Aluminium Induced Intrauterine Growth Retardation - an experimental study

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Present study was carried out to determine the effect of aluminum containing antacid on the intrauterine growth and development of fetus. The duration of exposure was also correlated with the effects on fetal morphology and their weights. Seventy-two pregnant mice were given a daily i.e., dose of 0.7mg/100g of aluminum sulphate for various periods according to the grouping of experimental design. This dose was equivalent to maximum therapeutic dose of aluminum salt for a 70 kg man i.e. 5000mg aluminum/day. Fetal examination was performed on day 20 of gestation. The number of live and dead fetuses in the treated animals was not significantly different from the control groups. Therefore embryo lethality of aluminum cannot be induced. However there was a decrease in fetal body weight that was directly related to the duration of exposure to aluminum sulphate solution. Dissecting microscopic examination showed, the development was arrested in the groups exposed to drugs for longer periods. These results revealed that aluminum is a type of heavy metal, which is teratogenic for mammals even in doses, which are nontoxic for adults.

Key Word: Aluminum, teratogenicity, Growth retardation

Until first half of the 20th century, it was assumed that the development of embryo was dependant entirely on hereditary factors, but the observation made by Gregg in 1941 regarding association of congenital cataract with Rubella infection in pregnancy opened up a new field of research in human developmental defects as a result of exposure to environmental factors[1] Russof and Gaddum in 1937 detected for the first time that Aluminum crosses the placenta and it may reach the fetus in sufficient concentration to influence its development[2] In 1960, Schroeder hard reported a positive correlation of the hardness of drinking water to lethal congenital malformations, suggesting that metal content of water may be a contributory factor[3] There is sufficient evidence now available suggesting that maternal intoxication with certain metals including Aluminum in both man and laboratory animals may adversely affect pregnancy and development of conceptus[4].

In 1975 Benett et al[5]. found aluminum highly teratogenic in rats. They observed significant growth retardation as well as skeletal defects. In addition the incidence of fetal deaths and resorption was significantly increased In contrast to this Mc Cormack et al[6], 1978, found no significant effect on fetal weight or length, resorption rate or incidence of soft tissues or skeletal abnormalities. They suggested that aluminum might not be teratogenic in rats.

Yokel[7] observed the effects of aluminum exposure during lactation in rabbits in 1984. He found that prolonged systemic exposure to soluble aluminum in lactating mothers produced toxic effects including weight loss, decreased milk production, postural changes and lethality. Again in 1985 Yokel[8] in another experiment demonstrated aluminum highly toxic during gestation in rabbits. Domingo et al[9]., 1987 in their study administered aluminum nitrate by gavages to four groups of pregnant rats from the day 14 of gestation through 21 days of lactation at doses of 0, 180, 360 and 720mg/kg/day. These doses did not produce overt fetotoxic effects. However the growth of the offspring's was significantly less from birth and during all the period of lactation for the higher doses of aluminum nitrate.

Toxic effects of aluminum on brain and bone tissue are now described in a large number of clinical reports[10&11]. This study has grown rapidly during the last two decades because of the dramatic demonstration that aluminum loading could cause a lethal neuronal syndrome, "dialysis encephalopathy" and a unique form of osteodystrophy, among some patients with kidney failure[12].

Since normal embryonic development is characterized by critical periods of protein synthesis during cell division and differentiation, it is obvious that these periods represent a time of optimal enzymatic activity, and it is not surprising that many of these enzymes may be sensitive to toxic levels of aluminum[13]. The extent of cellular damage correlates with the dose and duration of aluminum loading[14].

Thus the purpose of this study was to establish the role of aluminum-containing compounds in producing embryo toxic and teratogenic effects if any, when their mothers use these compounds during pregnancy for various periods. In view of the results obtained, future guidelines for precautionary measures to be taken by the mothers during pregnancy could be formulated. Breast-feeding could be stressed upon if positive results are obtained because when it proves to be teratogenic, it must be harmful to infants and growing children fed on infant formula.

Materials and methods

72 female and 36 male albino mice were used for the present study. Animals were kept (in the animal house of Postgraduate Medical Institute, Lahore) in separate cages and fed with commercially prepared chick-feed No.3 and water ad labitum. Care was taken regarding maintenance of optimum light and temperature in the animal room. Mating was allowed in dark. Presence of vaginal plug was considered as a sign of conception and the day was taken as day 1 of pregnancy.

