May-July.2012, Vol.2.No.3, 1312-1318. e- ISSN: 2249 –1929

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

Available online at www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT

Research Article

Histopathological alterations in developing duodenum of Swiss mice, exposed to lead acetate

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Received: 5 April 2012; Revised 25 April 2012; Accepted 12 May 2012

ABSTRACT

In the present investigation, Swiss albino mice aged 1, 2, and 3 weeks were studied for the effects of lead acetate on duodenum development. The major objective is focused on the histopathological alterations in duodenum due to lead intoxication. Inbred healthy pregnant females were selected and exposed with lead acetate, 16 mg/animal (533 mg/kg BW) during gestation and lactation through canula. At 1, 7, 14 and 21 day of birth, litters were sacrificed, and their gastrointestinal tracts were fixed. Duodenum was evaluated for the developmental changes and histopathological alterations. Our results suggest that early life exposure may induce changes that will become apparent much later in life. Pups from lead exposed mothers, resulted changes in their developing duodenum and these changes were age related. With the advancing age of pups, the magnitude of damage in developing duodenum was increased. It is concluded that lead is an important toxicant which cause marked changes in duodenal cell proliferation and differentiation during postnatal period.

INTRODUCTION

In the modern society, thousands of hazardous chemicals and heavy metals are being produced and used in a wide variety of work places all over the world. They are taken into the body via inhalation, ingestion and skin absorption.

Lead is one such metal, which is generally considered one of the most toxic to human beings as well as animals. Exposure to lead is more dangerous for young and unborn children. Unborn children can be exposed to lead through their mother. In neonates, absorption may be different because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight[1, 2]; the gastrointestinal absorption of lead is greater in infants and young children[3].

Lead and other heavy metals create reactive radicals which damage cell structures including DNA and cell membrane[4]. The most critical effects of lead toxicity occur among children exposed during fetal

development, postnatal development or both. The postnatal maturing gastrointestinal tract undergoes several changes that may significantly increase the risk of toxicity. Gastrointestinal absorption of lead is influenced by dietary and nutritional and iron status. The GI tract has a uniform general histology with some differences, which reflect the specialization in functional anatomy. During the immediate postnatal period, the GI tract undergoes profound growth, morphological changes and functional maturation.

The extent of lead absorption in the GI tract depends on numerous factors including nutritional factors and the presence or absence of other metals, which interact with lead. Lead is poorly absorbed from the GI tract; however, toxic effects can result from the relatively small amount of lead that is absorbed. A variety of other factors are known to influence the absorption of ingested lead, including the chemical form of lead, the presence of food in the GI tract, diet and nutritional status with respect to Ca, vitamin D and iron[5]; however, for the most part, the mechanisms by which these interactions occur and induce histopathological changes are not fully understood.

The small intestine plays an important role in gastrointestinal absorption of drugs/compounds[6]. Development of the small intestine is comprised of three stages, (1) morphogenesis and cell proliferation, (2) cell differentiation and (3) functional maturation. Cellular proliferation has been studied extensively in animals and in man, usually in the adults[7-9].

The most common acute effect of lead is gastrointestinal colic. This is characterized by nausea, anorexia, vomiting and diffuse abdominal pain. Acute exposures have also resulted in malaise, convulsion, coma, encephalopathy and bradycardia[10-12].

Lead induced oxidative stress contributes to the pathogenesis of lead poisoning for disrupting the delicate antioxidant balance that exists within mammalian cells.

MATERIALS AND METHODS

Random bred Swiss mice were used for this study. Sexually mature males and females, weighing 30 gm were put in breeding cages in the ratio of 1:4 and provided standard diet and water *ad libitum*. The cages were checked every day in the morning and females showing vaginal plug were isolated. The selected pregnant females were divided into two groups and exposed orally by lead acetate (533 mg/kg BW) with the help of Canula.

1. Group 1: Control (distilled water only)
2. Group 2: Exposed of lead acetate (533 mg/kg BW) from 10^{th} day of gestation up to 21^{st} day of lactation.

During the respective tenure of experiment, histopathological alterations in duodenum were observed on postnatal day 1, 7, 14 and 21 from the control and lead treated group.

RESULTS

Control: The duodenum in the mice is structurally and functionally immature at birth and postnatal maturation involves changes in the cell population covering the villi and replacement of the immature epithelium with the mature epithelium. Postnatal maturation of the intestinal mucosa in mice occurs during the first 3 weeks after birth.

In the newborn mice, villi project into a distinct lumen on the surface of mucosa and crypts did not develop until after birth. Submucosa layer was thin. The muscularis externa was thin with connective tissue containing vessels. The outer serosa layer of the wall was represented only by a single layer of myoblasts. At 7^{th} day after birth, villi were increased in size and crypts were formed. Goblet cells and paneth cells were present but they did not comprise a significant population in the intestinal epithelium. The submucosa and muscularis externa showed further development. Villi showed continued growth and differentiation at 14^{th} day of postnatal period, resulting in a decrease in the area that was occupied by the primitive intestinal floor.

