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Lead Toxicity and Postnatal Development of Gastrointestinal Tract

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Abstract:

The present review deals with the maternal transfer of lead through the placenta during pregnancy and its deleterious effects on developing gastrointestinal tract. Exposure to lead is more dangerous for young and unborn children. It is well known that lead passes through the placenta of mother to fetus and accumulates in fetal tissues during gestation and it can be obtained through the milk during lactation .Lead may be released from maternal bone reserves during pregnancy and thus it becomes a major source of intoxication for the fetus. Prenatal exposure to low lead levels may increase the risk of reduced birth weight and premature birth. Lead is poorly absorbed from the GI tract; however, toxic effects can result from the relatively small amount of lead that is absorbed. There have been very few studies designed to evaluate consequences of lead toxicity during developmental stages, although this is the most sensitive period. The absorption of lead is influenced by many factors such as chemical form of the lead, diet, presence of food in GI tract, calcium status, vitamin D and iron. The mechanisms by which these interactions occur and induce histopathological changes in developing fetus are not fully understood. This reflects a lack of understanding of the mechanism by which lead is absorbed in the GI tract of mother and influence the developing fetus during gestation and lactation. Although threshold levels have been derived from the animal data, factors such as size, relative difference in consumption in proportion to size especially during infancy, and variable histopathological changes in GI tract are not well investigated during different developmental stages.

Keywords: Swiss mice, development, lead acetate, histopathology and gastrointestinal tract

1.0 Introduction:

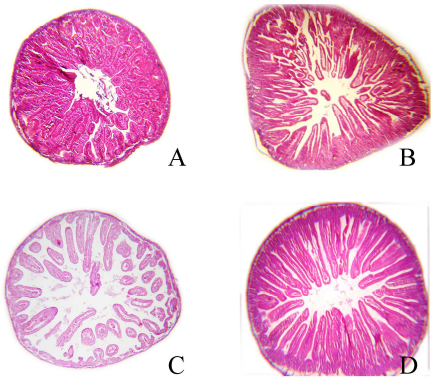
Environmental toxins are chemicals and other materials created largely from industry and carelessness. While a very large number of environmental toxicants are potentially harmful to health, the most commonly studied ones can be divided into three major categories: heavy metals, air pollutants, and pesticides. Environmental toxins that are internalized by skin absorption or by inhalation may be secreted into the lumen through the biliary system and lead to toxicity. Toxins suspended in air make their way into the intestinal tract by drainage from the sinuses into the pharynx and esophagus. Exposure to chemical agents at critical periods of development may cause some permanent change in the histology and physiology of various organ systems in organisms (Sharma and Mogra, 2014). A thin preepithelial water layer ("unstirred water layer") and a mucous layer cover the intestinal mucosa and limit absorption to toxins that can diffuse. The rapid turnover of the intestinal mucosa helps to protect the mucosa and the body against toxic injuries. The lower two thirds of the crypts form the proliferative compartment of the mucosa and, because of their location, are protected from the reach of toxic substances. The rapidly growing fetus is susceptible, but the placenta acts as barrier. Although transplacental transport of environmental toxins, such as lead and mercury, is recognized, toxins in amniotic fluid, such as nicotine and cotinin, have been poorly studied for possible absorption by either the skin or the GI tract (Van-Vunakis et al., 1974). The disposition of drugs and other environmental toxins varies at different stages of child development.

Distribution of lead in the environment has potential to affect a large number of people thus, its

developmental toxicity and neonatal histopathological investigations are of great importance. It is important to recognize that dose-response relationships in developmental toxicity can be intricate by multiple competing endpoints, such as gestation length, litter size, reduced fetal weight, disruption of fetal development and fetal death (Sharma and Mogra, 2013). Apart from these developmental disorders lead also affects the cellular structures of all growing organs leading to physiological disturbance in the body. One of the most important systems which are affected by lead is GI tract because ingested lead is directly absorbed through it. Lead causes significant changes in duodenal cell proliferation and differentiation during development (Sharma and Barber, 2012).The present study focused on the impediment in development of gastrointestinal tract in animals, exposed to lead through gestation and lactation.

2.0 Lead toxicity:

Most heavy metals are not physiologically or biochemically essential to an organism. In many cases they are extremely dangerous, as they are easily absorbed and remain in tissues for a long time (Amdur et al, 1991).Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The toxicity of metals changes with their form, solubility, and ability for transformation.



T.S. of duodenum in Swiss mice.

A. Control group on postnatal day 1
B. Control group on postnatal day 21
C. Lead treated duodenum on postnatal day 1
D. Lead treated duodenum on postnatal day 21

Figure 1

The ability for accumulation is strictly dependent on the presence of binding sites in the tissue and the metal's ability for binding to legends present in the cells. The degree to which a system, organ, tissue, or cell is affected by a heavy metal toxin depends on the toxin itself and the individual's degree of exposure to the toxin. In recent years, a wide body of evidence involvement of oxidative stress in metall-induced toxicity has been reported. The enhanced generation of highly reactive oxygrn species, such as hydroxyl radical (HO), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and lipid peroxides (LPO), between heavy metals is known to cause lession in various cellular components including lipids, proteins, and DNA (Halliwell et al., 1989).

