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Assessment of teratogenecity and embryotoxicity of sludge from textile industries at Pali (India) in Swiss albino mice exposed during organogenetic period

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Abstract: *The present investigation was carried out to assess the teratological effects of in-utero exposure of sludge leachate from textile and dyeing industries located in Pali, Rajasthan. Sludge was collected at the combined effluent treatment plant (CETP). Two groups of 10 pregnant Swiss albino mice each, were given sludge leachate of 1/10 and 1/100 dilutions with water ad libitum from 6th day to 15th day of gestation covering the critical period of organogenesis. Cesarean sections were performed on day 18 of gestation and all foetuses were examined for reproductive and teratological tests. Sludge induced maternal toxicity was evidenced by significant increase in leachate consumption, reduction in body weight gain and reduction in fur of the body. Developmental toxicity was evidenced by a significant decrease in foetal weight per litter, increase in the number of resorptions and an increase in total number of foetuses showing bone retardation and skeletal variations (specially of skull, sternebrae and vertebrae). The leachate of the sludge that is being dumped in the open areas of the town Pali seems to elicit teratogenic as well as embryotoxic potential as indicated by the findings of the present investigation.*

Key words: *Teratology, Sludge, Foetus, Embryotoxicity*
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Introduction

Industrialization is believed to cause inevitable problems, such as pollution of air, water and soil. Water pollution due to industrial processes has attained serious dimensions in India (Bhosale, 1985). Both, the quality and quantity of ground water is severely threatened by industrial sewage. Among the industries, textile industry plays a major role in modern civilization. Textile mill operations consist of weaving, dyeing, printing and finishing. Many processes involve several steps, each contributing a particular type of waste, which may invite many diseases: both occupational and general (Soni *et al.*, 2006; Mathur and Bhatnagar, 2007) and consequently escalating the economic cost. Textile industry has long been known to pose health risks causing respiratory diseases from cotton dust exposure (Kilburn, 1983) and noise induced hearing loss (Mass, 1975).

The above situation can be well depicted in Western Rajasthan, in India, on both the sides of river Bandi (located in Pali) that is considered as the lifeline of people living there. It houses a number of industries (textile and dyeing) that have seen a phenomenal growth during the last two decades. Studies conducted by Mohnot and Dugar (1987) and Mohnot and Durve (1989) have reported that various industrial units located in the three towns of Jodhpur, Pali and Balotra use about 77000 - 80000 tonnes of chemicals annually. However, potentially hazardous agents and situations are encountered in this industry, some of which might even influence the reproductive health (Suryavathi *et al*., 2005). These include solvents, dyes, noise, heat, vibrations, and prolonged standing, heavy metals *etc.* (Lu *et al*., 1994). Humans may also be affected from exposure to agents that interfere with ovulation or spermatogenesis (Olshan and Faustman, 1993). Exposure to such chemical compounds can produce a spectrum of adverse reproductive effects including chromosomal changes, mutations, sperm abnormalities, early or late foetal loss, still births, decreased birth weights, altered sex ratio, birth defects and childhood malignancies.

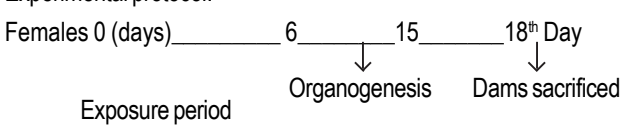
In the present study, an attempt has been made to study the effect of leachate of sludge obtained from CETP which receives effluent exclusively from textile and dyeing industries located at Pali, when administered during the organogenetic period (6th to 15th day of gestation) to Swiss albino mice.

Materials and Methods

Study area: The industrial effluent affected area is located along the river Bandi. In Pali town there are about 767 dyeing and printing units. Approximately 36 million litre per day (mlpd) industrial effluents containing high pH, chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solid (TDS), total suspended solid (TSS), sulphates and sodium are generated and discharged every day in the Bandi river (Gupta,1992; Chhoakar *et al*., 2000).

Test substance: Test substance used is sludge, collected from drying beds of Combined Effluent Treatment Plant (CETP), Pali. The biologically and Chemically treated sludge was collected, dried in oven, powdered and mixed in the ratio of 1:1. It was diluted 10 times with water and then homogenized and filtered. This filtrate served as 100% leachate, which was then diluted with water according to dose concentration of 1/100 and 1/10 respectively.

Experimental protocol:



Five to six weeks old Swiss albino mice, weighing about 20 g, were paired in the ratio of 3 females: 1 male. The females were checked for the presence of vaginal plug every morning. The day a vaginal plug was seen was taken as day 0 of gestation and the female was presumed to be pregnant. Such females were caged singly and were assigned randomly to the different dose groups. They were given dose concentration of leachate according to the group 1/10 and 1/100 and the control group received simple tap water daily from day 6 to day 15 of gestation *i.e*. during the period of organogenesis. For teratological observations, all the dams were sacrificed / laperotomised on day 18 of pregnancy and their uterine horns were examined for the number of implantations, number of living, dead or resorbed foetuses, number of stunted foetuses, sex ratio and any gross abnormalities. The live foetuses and their placentae were immediately weighed. One third of the foetuses from each litter were fixed in 70% ethanol to study their skeletal malformations (Dawson, 1926). The remaining foetuses were fixed in Buoin's fluid to assess the visceral deformities by razor section technique of Wilson (1965). The data was statistically evaluated using Student's t- test (Ipsen and Feigl, 1970) and one tailed Mann - Whitney U - test (Sokal and Rohlf, 1973).