The salt of aluminum used for the present study was $Al_2(SO_4)$. 16H2O. 0.7mg/100gm body weight was the required amount, which was given intraperitonealy. The animals were weighed and average weight was found to be 50gm.

Experimental Design

Pregnant female mice were divided at random into various control and experimental groups, labeled and given intraperitoneal injections of distilled water and drug respectively (Table 1)

Table 1: Experimental Design

Control			Experimental		
Control Group	Dose	Period	Group	Dose	Period*
A	0.25ml of dist. water	1-6	A_1	0.25ml of $Al_2(SO_4)$ 3 solution	1-6
B	-do-	7-12	B_1	-do-	7-12
C	-do-	13-18	C_1	-do-	13-18
D	-do-	1-12	D_1	-do-	1-12
E	-do-	7-18	E_1	-do-	7-18
F	-do-	1-18	F_1	-do-	1-18

*Days of gestation

Recovery, Fixation and Preservation Of Embryos

On day 20 of pregnancy the animals were sacrificed and the two horns of uteri containing the embryos were dissected out. The embryos along with uteri were then fixed in buffered formalin. Forty-eight hours after fixation, weight of the embryos was measured in grams.

Results

Animals were weighed on an electric balance at the Anatomy Laboratory of Postgraduate Medical Institute Lahore. Detailed morphological study was carried out under a dissecting microscope using a magnification of 10x. Head, ear, eyes, limbs, trunk and tail were carefully seen and compared in different groups of embryos. (Table 2)

Statistical Analysis

The statistical analysis of results obtained regarding the weight of embryos recovered was done using T-Test and F-Test showing analysis of variance. (Table 3)

Table-3 Statistical Analysis of Weight of Embryos

Mean Group	Control	Experimental	Mean Difference from control	Standard error	Computed value of t	Result
A	1.140	1.084	0.056	0.034	1.627	Insignificant
B	1.024	0.987	0.037	0.075	0.492	Insignificant
C	1.006	0.953	0.053	0.056	0.944	Insignificant
D	1.089	0.762	0.327	0.090	3.649	Significant
E	1.013	0.574	0.439	0.057	7.646	Significant
F	1.098	0.081	1.017	0.066	15.485	Highly significant

Discussion

Dissecting microscope study of embryos recovered from the surviving mothers of experimental groups showed that they were negatively affected by this heavy metal only when it was used for prolonged periods during pregnancy especially in the periods of organogenesis. In other words the results obtained were related to the length of the period for which the drug was used. The dwarfism was more marked as the duration was increased. Similar was the case with the weights of the embryos. Weight decreased as the length of period of drug administration was increased.(Fig:1)

In group A_1 embryos neither any morphological abnormality nor drastic change in fetal weight was observed. Group B_1 showed reduction in fetal weight. In-group C_1 the development of ear is affected to some extent. The treatment period was last 6 days of gestation i.e. from day 13-18 of gestation. According to Rugh[15, 16] the development of ear in mouse starts on day 9 of gestation and the maximum development of pinna occurs between days 13 to 16 of gestation.

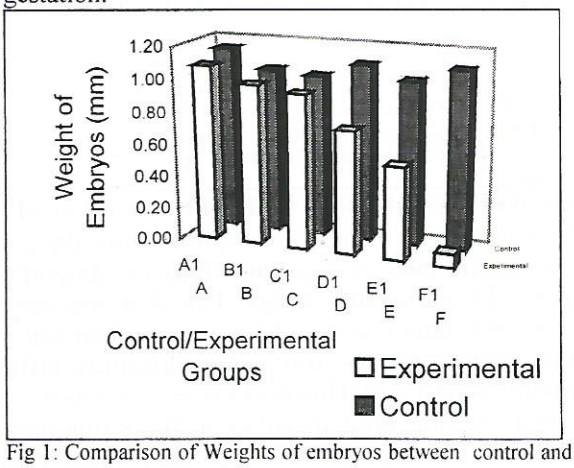

Fig 1: Comparison of Weights of embryos between control and experimental groups

The embryos of groups $D_1$and E_1 showed statistically significant effects on fetal weights. Gross development of eyes, ears and jaws was also affected because both these groups involve the main organogenic period (6^{th} to 12^{th}) of gestation. In none of the previous studies aluminium compounds were used through out pregnancy. In this regard embryos of groupF_1 provide additional information regarding prolong use of aluminium compounds in pregnancy. The animals of this group showed arrested growth and development of gross body features. The comparison of the effect on percentages of weights of various experimental group embryos with average weight of control group is shown in Fig 2.