Lead is an indestructible heavy metal that can accumulate and linger in the body. Although the problem of lead exposure has been reduced in the United States, minorities and disadvantaged individuals remain chronically exposed. In developing countries, occupational and environmental exposures still exist and are a serious public health problem. International groups, such as the World Health Organization, are working to increase international awareness of lead exposure issues and abatement programs. In 1998, the U.S. National Center for Environmental Health identified childhood lead poisoning as one of its five global priorities. According to the World Health Organization, fifteen to eighteen million children in the developing world have suffered permanent brain damage as a result of lead poisoning. The Occupational Safety and Health Administration identified over 120 occupations in which workers may be exposed to lead (WHO, 2000). Based on epidemiological and experimental data, the Working Group of the International Agency for Research on Cancer (IARC) concluded that inorganic lead compounds are probably carcinogenic to humans (group 2A) (DEFRA, 2002).Lead toxicity can affect organ system. Environmental lead exposure remains a serious concern for the growth and development of children. Exposure of lead during pregnancy is one source from which a fetus can be exposed to lead. Mice exposed to lead continuously beginning at approximately 6 days prior to birth, showed significant decrease in their blood lead level 2 weeks after weaning, despite continued exposure to adult. Their result suggests maternal transfer of lead is more efficient than oral adult exposure and

substantial lead transfer occurs both transplacentally and lactationally. Acute exposures may lead to nausea, vomiting, and diarrhea and may be difficult to identify, as infectious causes are more common. However, additional features, such as excessive drowsiness, involving other organs should raise suspicion.

3.0 Effects of lead:

The reproduction, development, growth and survival of most species under controlled conditions are adversely affected by lead but its effects are modified by several physical, chemical, and biological factors. The most dangerous effects of lead toxicity occur among children whose mothers exposed to this heavy metal during pregnancy and lactation. The incomplete development of blood brain barrier and greater intestinal absorption of lead makes children more vulnerable to toxic effects of lead as compared to adults. The other important factor is small percentage of dense bone tissue in children due to this the lead remains in soft tissues and does not transfer to bones (knodel,1995).The pregnant female should remain away from lead sources as gestational exposure is more dangerous. It is well recognized that exposure to lead acetate in Swiss mice from 10^{th} day of gestation caused significant decrease in body weight of their pups (Table 1). Several reports have indicated that lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to lead (Mirhashemi et al, 2010). Lead may cause a transient disturbance in the blood-brain barrier during early postnatal growth of rats. This effect is associated with the presence of hemorrhagic lesions, suggesting focal damage to the vessels as an important event in the pathogenesis of lead encephalopathy to suckling rats (Sundstromet al, 1985).Effects in children generally occur at lower BLLs than in adults. There is a wide range of neurological effects associated with lead exposure, some of which may likely be irreversible. Lead exposure can lead to renal effects such as Fanconi-like syndromes, chronic nephropathy, and gout. In a study, conducted by Banu et al (2006) lead induced chronic glomerulonephritis and atrophy in renal tubules in Swiss mice. Most lead-associated renal effects or disease are a result of ongoing chronic or present high acute exposure or can be a latent effect of chronic past lead exposure. Other potential health effects of lead are currently being studied. Lead toxicity can manifest itself with GI effects. The gestational and lactational exposure of lead induces alteration in the basic precursors of developing GI tract which in turn interfere with the absorption of food in early stages (Sharma and Barber, 2012). Increased levels of lead affect the smooth muscle of the GI tract, producing a vague abdominal syndrome, which is manifested by anorexia, nausea, cramping, and a metallic taste in the mouth.

Table 1: Effects of various levels of lead exposure on body weight of pups during postnatal period

Treated groups	Postnatal days			
	1	7	14	21
Control	1.995±0.1338	5.1±0.1364	8.955±0.1877	12.9075±0.6869
8 mg	1.6025±0.0737 An.s.	3.155±0.6320 A**	6.29±0.2421 A**	8.17±0.2152 A**
16 mg	1.24±0.0548 A*	2.755±0.1473	5.98±0.4824 A**	6.5575±2.9892 A**
32 mg	1.0925±0.0754 A*	2.705±0.1593 A**	4.86±0.1766 A**	4.86±0.2152 A**
Vitamin C	1.67±0.2020 An.s.	4.19±0.1449 A*	8.36±0.1817 A**	11.0975±0.261 A**
Vitamin E	1.7275±0.1996 An.s.	4.545±0.1767 An.s.	8.605±0.1251 An.s.	11.2775±0.314 A**
8 mg + vitamin C	1.43±0.1802 An.s., Bn.s., En.s.	4.025±0.2296 A**, B*, En.s.	7.9375±0.290 An.s., B**, En.s.	10.3775±0.337 A**, B**, En.s.

