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EFFECTS OF SILVER AND LEAD NITRATES ON IMPLANTATION INTERFERENCE, MISCARRIGE AND TERATOGENICITY: A PRELIMINARY REPORT

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ABSTRACT
The present work elucidates the menacing effects of silver and lead nitrates on both the pregnant mice and developing embryos. Consequent upon the subcutaneous injection of different concentrations of $AgNO_3$ and $PbNO_3$, the inseminated females were invariably confronted with episodes like preterm delivery, gastrointestinal complications, fetotoxicity and precocious parturition of congenitally malformed embryos.These stillborns, unlike the control embryos of the same age group, were destitude of eyes, ears and hair follicles. They ultra structurally displayed the presence of aberrant hepatocytes and renal corpuscles. The oral administration of these compounds to mated females generated a panoplyof augmented phenomena including implantation failure and disanchorage of placenta. Several mated mice developed intestinal neoplasia, lung tumor and other visceral anomalies.

INTRODUCTION

Occupational and environmental exposure to and dietary intake of various compounds not only affect the maternal reproductive physiology, but also generate congenital malformations of embryos (Fraser, 1990). Mounting incidences of miscarriages, stillbirths and parturition of structurally deformed and mentally retarded children are noticed among women who work in industries handling textiles, dyes, lead, mercury, cadmium, etc (Gilbert, 1997). Such maternally intruded materials, which inflict injury upon the developing embryos,are known as teratogens (Moitra *et al.*, 1997). The Greek word '*Teras*' means a prodigy or a monster. Although only some 10% of congenital abnormalities are thought to be caused by teratogens (Brent, 1995), yet heavy metals, such as silver and lead, are widely used in our day-to-day life from medicines to commodities.

Silver salt containing drops are instilled into the eyes of the neonates for preventing them from contracting gonorrhoea from affected mothers, whereas lead compounds for the treatment of a variety of neurological and gastrointestinal complications (Boericke and Boericke, 1927). From the perspective of reproduction, detrimental effects of lead, as also silver, include infertility, depressed libido, miscarriages and premature delivery in human females besides embryotoxicity and foetal malformation (Winder, 1993). Moreover, concomitant with the increase in the concentration of lead in blood among the first trimester carrying women, there is a higher risk of preterm birth (Vigeh *et al.*, 2009).

Interestingly enough, from their investigation assessing comparatively the effects of four essential (Cu, Mn, Fe and Zn) and eight nonessential (Cr, Hg, Al, V, As, Cd, Pb and Ag) metals on mouse embryonic development .Hanna *et al.* (1997) reported the embryotoxic nature of these metals and consequent triggering of abnormal development through metal binding to "intracellular ligands, including protein" of less known identity.

All these information prompted us to design experiments on murine model for studying the effects of oral and subcutaneous administration of Ag and Pb nitrates on (a) maternal reproductive physiology, (b) embryo toxicity and(c) the appearance of novel protein, if any.

MATERIALS AND METHODS

Mature virgin inbred mice (110-125g) were allowed to mate with males at the University animal house ($23 \pm 2^{o}C$) overnight for they copulate at night. Ovulation occurs within a few hours thereafter with consequent fertilization. The age of individual embryos was calculated in accordance with Thelier's convention (Slack, 2006). Each inseminated female was singled out next morning on the basis of the criterion of the presence of a "vaginal plug", a solid white deposit over the external genitalia.