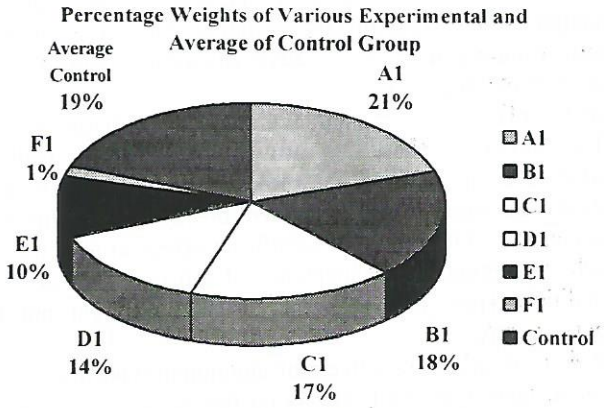

Fig 2: PI diagram showing comparison of the effect on percentages of weights of various experimental with average weights of control group embryos

Conclusion:

The conclusion drawn from the present work is that, the aluminum containing compounds are very hazardous to mammalian embryos specially when they are used during organogenic period. Development of the infants and growing children could also be badly affected by the use of infant formulae as almost all of these contain large quantities of aluminum in their contents.

Table-2 Morphological and Morphmetric Observations:

Division into, head,neck and tail	A	A1	B	B1	C	C1	D	D1	E	E1	F	F1
	Distinct	Distinct	Distinct	Distinct	Distinct	Distinct	Distinct	Distinct	Distinct	Distinct but neck properly formed	Distinc	Distinct but neck properly defined
Head												
Forebrain bulge	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Moderate	Absent	Very prominent
Midbrain bulge	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Moderate	Absent	Very prominent
Hindbrain bulge	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Moderate	Absent	Very prominent
Fontanellae	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Small	Absent	wide
Eyes												
Apertures	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical	Round	Elliptical	Round
Eye lids	Well developed	Well developed	Well developed	Well developed	Well developed	Well developed	Well developed	Well developed	Well developed	Not fully developed	Well developed	Rudimentary
Lens	Large	Large	Large	Large	Large	Large	Large	Large	Large	Medium	Large	Small
Ears												
External auditory meatus	Well formed	Well formed	Well formed	Well formed	Well formed	formed	formed	formed	formed	Rudimentary	Formed	Rudimentary
Pinna	Well developed	Well developed	Well developed	Well developed	Well developed	Not fully developed	Well developed	Not fully developed	Well developed	Developed in the form of thickened flap	Formed	Poorly developed in the form of thickened flap
Snout												
Nostrils	Anteriorly placed	Anteriorly placed	Anteriorly placed	Anteriorly placed	Anteriorly placed	Anteriorly placed	Anteriorly placed	Anteriorly placed	Anteriorly placed	Laterally placed	Anteriorly placed	Laterally placed
Lips	Well formed	Well formed	Well formed	Well formed	Well formed	Well formed	Well formed	Well formed	Well formed	Well formed but upper is protuberant	Well formed	Well formed but upper is protuberant
Jaws	well developed	well developed	well developed	well developed	well developed	well developed	well developed	well developed	well developed	developed	well developed	Less well developed
Trunk												
Heart bulge	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Distinct	Indistinct	More distinct
Liver bulge	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Absent	Present
Limbs												
Distinction into 3 parts	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Interdigital clefts	Deep	Deep	Deep	Deep	Deep	Deep	Deep	Deep	Deep	Small	Deep	Very small
Claws	Formed	Formed	Formed	Formed	Formed	Formed	Formed	Formed	Formed	Formed	Formed	Formed
Tail	Almost straight Almost straight	Almost straight	Almost straight	Almost straight	Almost straight	Almost straight	Almost straight	Almost straight	Almost straight	Cranially directed reaching upto snout	Almost straight	Cranially directed reaching upto face

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