced abnormalities and thus showed protective effects against tolbutamide induced abnormalities in fetuses (Figure 2).

This experiment can be used as initial steps to study anti-teratogenic effect of various natural, artificial compound and their derivates to maintain therapeutic value of various popular drugs used in human and veterinary medicine.

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