Subcutaneous injection experiment

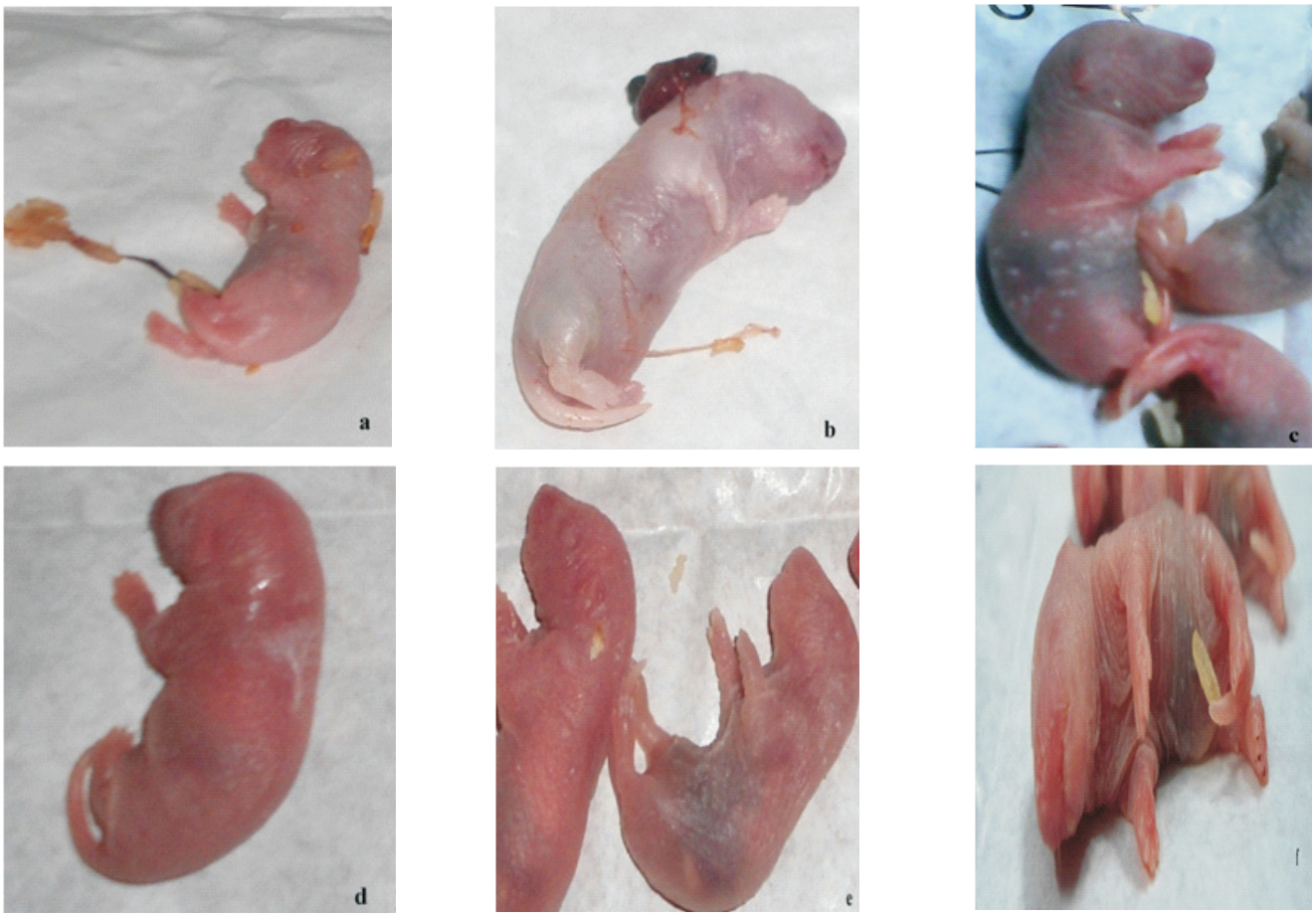

Figure 1: Silver (a, b and c) and lead (d, e and f) nitrate exposed 16-day old embryos.Maternal exposure to $AgNO_3$ (a) at high dose generated stillborn embryo with intact placenta attached to umbilical cord, (b) at intermediate concentration produced embryo with flipper like blunt limbs and slender umbilical cord detached from placenta and (c) at low dose demonstrated palpable impression of digit formation, with hitherto incomplete apoptosis (syndactyly)in the conceptous. (d) high dose lead produced embryo with blunt snout and completely detached umbilical cord. (e) intermediate lead concentration was instrumental to phalanx development in limbs. (f) mild hydrocephaly, besides multiple skin creases (*cf.* Table 1 and Figure 2c). mimicing "michelin tire baby syndrome", characterize the low Pb exposed embryo