16 mg + vitamin C	1.3525±0.1992 An.s., Cn.s., En.s.	3.8275±0.301 A**, C**, En.s.	7.795±0.2544 A**, C**, En.s.	10.535±0.3315 A**, C**, En.s.
32 mg + vitamin C	1.2725±0.1943 An.s., Dn.s., En.s.	3.68±0.3346 A**, D*, En.s.	7.46±0.3140 A**, D**, E*	9.59±0.5074 A**, D**, E**
8 mg + vitamin E	1.615±0.1025 An.s., Bn.s., Fn.s.	4.055±0.211 A**, B*, Fn.s.	8.0175±0.206 A*, B**, Fn.s.	10.38±0.3174 A**, B**, F*
16 mg + vitamin E	1.5025±0.1582 An.s., C**, Fn.s.	3.9±0.3201 A**, C**, Fn.s.	7.7925±0.3217 A**, C**, F*	9.9025±0.8418 A**, C**, F**
32 mg + vitamin E	1.265±0.1930 An.s., Dn.s., Fn.s.	3.685±0.263 A**, D*, Fn.s.	7.58±0.4458 A**, D**, F**	9.5925±0.5770 A**, D**, F**

Values are expressed as means ± S.D. for six female Swiss mice/group, P value >0.05 = non significant (n.s.), <0.05 = significant (*) and <0.01 = highly significant (**).
A = compare with control, B = compare with 8 mg lead, C = compare with 16 mg lead, D = compare with 32 mg lead, E = compare with vitamin C and F = compare with vitamin E.

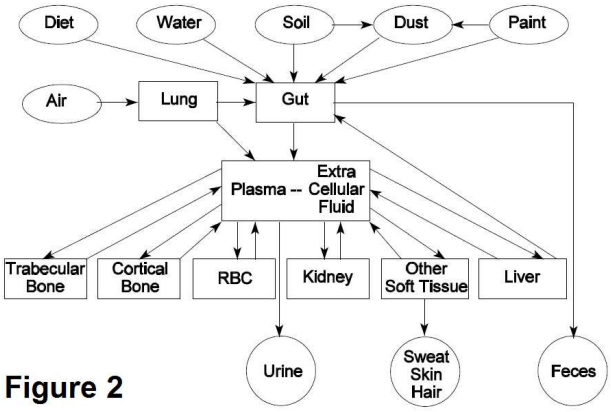


Figure 2

Conceptual diagram: Movement of environmental lead into and through the human body. Source: (EPA, 1986)

4.0 Development of gastrointestinal tract:

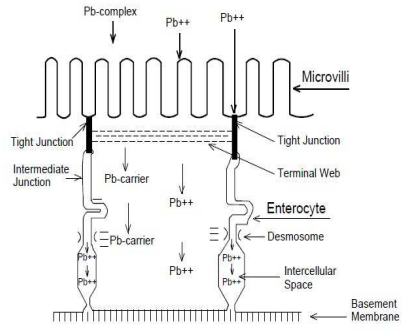
The stomach is divided into glandular and non-glandular components. Non-glandular stomach is lined by keratinized, stratified squamous epithelium and is distinctly separated from the glandular stomach by the limiting ridge. Glandular stomach is composed of rudimentary glandular buds, containing scattered, small cells and mature gastric epithelial cells are not apparent. Although the cells in the rudimentary gastric glands of the perinatal mouse appear immature there is modest differentiated cell function. Gastric gland morphogenesis takes place after birth with secretory function maturing during the first few postnatal weeks (Henning, 1981; Johnson, 1985; Karam et al., 1997; Kataoka et al., 1984). Mucous neck cells migrate through the neck region over a two-week period into the base where upon they differentiate into digestive enzyme secreting zymogenic cells with a life span of several months (Karam and Leblond, 1993).

Morphogenesis of the mouse intestine, however, is not completed at the time of birth but continues into the third postnatal week. During the first 2 weeks after birth the intervillus epithelium develops into crypts, which contain dividing epithelial stem cells at their base. After limited proliferation descendants of crypt cells differentiate into the four principal epithelial cell lineages, Paneth cells, enterocytes, Goblet cells and enteroendocrine cells, which migrate up the villus in a proximo-distal direction until they are extruded near the villus tip (Schmidt et al, 1985). Increased production of epithelial cells results in lengthening of villi until the rates of cell production and cell loss at the apical extrusion zone reach equilibrium in the third postnatal week. The migration of epithelial cells in ordered vertical stripes along the crypt-villus tip axis is also established during this postnatal period (Gordon and Hermiston, 1994). In our own study it was observed that at the time of birth epithelial cells of developing villi were not arranged in regular pattern but with advancing age cells became synchronized. During postnatal development rapid proliferation occurs in crypts and they increase in length along with villi (Fig.1). The cellular changes are apparent from birth to weaning (Sharma and Barber, 2012).Thus, crypts and associated villi constitute the anatomical and functional units in the mature epithelium of the small intestine. The mechanisms underlying intestinal epithelial cell proliferation during normal development are likely to be different from those operating in the proliferative response to injury in the adult intestine. Lipopolysaccharide (LPS), which is sufficient to protect against dioctyl sodium sulfosuccinate (DSS) toxicity associated with a defective proliferative response in the sterilized mouse intestine (Rakoff-Nahoum et al, 2004), and