Results and Discussion

In the present investigation, the treated sludge leachate from CETP, Pali at the tested dose levels produced maternal toxicity to certain extent. The toxic symptoms in mothers were muscular tremors, ataxia, convulsions, hypersalivation, lacrimation and restlessness. The higher concentration of leachate caused a reduction in the average weight gain by dams during gestation (Table 1), but no mortality of the dams occurred. The lower dose of sludge leachate elicited lesser number of implants and reduced litter size while the higher dose caused significant reduction in the weights of foetuses and placenta. The higher dose also caused a slight increase in the number of dead and resorbed foetuses (Table 2).

The foetuses when examined for external malformations showed subcutaneous edema and open eyelids. Free hand razor sections of the brain region showed hydrocephaly, involving chiefly the lateral ventricles. Alizarin stained skeletons of the foetuses of both the dose groups elicited reduced ossification of skull bones, reduced number of ribs and sternebral defects (Table 3).

The high dose of sludge leachate was more effective in lowering the maternal weight gain. This could probably be associated with the decrease in the litter size, which may be due to implantation failure and increase in the number of resorptions. Similar association has also been reported by Kavlock *et al.* (1981) and Rands *et al*. (1982). Aluminium induced maternal toxicity was evidenced by significant reduction in body weight gain by Albina *et al*. (2000).

The dams exposed to the lower dose of leachate showed reduced litter size as compared to those that were administered the higher dose. This may be attributed to the lower number of implantation sites in the low dose group. Adverse effects on litter size have earlier been reported with Trypan Blue (Beck, 1983) Tributyltin Chloride (Ema *et al*., 1997), Cadmium (Hovland *et al*., 1999) Arsenic (Hunter, 2000) and Aluminium (Albina *et al*., 2000)

Table - 1: Effect of leachate on maternal weight gain

Mother No.	Weight in grams		
	Control	1/100 concentration of leachate	1/10 concentration of leachate
1	7.80	4.69	6.96
2	19.60	8.01	7.92
3	11.70	9.90	6.40
4	10.40	8.00	3.91
5	11.20	7.71	5.20
6	9.72	7.79	7.52
7	18.09	7.57	3.71
8	14.69	6.40	2.48
9	12.04	5.73	2.84
10	16.24	4.95	6.40
Avg.	13.14 + 1.96	7.07 + 1.26	5.33 + 1.40*

* = Significant difference ($p<0.05$)

Administration of high dose of leachate *i.e*. 1/10 showed higher number of resorptions while dose 1/100 given to pregnant females showed less number of resorptions (Table 2). The litter size is the function of foetal mortality, including resorption of the embryos. Thus the evident decline in the number of alive foetuses per dam may be attributed to the increased percentage of the resorbed foetuses. Reduced litter size due to increased foetal resorptions in Benomyl and Phosphamidon treated rats and mice respectively has also been reported earlier by Kavlock *et al*. (1982) and Bhatnagar and Soni (1988).

A dose related decline in the foetal weight was observed with leachate (Table 2). At the same time a decrease in maternal weight gain was also evident. A similar relationship between maternal and foetal weight loss was observed by Hovland *et al*. (1999) after exposure of mice with cadmium. The probable cause for such an association may be due to administration of leachate that contains various heavy metals, organochlorines, dyes, inorganic compounds, sulphates, acids and alkalis. The maternal organism is put under stress and this in turn might affect the growing foetus leading to its growth retardation and hence reduced foetal weight.

Decreased foetal weight was accompanied with reduced degree of foetal ossification. This reduction in ossification may lead to light weight skeletal system, decreasing the average body weight. Similar relationship has earlier been observed by other workers (Murray *et al*., 1979; Verma *et al*., 1987). This relationship between foetal weight and malformations in developmental toxicity studies has been well documented by Kavlock *et al*. (1981).

Occurrence of skeletal and visceral abnormalities in both the groups (*i.e*. 1/l00 and 1/10) treated with the leachate may also be seen as an indication of growth retardation, leading to reduced weight of foetuses. The same is also suggested by Lu *et al*. (1979) after conducting studies with nickel chloride on mice.