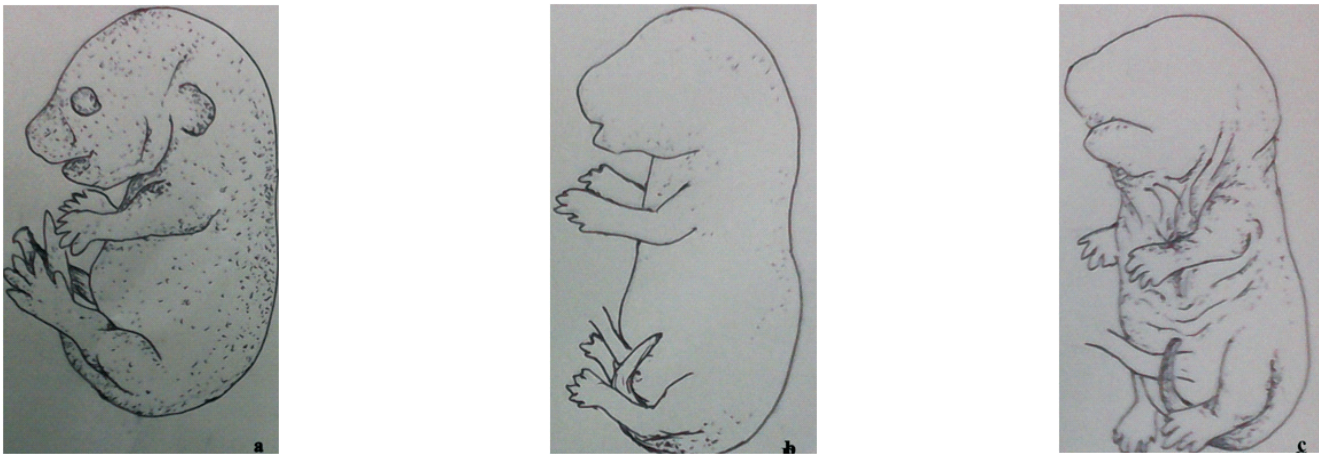

Figure 2: Comparative depiction of 16-day embryos in sharp contrast to (a)the control,both (b) silver and (c) lead nitrate exposed conceptuses are destitute of eyes,ears,nasal pits and hair follicles. Epidermolysis bulbosa, manifesting loose skin creases(*cf.* Figure 1f and Table-1), characterizes $PbNO_3$ exposure.

Inseminated females (total 28) were divided into 3 groups: control (4), $AgNO_3$ exposed (12) and $PbNO_3$ exposed (12). Three different concentrations: high, intermediate and low, were made by dissolving 30, 20 and 10 mg respectively of

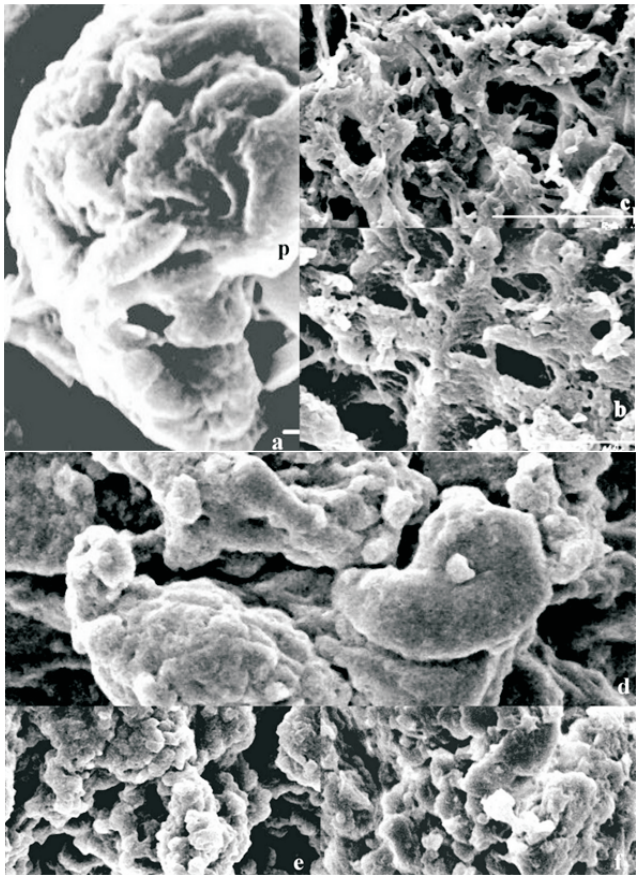

Figure 3: Scanning electron micrograph of 16 day foetal tissue following the maternal exposure to silver and lead nitrates (a) Control,a renal corpuscle with podocyte (P) and surrounding urinary space (white arrow). (b) The comb like structures that come in contact with basal lamina represents interdigitating pedicles , called filtration slits. Podocytes are slightly distorted and confluent ($AgNO_3$). (c) $PbNO_3$ obliterated many filtration slits, juxtaposing several podocytes together. (d) $AgNO_3$ treatment reduced volume of individual glomerulus, increased spaces in renal cortex exposing several displaced interwoven convulsed tubules (T). (e) Silver salt treated liver showing increase in spaces within hepatic parenchyma, though hepatocytes are apparently less affected. (f) Lead treatment often adheres neighbouring Hepatocytes (H) together

$AgNO_3$ in 100 mL Mili –Q water separately. The same individual dilutions were also made for $PbNO_3$ also.