which induces inflammatory responses in the zebrafish intestine (Bates et al, 2007), did not induce epithelial cell proliferation. The mucosal epithelium of the small intestine rapidly renews and adapts itself after injury (Blikslager and Roberts, 1997). Crypt cell proliferation leading to intestinal growth and promoting re-establishment of mucosal integrity after injury are essential processes for the differentiation, maintenance, and repair of the intestinal epithelium (Quaroni et al, 1999). Epithelial cells of the GIT experience permanent renewal that includes cell proliferation, migration, differentiation, apoptosis, and cell shedding into the intestinal lumen (Bjerknes and Cheng, 2005).

5.0 Absorption of lead:

There are various cellular mechanisms described for the absorption of lead (Fig.3). The first mechanism of lead absorption may be diffusion through lumen of the gut driven by concentration gradient generated by lead ions (Pb2+). It is a passive diffusion and this passive diffusion process requires no energy. It involve either intracellular or paracellular movement of lead across the wall. Paracellular transport occurs across the region between cells called" tight junction". In the second possibility, the lead may enter the gut tissue by pinocytosis. In pinocytosis lead bearing media in liquid micro region of the gut are engulfed by enterocytes. These methods of lead absorption were described by Mushak (1991).



Schematic drawing of the enterocyte showing possible mechanisms for lead absorption. Possible mechanisms include: (1) an active or facilitated component; (2) a transcellular component perhaps involving pinocytotic mechanisms; and (3) a diffusion-driven paracellular route across tight

Figure 3: Source: Mushak, 1991, adapted from Marton et al. (1985).

Lead is more readily absorbed in fasting individuals (up to 45% for adults) than when ingested with food. Absorption is also increased in children suffering from iron or calcium deficiencies. Inorganic lead absorbed into the mammalian body enters the bloodstream initially and attaches to the red blood cell. There is a further rapid distribution of the lead between blood extracellular fluid and other storage sites that is so rapid that only about half the freshly absorbed lead remains in the blood after a few minutes. Inorganic lead ion is not metabolized in the body; it can be conjugated with glutathione (ATSDR, 1993). The concentration of lead in blood is used as a measure of exposure. Therefore, effects of lead cannot be described in terms of route specificity. The major pathway of lead intake, absorption and retention of lead from the gastrointestinal tract varies widely with age, sex, and diet of the organism. Generally, the presence of other metals reduced the magnitude of the lead effect. Reduced efficiency of food conversion may be an indication of improper absorption of nutrients from the gastrointestinal tract or of a metabolic defect at the cellular level. Reduction of ability to absorb the toxic compounds of lead would be accompanied by reduced ability to absorb nutritionally required elements as well. After lead is absorbed into the body, it circulates in t he blood stream and distributes primarily in the soft tissues (kidneys, brain and muscle) and bone (Fig2). Lead toxicity include gastrointestinal disturbances-abdominal pain, cramps, constipation, anorexia and weight loss-immunosuppression, and slight liver impairment (ATSDR, 1993; EPA, 1986a). Prenatal and postnatal development is compromised significantly by the presence of lead in the body. Its absorption may vary depending on dietary factors and the chemical form of the lead.

In neonates, absorption may be different because of the immaturity if their gastrointestinal tract and their large skin surface area in proportion to body weight (Morselli et al, 1980). The gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al, 1978). The gastrointestinal mucosa is very responsive to toxic insults at the time of birth it is highly affected by lead during gestation and neonatal period. The results of histopathological study conducted on pregnant Swiss mice showed that lead induced swelling of keratinocytes and hypertrophy in squamous mucosa with hyperkeratosis in squamous epithelium of the stomach in pups at the time of birth (Sharma et al, 2013). Gastrointestinal absorption in children may be only 30% for lead present in dust and dirt and 17% for lead in paint chips, compared with 50% for