Table - 2: Reproductive performance of mice treated with sludge leachate

Dose given	Treatment period	No. of autopsies performed	Total implants (Mean±SE)	Litter size (Mean±SE)	Alive foetuses			Dead foetuses (Mean ± SE)	Resorbed foetuses (Mean ± SE)	Placental weight (Mean ± SE)
					Foetal body weight (g) (Mean ± SE)	Sex ratio	Immature or stunted (Mean ± SE)			
Control	6-15 days	10	4.6±0.77	4.5±0.76	1.20±0.07	52.3% : 54.7%	0	0	0.4±0.07	0.13±0.01
1/100	of gestation	10	3.2±0.68	1.9±0.67	0.96±0.21	57.8% : 42.1%	0.4±0.23	0.4±0.23	0.5±0.23	0.09±0.02
1/10		10	5.5±0.91	3.0±0.85	0.54±0.17*	56.6% : 43.3%	1.0±0.65	1.0±0.85	1.4±0.85	0.06±0.01*

Student's test: * = Significant difference ($p<0.05$)

Table - 3: Skeletal malformations in mice foetuses after exposure to sludge leachate

Treatment period	Dose given	Total no. of foetuses examined	% foetuses with partially ossified skull	% foetuses with reduced no. of ribs	% sternal defects	% partial ossified vertebral column	Reduced ossification of metacarpals	Reduced ossification of metatarsals	Reduced ossification of forelimb	Reduced ossification of hindlimb
6 to 15 days	1/10	21	95.24	90.48	100	42.86	33.33	38.09	47.62	47.62
or exposure	1/100	11	100	100	90.91	63.64	00	00	81.82	81.82
	Control	23	00	00	00	00	00	00	00	00

Significant reduction was found in average placental weight of dams treated with high dose of leachate. A similar influence on the placental weight has been reported in mice, guinea pigs and rabbits (Dawes, 1968).

Sex ratio of the foetuses did not reveal much difference after treatment with the sludge leachate. This is in concomitance with the observations of Soni and Bhatnagar (1989), Deacon *et al*. (1989) Mathur and Bhatnagar (1991), Albina *et al*. (2000).

Sludge leachate when administered during days 6 – 15 of gestation, induced external anomalies such as subcutaneous edema and open eyelids in foetuses. The edema in foetuses may be attributed to hyperprotonemia, which causes water movement from capillaries into the tissues, as suggested by Grabowski (1981).

The partial ossification of skull bones may have affected the diameter of the eye orbit. This could result in changes in the attachment of eye muscles, thereby leading to the stage of open eyelids where the eyes remain open. Open or non closing eyelid was seen in foetuses of both the treated groups. Sehgal *et al*. (1995) while studying the effect of cadmium in Swiss albino mice also reported eye abnormalities in the form of open eyes, slit eyes, exopthalmia, micropthalmia and anopthalmia. The commonest among them were open and slit eyes in all the treatment groups. Ottolenghi *et al*. (1974), reported open eyes in hamster foetuses with endrin administration and regarded this phenomenon as an indicator of growth retardation.

The occurrence of enlarged cerebral ventricles in a number of foetuses was observed. Hydrocephalus chiefly involved the lateral ventricle with little or no third ventricle enlargement. In rat foetuses, hydrocephaly has been documented after exposure to a wide variety of teratogens applied at every stage of development but the precise pathogenesis for its cause is unclear (Hockwald, 1985; Eliss *et al*., 1987). Fluid accumulation in the lateral ventricles might be the leading cause of their becoming enlarged.

Reduced ossification of skull was a common feature in the foetuses due to leachate treatment (Table 3). It may be possible that the chemicals present in the leachate, affected the concentration of calcium and magnesium in treated mothers and thereby decreased their supply to the growing foetus and hence affected its skeletal development. Delayed or reduced ossification of the foetal skeleton has been reported in various animals by different authors. (Bhatnagar and Soni, 1988; Deacon *et al*., 1989; Driscoll *et al*., 1998; Mishra *et al*., 2003). Short and rudimentary ribs were observed in the foetuses of experimental groups. The occurrence of both these anomalies can be explained on the basis that growth being slowed down due to leachate treatment, the rib formation is initiated but its complete development is inhibited. Occurrence of wavy ribs, though sporadic, was witnessed in some of the foetuses of treated groups. It may be related to the arrested calcium deposition in the ribs. Such ribs then lack rigidity and may bend in response to muscular tensions or other physical stress.

A large number of foetuses with reduced number of sternebrae were seen in both the treated groups. In the high dose group 100% foetuses exhibited absence of one or more sternebrae. Hence they are regarded as terata caused by leachate treatment. Disfiguring of the sternum included cleaved sternebrae, rudimentary sternebrae, dumbell shaped sternebrae, and cleaved / absent xiphisternum. All these sternal defects may possibly be a result of retarded foetal growth.

Alizarin preparation of the treated foetuses also exhibited reduced number of ossification centres in the fore and hind limb paws. These may occur due to maternal stress as a result of leachate administration. The maternal stress might lead to reduced blood flow through the placenta and produce a deficit of circulating nutrients leading to retarded growth, an expression which could lead to these phalangeal ossification defects. This is in accordance with the observations of Bhatnagar and Soni (1988).

From the present investigation, it appears that the leachate when administered during the organogenetic phase caused a dose related reduction in the average weight gain of the dams but caused no maternal mortality. Foetotoxicity, as evidenced by the increased occurrence of several skeletal variants and decreased foetal body weights, increased number of resorbed, dead and stunted foetuses, was noted at the higher dose level. This seems to be suggestive of the potential of leachate to cause dysmorphogenic effects.

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