As regards the subcutaneous $AgNO_3$ investigation for each concentration each of the four mice was injected subcutaneously with 0.1mL carrier solution of $AgNO_3$ daily for 14 days. The same injection regimen was adhered to for $PbNO_3$, too for all the three concentrations,

The experiment was terminated on day 16, except where miscarriage had occurred prior to, or on day 16.The females were anesthetized and killed for visceral examination. The fetuses, irrespective of stillborn or alive, were recovered for investigation (Table 1).

Oral administration experiment

Three different concentrations,*viz.*, 80, 50, and 20 μg/dl of $AgNO_3$, as also $PbNO_3$, respectively were prepared. The concentrations of silver and lead salts were calculated on the basis of the embryo toxicity test employing twelve metals on peimplantedmouse embryos (Hanna *et al.,* 1997) with a view to achieving implantation failure.

Each of the 22 females was fed by buccopharyngeal insertion of blunt pipette draining 0.1ml of carrier solution gently on day 2, day4, and day 6 after coitus. Since the uterus is competent to accept embryos for implantation within a short period of 4 days from mating (Slack 2006), all treated females were sacrificed on the day 7 postcopulation to ascertain whether the implantation had occurred or not. In the event of death of animals, the respective abdomen was cut open for examining the viscera within and the implantation status (Table 2).

Scanning electron microscopy

Fetal renal and hepatic tissues were fixed in 2.5% glutaraldehyde in 0.2M cacodylate buffer (4 hours), thereafter in 1% OsO_4 (4hours), dehydrated (including CPD), stub mounted, gold coated and finally examined under scanning electron microscope at the University of Burdwan Science Instrumentation Centre(Hoppe, 1981).

SDS PAGE and protein profiling

For analysing the protein mixtures obtained from liver of mated females with implantation failure (Table 2) along with those from the control ones, the proteins obtained from the liver were individually boiled and treated sample buffer containing SDS and â-mercaptoethanol. The samples were loaded along with molecular weight markers (protein ladder) in gel and electrophoresed. The proteins were visualised with Coomassie Brilliant Blue R-250 (CBB) stain (Wilson and Walker, 1997; Koner *et al.,* 2012).The Ag and Pb induced proteins, against the control ones (Fig. 5) were analysed in GEL DOC. The Rf value was plotted on semi-log paper. Unlike other bands, the proteins induced by both silver and lead nitrates are unique for having a molecular weight of 64.5 KD (Table 3)

RESULTS AND DISCUSSION

Despite their massive uses in pharmaceutical, aesthetic and dyeing industries, both silver and lead are implicated in occupational hazards (Gilbert, 1997: Moitra *et al.*, 1997). Their unscrupulous human applications on one hand suppress libido, reduce sperm motility and number, and cause chromosomal aberrations and on the other hand give rise to infertility, premature delivery, miscarriage, implantation failure, pre-eclampsia, stillbirths, fetotoxicity, and even the parturition of malformed babies (Winder, 1993). We report the detrimental effect of silver and lead nitrates on maternal gestational physiology and teratogenicity.

Subcutaneous Injection Experiment

In contrast to the normal 21 day gestation period, the subcutaneous injection of different concentrations of each salt (Table 1) into inseminated females (i) causes preterm birth between 14 and 16 days post fertilisation and (ii) generates congenitally malformed embryos. The higher concentrations cause greater prenatal mortality whereas the low dosage litters are born to die in about an hour postnatally in the present experimental set up exemplifying dose-response relationship (Paul *et al.,* 1999).