lead in food and beverages. Gastrointestinal absorption in laboratory animals is similar to that of humans. The percentage of lead absorption is higher in case of young in comparison to adult animals and the rate of absorption is influenced by various dietary factors. The laboratory animals absorb lead from the respiratory tract as competently as humans and that the absorption rate is not affected by chemical form or concentration of lead in the air (EPA, 1986a), studies on this subject are available but the data is very limited. The chemical nature of lead does not affect the rate of absorption; either the lead is incorporated in the form of dust, dirt paint or through diet .Lead absorption may increase due to nutritional deficiency and may enhance toxic effects of lead (Mahaffey, 1981). Supplementation with essential minerals may compete for lead during intestinal uptake and thereby decreasing absorption and in turn toxicity of lead. Essential metal and toxic metal often have comparable characteristic, and therefore may take advantage of the transport and binding proteins for essential metals in order to gain right of entry to enterocytes and other target cells (Bridges and Zalups, 2005).Many studies on animal and human beings haves investigated the impact of mineral nutrition on lead absorption and toxicity but the molecular mechanisms of intestinal uptake and transport of lead remain unknown. It has also been suggested that lead uptake may accompany iron absorption through a common gastrointestinal pathway (Tandon et al, 1994). Gastrointestinal lead absorption is thought to be influenced by meal size and total feed intake, as well as nutrient composition (Juberg et al, 1997). The extent of lead absorption in the gastrointestinal tract depends on numerous factors including nutritional factors and the presence or absence of other metals, which interact with lead. In children gastrointestinal absorption of lead is higher and may be a much as 40% gastric acid solubilizes lead salts and lead absorption occurs in the small bowel by both active and passive transport (Ellenhorn and Barceloux, 1988; Tsuchiya, 1986). Poisindex (1994) reported that absorption of lead may be amplified in individuals, who are suffering with iron, zinc or calcium deficiency. Ingested lead probably enters sequentially from gut, to blood, to bone and soft tissue, and by way of the bile to small intestine and finally get out of the body with fecal excretion (De-Michele, 1984) as bile is one of the important routes of excretion. There are many studies in experimental animals which support the hypothesis that gastrointestinal absorption of lead is age related. The mice pups absorb 40-50 times more lead via the diet than the adult mice (Forbes and Reina, 1972; kostial et al, 1978). In mice, iron deficiency increases the gastrointestinal absorption of lead, possibly by enhancing binding of lead to iron binding proteins in the intestine (Barton et al., 1978b; Morrison and Quatermann, 1987). About 90% of ingested lead is eliminated unabsorbed through feces (Tepper and Levin, 1972) and approximately 76% is excreted mainly in the urine. The other routes of lead excretion are gastrointestinal secretions [about 16%] and hair, nails and sweat [<8%].

6.0 Lead and gastrointestinal tract:

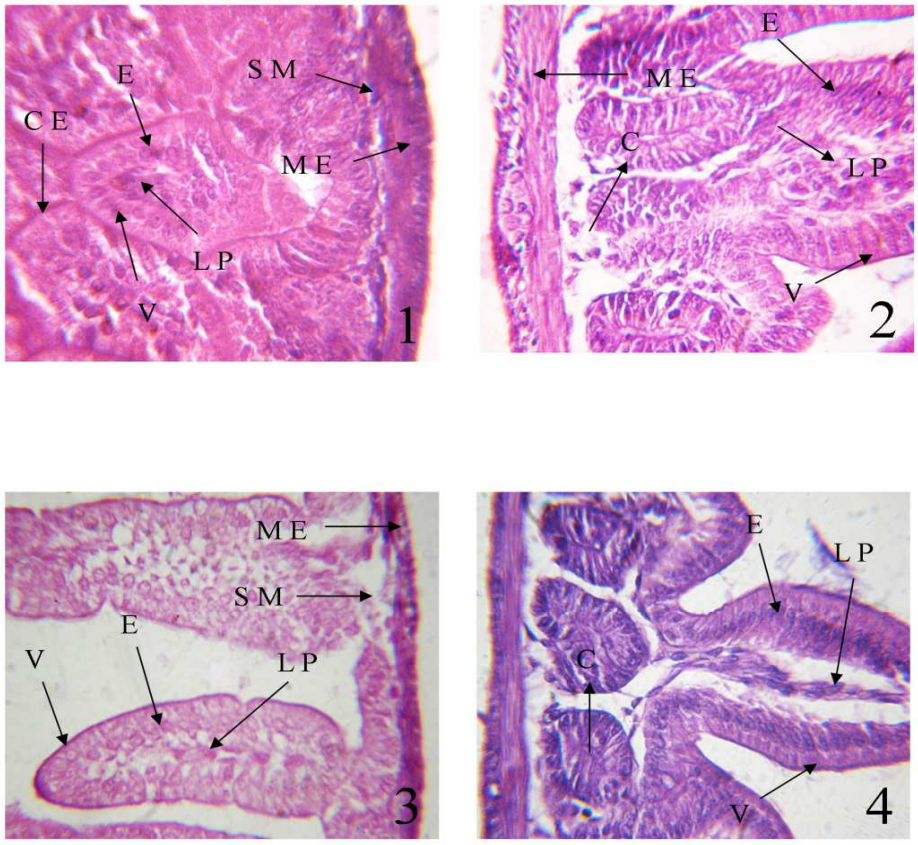
In vitro analysis of the bioavailability of lead via the gastrointestinal tract of the rainbow trout, lead accumulation, composed of lead binding to the mucus, lead in the mucosal epithelium, and lead transported into the blood, was also much lower in stomach than that in intestine. (Ojo and Wood, 2007 Studies in rats indicate that the epithelial cell loss in the healthy GIT is mainly due to apoptosis and not to cell shedding (Hall et al., 1994). Reduced cell shedding (without apoptosis) in the SI of epithelial cells from villi into the gut lumen seems unlikely because of the abrasive effect of nutrient components, which would expectedly have an opposite effect on villus size. The epithelium of the gastrointestinal tract has a highly stereotyped organization with a continuous high level of cell proliferation (Wright and Alison, 1984).