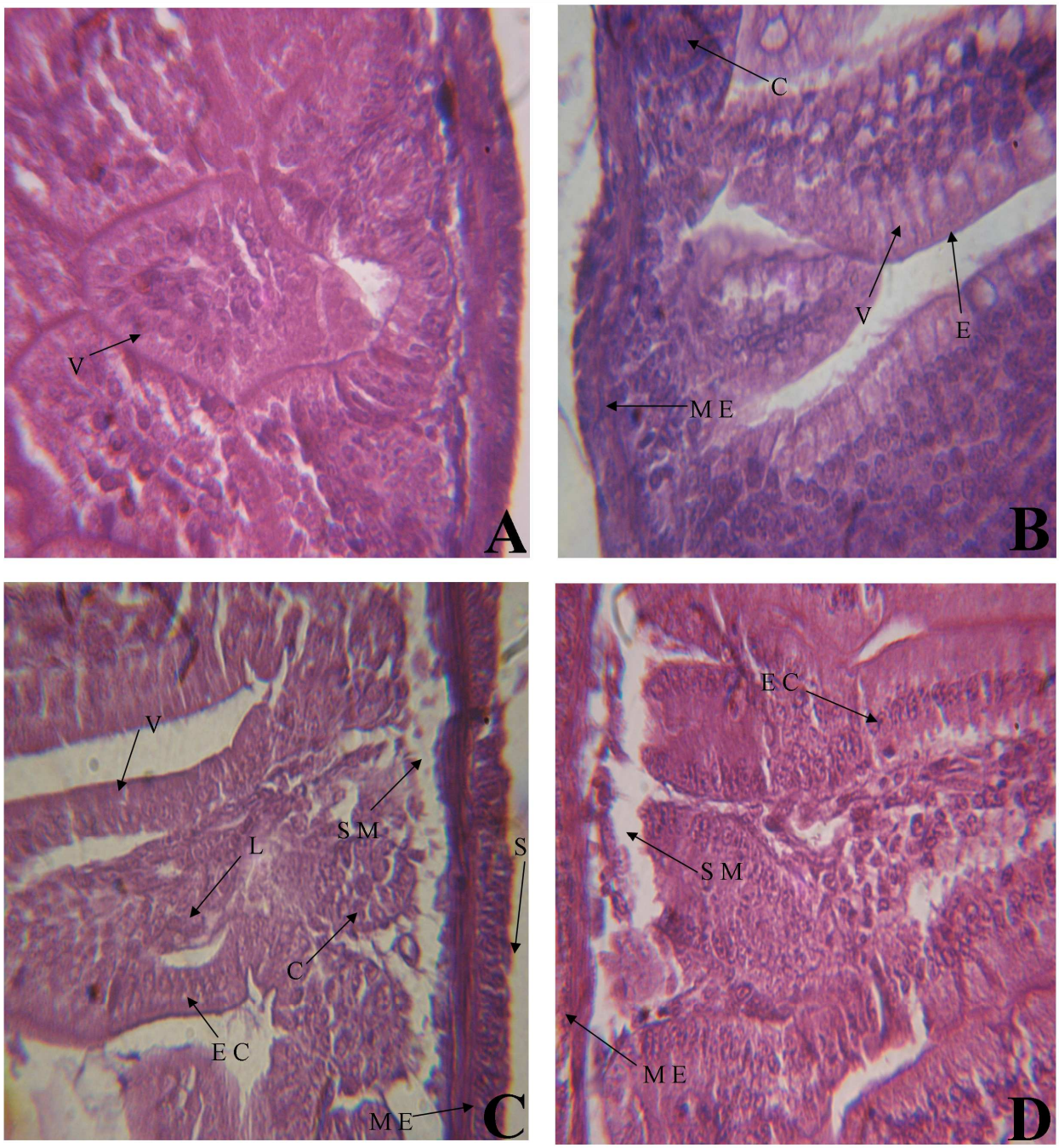

Fig-1: T.S. of control duodenum of Swiss mice. **A.** Postnatal day 1, showing undifferentiated layers of mucosa with villi (V), submucosa, muscularis externa and serosa. **B.** Postnatal day 7, showing villi which covered with epithelium (E), developing crypts (C) formed from the invagination of villi and differentiation of circular and longitudinal layers in muscularis externa (M E). **C.** Postnatal day 14, villi with epithelial cells (E C) shows normal distribution of lacteals (L). Crypts (C), submucosa (S M) , muscularis externa (M E) and serosa (S) also clearly formed. **D.** Postnatal day 21, showing normal architecture of villi with epithelial cells (E C), submucosa (S M), muscularis externa (M E) with longitudinal and circular muscles and serosa layers.