Table 1: Effects of maternal subcutaneous injection of solution of silver and lead nitrates on inseminated females and developing embryos

	concentration of solution	Day following Coitus	Attributes of developing embryos, f etus and pups
Control (4)		16 (2)^{k}21 (2)	Normal growth, eye, external ear, nasal pits, fingers and toes formed typically (Figure 2a)The litters were allowed to grow to term, normal pups.
$AgNO_3$ (12)	High (4)	15 (3)^{s}16 (1)^{s}	Stillborn with completely dislodged placenta with umbilical cord attached to foetus (Figure 1a)Foetus with complete placenta and umbilicus, but without eye, pinna and nasal pits, reduced glomerulus (Figure 3d)
	Intermediate (4)	15 (3)^{s}16 (1)^{s}	All these fetuses mimic the high concentration situation, the termini for and hind limbs exhibit skewed apoptosis with serration (Figure 1b), Confluent hepatocytes (Figure 3e)
	Low (4)	15 (1)^{s}16 (1)^{s}16 (2)^{k}	All foetus posses stumpy flipper like limbs with the impression of phalanx development, but yet maintaining syndactyly (Figure 1c). Mild posterior regression (Figure 2b)Several hepatocytes are coalescent (Figure 3f)
$PbNO_3$ (12)	High (4)	14 (1)^{s}15 (1)^{s}16 (2)^{s}	Preterm birth (shortening of gestation period) with complete detachment of umbilical cord (Figure 1d). Due to retrograding development other external features mimic normal 12d embryos, ultrastructurally the renal cortex indicates filtration constrain (Figure 4c)
	Intermediate (4)	15 (1)^{s}16 (1)^{s}16 (2)^{s}	Limb-lengthening nominal, but the distal ends with stumps of phalangeal ends (Figure 1e)
	Low (4)	16 (1)^{s}16 (3)^{k}	Torso with ventrally preponderant loosened skin folds (epidermolysis bulbosa) (Figure 1f). Blunt snout, mildly hydrocephalic (Figure 2c)

Number of animals/foetuses is within parenthesis. The superscripts 's', 'k' and 'p' stands for specific day post-insemination when still birth, killing and parturition respectively had occurred.

Table 2: Effects of oral administration of silver and lead nitrates on inseminated female mice and the implantation status.

	Control/concentration of salt solution	Effects on experimental females	
		Died on day 7	Killed on day 7
Control(4)		None	None
$AgNO_3$(9)	High(3)	2 died on day 4 NAD	1 showing lung tumour (Fig. 4a)
	Mid (3)	1 died on day 4 with cyst in the liver (Figure 4c)	2NAD
	Low(3)	None	3 NAD
$PbNO_3$(9)	High(3)	1 died on day 4 with neoplastic growth in intestine (Fig.4b).1 on day 6 NAD	1 with slight hepatic enlargement.
	Mid (3)	1 died on day 6 with mild liver enlargement.	2NAD
	Low(3)	1 died on day 6 with hepatic enlargement.	2 NAD

All the animals received injection on day2, day4, and day 6 postcoitus. There was implantation failure in all the experimental animals. The control ones sacrificed on day 7 had normal implantation. NAD stands for "No apparent defects".

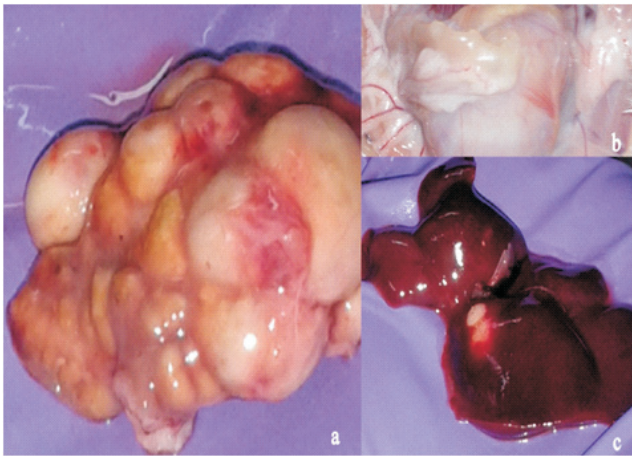

Figure 4: Effect of oral administration of silver and lead nitrates on the viscera gravid female. (a) Tumour formation near lung (Ag treated). (b) Neoplasic growth near small intestine (Pb treated). (c)Cyst formation in liver (Ag treated).