Gastrointestinal absorption of cadmium changes considerably from single to repeated exposures. Exposure to cadmium causes a significant increase of this metal concentration in intestinal mucosa and also an increase in the intestinal MT (Reeves and Rossow, 1996). The results obtained by Groten et al., (1991) supported the hypothesis that ionic cadmium after CdCl2 intake is bound to endogenous MT in the intestine and then transported to the kidney.Usually, the level of cadmium in the intestine wall is not increased much because the epithelium is continuously sloughing (Renata Swiergosz, 2001). In cadmium treated rats the duodenums were enlarged and there was a significant reduction in the percentage of crypts containing Paneth cells. Remaining Paneth cells appeared vacuolated. By both light and electron microscopy changes were noted in the epithelial cells covering the villus tips. It is suggested that these histopathological

appearances will be seen in chronic dietary exposure to cadmium (Phillpotts, I986). Tarasub (2009) treated rats once daily by oral gavage for five days with Cd acetate 200 mg/kg BW. The results showed that Cd treatment could induce the mucosal damages of stomach, villus damage of small intestine and infiltration of inflammatory cells into the lamina propria. These results demonstrate that the gastrointestinal tract was involved in the toxic effects of oral exposure to Cd.

An autoradiogram of the entire small intestine showed that most of the retained Cd was located in the proximal bowel, and distributed uniformly throughout the mucosa and submucosa (Sullivan et al., 1984). Necrosis leading to cell death in the columnar epithelial cells at the tips of the villi in the anterior intestine, and necrosis and shortening of villi in the posterior intestine, were evidently seen in M. tengara due to Cd toxicity (Kothari et al., 1990). More or less similar histological changes due to low concentration of lead acetate on the apical surface of the jejunal enterocytes were studied. The shape of the jejunal villi in poisoned rats was similar to that in non-poisoned rats. A marked feature of the rats' jejunum exposed to heavy metal for 30 days was a rough appearance of the surface villi, probably associated with distortion of the glycocalyx layer. It was sssconcluded that the pronounced toxic effects of lead were related to modification of biochemical properties of the surface coat of enterocytes (Tomczok et al., 1988). Since the gastrointestinal mucosa is the first target organ of lead exposure and intestinal inflammatory cells are responsible for providing protection against pathological damage caused by the toxicity. The developing GI tract is more venerable to lead exposure; in an experimental study conducted on pregnant mice it was observed that there was profound alteration in the developing pattern of duodenum villi during postnatal development in mice (Fig. 1ss and 4). There are many studies which support that mature GI tract is more resistant to lead in comparison to immature. According to Poleksić et al (2006) histological structure of the digestive system is basically, A: Mucosa, which consists of the 1. Lamina epitelialis made up of simple columnar and glandular epithelium, 2. Lamina propria made up of connective tissues, B: Submucosae, consisting of two layers 1: stratum compactum, 2. Stratum granulosum, C: Muscular layer and D: Serosa. This histological structure is not altered even in experiments in which fish were fed with food containing heavy metals (Kruatrachue et al, 2003).

The histological alterations in developing GI tract were studied by Sharma and Barber (2012) during pregnancy in Swiss mice. They observed that on 1 day, the epithelial cells of developing villi were not arranged in regular pattern but with advancing age, these cells and there nuclei were present in synchronized fashion but in lead exposed group the epithelium could not be identified clearly. At postnatal day 21, villi vary in height and appeared more closely packed than in previous stages while in lead exposed group the columnar epithelium which covered villi was not clearly seen and epithelial cells were intermingled with each other. Crypts architecture was largely destroyed and submucosal layer was thickened compared to control and components in both circular and longitudinal layers of muscularis externa were not identified. Sharma et al (2013) also observed degenerative changes in submucosa and distorted muscularisexterna in the stomach during postnatal period in lead exposed Swiss mice. It is also inferred from the present results that exposure to lead acetate during the period of gestation and lactation resulted in teratogenic effects on the developing stomach of neonate Swiss mice and with that of higher dose were more marked. The stomach of lead acetate exposed mollies exhibited irregularity, shrinkage and fusion of stomach microvilli as well as atrophy of the submucosal zone (Mobarak and Sharaf, 2011). It was suggested that lead increases the formation of gastric ulcers by interfering with the oxidative metabolism in the stomach that increased the incidence of gastric ulcer (Olaleye et al, 2007). The implication of this is that lead causes an increase in the formation of free radicals, which, if not mopped up by free radical scavengers, will expose the stomach to inflammation and gastric mucosal damage. These adverse effects of lead as well as its inhibition of enzyme activities (Dai et al, 2009; Abdallahet al, 2010) might be the main inducer of the obtained intestinal histopathological damage of the exposed mollies.