As compared to normal 16-day embryos (Fig. 2a) characterized by the presence of eyes , external ears , nasal pits , fingers and toes and prominent hair follicles, the Ag and Pb exposed ones (Fig. 2a and 2b) of the same age are devoid of all of these features. The teratogenic effects of these extrinsic chemicals are responsible for decelerating morphogenesis (bradykinesia). This procrastinating apoptosis is apparent since dactylogenesisis yet incomplete in their limbs. The Ag exposed embryos possess smooth skin whereas the Pb ravaged ones have several skin folds particularly on the torso ventrally (Fig. 1f and 2c).

Scanning electron microscopy reveals considerable ontogenic anomalies in the fetal kidney (Brenner, 2008) and liver inflicted by these metallic compounds. Both these agents produce ill developed glomeruli (Figures 3b and 3c) vouching for hypoplastic renal corpuscles which are surrounded by increased urinary spaces (Figure 3d).The hepatocytes exhibit kinky outline. These may be scattered or regionally confluent along with hollow spaces in liver parenchyma (Figures 3e and 3f).

These two chemicals, as such, not only lead to still birth or abortion , but also do indeed exert teratogenic influence on generating dysmorphogenesis in embryos,with the incidental birth of malformed pups.

Oral administration experiment

This satellite investigation was geared for exploring if the oral

Table 3: Estimation of molecular weight of individual bands on the SDS PAGE

Log of molecular	weight	Rf values	Antilog molecular	weight	(actual molecular weight)
·	1.72	·	0.357	·	52.48
·	1.20	·	0.732	·	15.99
·	1.81	·	0.290	·	64.50*
·	1.34	·	0.625	·	21.88
·	1.67	·	0.393	·	46.77
·	1.54	·	0.482	·	34.67

* induced protein / band

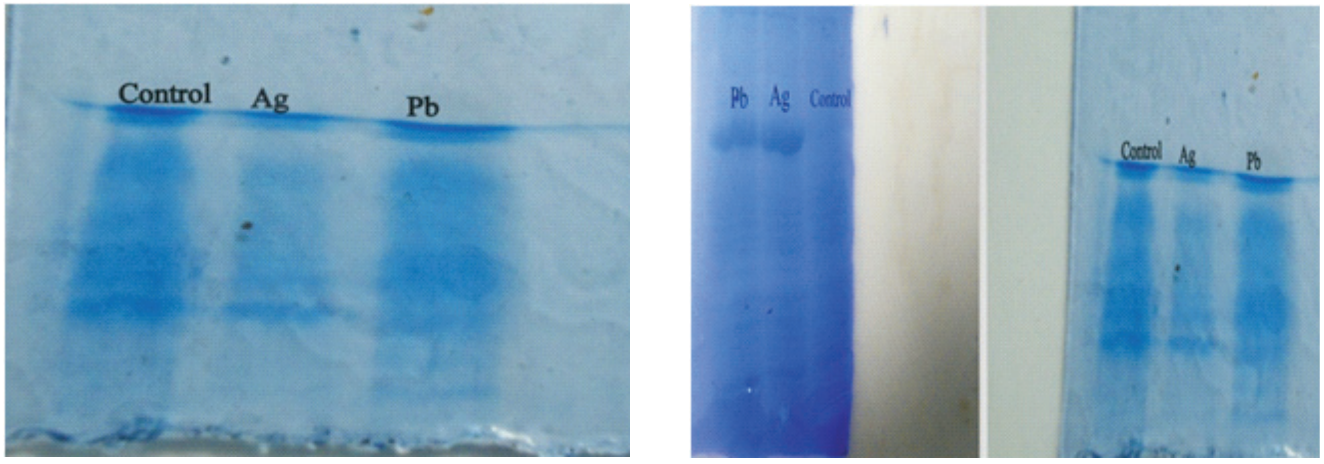

Figure 5: Pb-Induced protein band, Ag Induced protein band, Control-Absence of induced protein

engulfment of these chemicals by the inseminated mice mimics human females as regards faulty implantation along with the presumable production of induced protein.