T.S. of duodenum in Swiss mice. **Fig. 1:** Control group on 1 day shows immature villi (V) covered with columnar epithelium (C E), arranged enterocytes (E), undeveloped lamina propria (L P), submucosa (S M) and muscularis externa (M E) are also observed in developing state. **Fig. 2:** Control group on 21 day shows closely packed villi (V), arranged enterocytes (E) and crypts (C) and well developed lamina propria (L P) and muscularis externa (ME). **Fig. 3:** Lead treated duodenum on 1 day shows thickened villi (V) and hypertrophy in enterocytes (E) lamina propria (L P) in narrow space, disrupted submucosa (S M) and muscularis externa (M E). **Fig. 4:** Lead treated duodenum on 21 day shows leaf like villi (V) and shrinkage in lamina propria (L P), necrotic enterocytes (E) and destroyed crypts (C)

Figure 4

7.0 Lead Uptake and Other Factors:

Although lead may be utilize paracellular and transcellular pathways to cross the intestinal epithelial barrier in vivo. Because lead is a toxic, nonnutritive heavy metal, it is unlikely that intestinal absorptive cells possess mechanisms specific for lead uptake; lead most likely uses mechanisms for nutritive heavy metals, such as zinc, to enter the intestinal epithelium. The decrease in total cellular lead content of cells introduced to lead simultaneously with increasing concentrations of zinc suggest that lead and zinc may be competing for the same binding sites on the apical membrane. Additionaly, this decrease in lead accumulation occurred (to 52 and 40% of control for 5 and 10 µM lead, respectively) when cells were exposed to equimolar concentrations of lead and zinc, suggesting a specific mechanism of inhibition. Uptake of lead is time and temperature dependent, involves sulphydral groups, and is decreased by the presence of Zn. Calcium and phosphorus added to rat diets decreased the accumulation of lead in femurs and kidneys (Sobel et al., 1940; Six and

Goyer, 1970). Conversely, low dietary iron has been shown to enhance lead uptake from the gastrointestinal tract in rats (Six and Goyer, 1972). Protein has also been found to affect lead absorption and toxicity, so that rats fed both low-protein and high-protein diets had an increased lead uptake (Milev et al, 1970).

Vitamins C and D may each modify the absorption of lead. Thus a low intake of Vitamin C may enhance the severity of plumbism (Pillemer et al., 1940). Vitamin D added to the diet will increase lead toxicity (Sobel et al., 1940) although this has been denied (Tompsett, 1939). In our own study conducted on Swiss mice it was observed that administration of vitamin C and E with lead during gestation and lactation significantly increase the body weight of pups in comparison to lead intoxicated pups (Tab.1).This may be due to less absorption of lead through gastrointestinal tract of mothers. In the body, calcium binds to lead and inhibits its absorption; therefore, dietary calcium interferes with the absorption of lead through the intestinal mucosa. Dietary deficiencies in calcium, zinc, iron, vitamin E, copper, thiamin, phosphorus, magnesium, fat, protein, minerals, and ascorbic acid increased lead absorption and its toxic effects (EPA, 1985). In contrast to this inference Sharma et al (2013) studied the hematopathological changes in developing red blood cells of neonates of Swiss mice exposed with lead acetate and vitamins through their lactating mothers. They reported that supplementation of vitamin C and E during pregnancy and lactation incapable to ameliorate the alterations in red blood cells generated by lead but they induced negative impact on developing blood cells. Under dietary heavy metal exposure, intestine always exhibited higher burden than stomach. Doses of lead acetate greater than 200 µg/ day decreased the transfer of calcium across the duodenal wall in rats (Gruden et al, 1974). The presence of food alone can impact the absorption lead. Lead ingested on an empty stomach is more thoroughly absorbed than if food is also present. In fasting adults, up to 70% of ingested lead is absorbed; whereas only 10-15% of the lead is taken up when food is co ingested. The increased stomach acids present in the absence of food may be responsible for the increased lead uptake (Hemphill et al, 1991).Protection against various toxic effects of ingested lead was provided by measured dietary supplements of calcium, iron, zinc, ascorbic acid, and vitamin E. Although much is known about the effects of lead toxicity throughout the body, the mechanisms of lead uptake and transport through intestinal absorptive cells are not clear.

8.0 Oxidative stress:

Lead affects mammalian systems by directly lowering antioxidant reserves and generating ROS. These ROS alter cellular membranes and tissue, resulting in vascular, neurological, and genetic damage. Due to lead toxicity in mammalian systems reactive oxygen species (ROS) generates and antioxidants reserves deplete. These ROS manifest in various neurological and genetic disorders especially due to cell membrane damage. The pathogenesis of lead toxicity is multifactorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl protein, alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body (Ercal et al, 2001). Various studies have demonstrated the tendency for lead to catalyze oxidative reactions and generate reactive oxygen species. These reactive oxygen species (ROS) restrain the production of sulfhydryl antioxidants, slow down enzyme reactions leading to impair heme production and cause inflammation in vascular endothelial cells. It also induces damage in nucleic acids and inhibits DNA repair, and ultimately initiating lipid peroxidation in cellular membranes. These extensive effects of ROS generation have been postulated to be major contributors of immense diseases related to lead toxicity. Reactive oxygen species (ROS) have been shown to be to be involved in the etiology of many inflammatory disorders of the gastrointestinal system (Perry et al., 1986; Schmassmann et al, 1997). Like other commonly found, persistent toxic metals – mercury, arsenic, and cadmium – lead damages cellular material and alters cellular genetics. The mechanism all of these toxic metals have in common involves oxidative damage. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage.

Oxidative stress has been shown to be a major causative factor for many diseases, including gastrointestinal ulcers. The hotspot of this study is to examine if the increased susceptibility of the stomach to ulcer after prolonged lead exposure can be explained (partly or totally) by an increased oxidative stress in the stomach (Olaleye et al, 2007).

In our own study conducted on pregnant Swiss mice (Sharma et al, 2013) we also observed degenerative changes in sub mucosa and distorted muscularis externa of stomach during post natal development in pups. The root cause may be the interference of free radicals with oxidative metabolism. Lead toxicity leads to free radical damage via two separate, although related, pathways: (NRCC, 1973) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide, and (Tsuchiya, 1979) the direct depletion of antioxidant reserves (Ercal et al, 2001).Apart from these free radicals nitric oxide (NO) is believed to be involved in the regulation of various gastric functions and in the modulation of gastric mucosal integrity. From diverse experimental reports it is clearly postulated that suppression of NO production by NO synthase inhibitor worsens gastric lesions induced by ethanol in rats (Konturek and Konturek, 1995; Gaboury et al.,1993). Free radicals and nitric oxide could induce damages of microvilli, tight junction between enterocytes and paracelluar junction, which would lead to increased intestinal permeability (Gong et al., 2002). The intestinal mucosa is also vulnerable to oxidative stress and reactive oxygen species (ROS) generated by several conditions, such as ischemia/reperfusion, inflammatory bowel disease (Halliwell et al, 2000). Free radicals or ROS generated during oxidative metabolism can inflict damage on all classes of cellular macromolecular components (e.g., mitochondria, endoplasmic reticulum, protein, etc.), eventually leading to cell death (Bergamini et al, 2004). These reactive oxygen species promote lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA and membrane damage, altered gene expression and apoptosis (Stohs et al, 2000).

Lipid peroxidation has been implicated in the etiology of damage to subcellular membranes and then injury in the cell. Lipid peroxidation (LPO), a result of the reaction of oxyradicals and polyunsaturated acids, has been suggested as an attack factor in the gastric mucosa (Guo et al, 2005). Sass (1970) first reported that lead poisoning in dogs is associated with perforating gastrointestinal ulcers. The acidified ethanol model has been used widely to produce gastric mucosal damage (Anadan et al., 1999). Wapniret al (1977) reported that juvenile rats fed a diet containing 1% lead acetate for 7 wk suffered from malabsorption of certain amino acids, as the intestinal absorption of glycine, lycine, and phenylalanine were decreased. The prevention of lipid peroxidation is essential for all aerobic organisms, and so the organism is well equipped with antioxidants that directly or indirectly protect cells against the adverse effects of xenobiotics, carcinogens and toxic radicals.

9.0 Conclusion:

From the above discussion it can be summarized that distribution of lead in the environment has the potential to affect large number of population thus its developmental and neonatal toxicity is of great importance to study. Lead affects mammalian system by two separate mechanisms, one by depleting the antioxidant reserve in the body and secondly by generating reactive oxygen species. There is direct association of early life exposure of environmental lead and appearance of various disorders in adulthood. As lead freely crosses the placental barrier its gestational exposure induces alterations in developing GI tract which in turn interferes in the absorption of food in early stages of development. The absorption of lead from GI tract and retention in the body varies with age, sex and diet. It can be concluded that lead exposure during the period of organogenesis is lethal to the postnatal development of gastrointestinal tract. Lead is suspected to cause disturbance in hypothalamic pituitary axis indicates that lead exposure during pregnancy places the animal at significant risk.

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