The present experiment (Table 2)deals with the oral feeding of $AgNO_3$ and $PbNO_3$ on day 2,day 4,day 5and day 6 and sacrificing these females on day 7 postmating.The chronology of this experiment was so designed because implantation occurs within 4 days of coitus (Slack, 2006). Apart from implantation failure, the chemicals are harmful to the female. Death ensued in many of them. Those few surviving ones exhibited pulmonary tumour, intestinal neoplasia and hepatic cyst formation (Fig. 4a, 4b, 4c).Interestingly enough, the exclusive emergence of heavy 64.5 KD proteins only in cases of " no conceiving status" of mated mice (Table- 3) may accuse this hitherto unknown protein of impeding implantation. Hanna *et al* . (1997) using four essential and eight non essential metals on mice have suggested that metal binding to some protein of unknown identity or ligands may disrupt the implantation of blastocysts to endometrium.

Global literature is replete with a wealth of information on maternal exposure to chemicals, viruses, bacteria and radiation leading to the malformed progeny (Gilbert, 1997). Although there is no growth retardation (Paul *et al.*, 1999), both the metallic salts drastically affect the development of sense organs.As compared to the controlled ones, all fetuses of the same age (day 16) are without eyes, external ears and olfactory pits (Fig. 2b and 2c). This delayed organogenesis parallels cortisone induced decelerated palatal closure leading to cleft-lip and cleft-palate in mice (Fraser, 1990). Numerous skin creases on the torso of Pb-afflicted fetal mice dramatically (Fig. 1f and 2c.) resemble those human beings affected with "Michelin tire baby" syndrome (Mange and Mange, 1994).Whether the aetiology of the comparable conditions crops from same genomic homology is a matter of contemplation and future investigation. Several clinical stigma of human Down syndrome caused by trisomy of chromosome 21, are produced by the chromosome 16 trisomy of mouse as the human and mouse genome projects corroborate (Weaver *et al.*, 2005). An insight into the phylogeny and homology of genomes paves way to a fascinating and emerging vista in developmental biology.

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REFERENCES

Boericke, W. and Boericke, O. E. 1927. Homeopathic Materia Medica; ISBN 0766183882.

Brenner, B. M. 2008. The Kidney. 8th edition Saunders, Philadelphia. Brent, R. L. 1964.

Brent, R. L.1964. Drug testing in animals for teratogenic effects: Thalidomide in pregnant rat. *J. Pediat*. **64:** 762-771

Fraser, F. C.1990. Of mice and children: Reminiscence of a teratogeneticist. In: Issues and Reviews in Teratology. **Plenum Press**,

New York. **5:** 1-75.

Gilbert, S. F.1997. Developmental Biology. *5th edition. Sunderland, Massachusetts.*

Hanna, L. A., Peters, J., Wiley, L., Clegg, M. and Keen, C. 1997. Comparative effects of preimplantation mouse embryo development in vitro. *Toxicology*. **116:** 123-131

Hoppe,U.1981. Threedimensionalelectronmicros copy, *Ann. Rev.* Biophysics. *Bioengineering.* **10:** 563-571

Koner, A, Nandi, N., Chakraborty, S. and Chakraborty, M. 2012. Effect of Homeopathic Potency using eukaryotic system. *WJST ISSN*: 22312587. **2(2):** 37-42.

Mange, E. J. and Mange, A. P. 1994. Basic Human Genetics. Sunderland, Massachusetts.

Moitra, P. K., Paul, K., Maity, C. R. and Ghosal, S. K. 1997. Teratogenic effects of uranylacetate on developing chick embryos. *Ind. J. Physiol. Allied Sci.* **51:** 65-71.

Paul, K., Moitra, P. K., Mukherjee, I., Maity, C. and Ghosal, S. K.1999. Teratalogy of arecolinehydrobromide on developing chick embryos: A preliminary report. *Bull. Environ. Contam. Toxicol.* **62:** 356-362

Slack, J. M. W. 2006. Essential Development Biology, 2nd. Edition. Blackwell. London.

Vigeh, M., Yokoyama, K., Seyedaghamiri, Z.,Shinohara, A., Matsukawa, T., Chiba, M. and Yunesian, M. 2010. Blood lead at currently acceptable levels may cause preterm labour. *Occup, Environ. Med (published online on 26th August).* **10:** 1136

Weaver, R. F. 2005. Molecular Biology 3rd edition, Mc Graw Hill.

Winder, C. 1993. Lead, reproduction and development. *Neurotoxicol.* **14:** 303-318.

Wilson, K. and Walker, J. 1994. Practical Biochemistry: Principles and Techniques. *4th edition. Cambridge Univ. Press.*