Inside each villus was a network of blood capillaries and a lacteal. Brunner's glands were found in the submucosa layer. The muscularis externa showed definite inner circular and outer longitudinal layers of smooth muscle. At postnatal day 21, villi vary in height and appeared more closely packed than in previous stages. The proliferation of crypt cells showed to be low until weaning commences. Occasional goblet cells were found only in limited numbers and scattered within the epithelium covering villi.

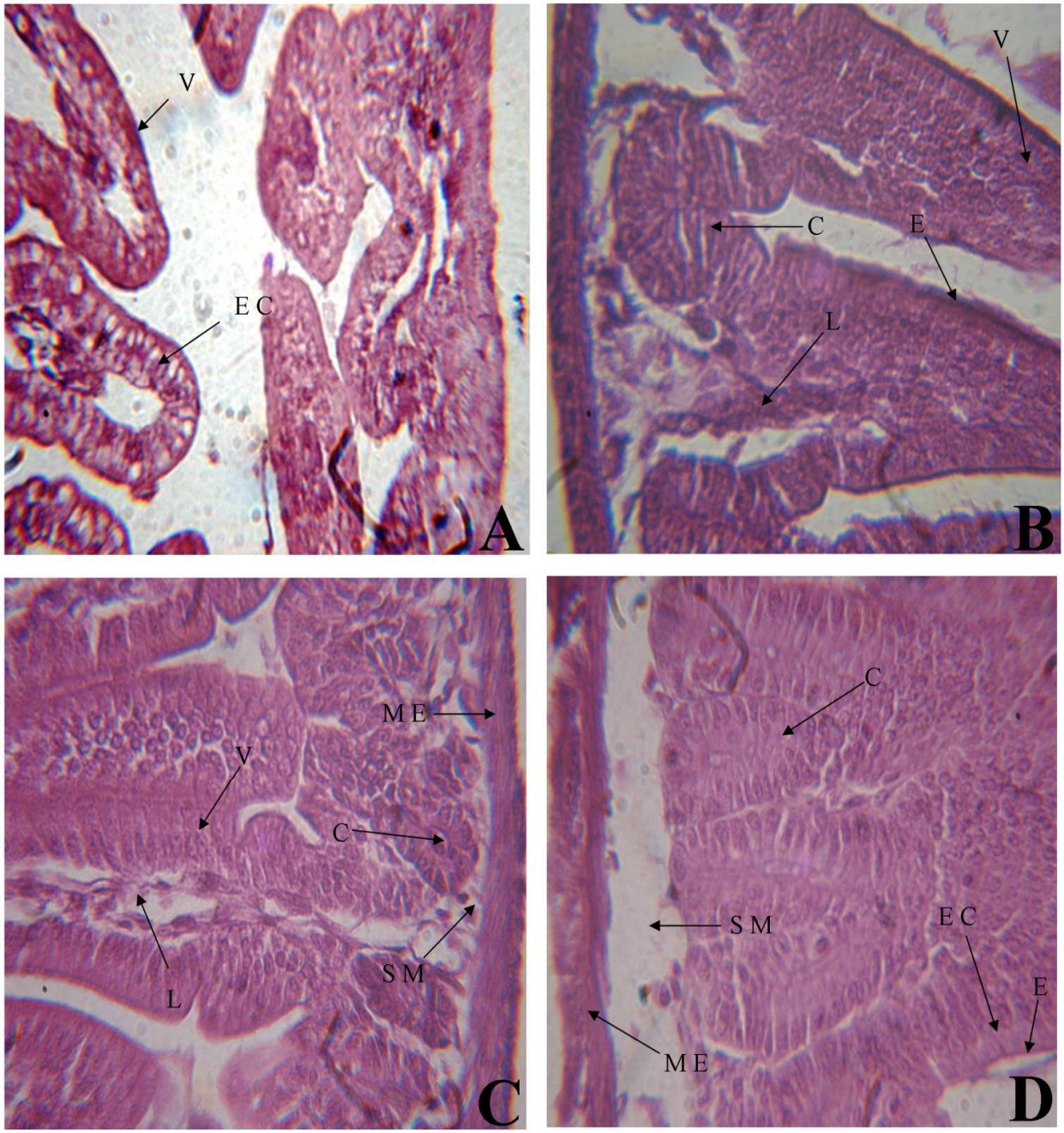

Fig-1: T.S. of lead treated duodenum of Swiss mice. **A.** Postnatal day 1, shows scattered villi (V) with vacuolated epithelial cells (E C) in mucosa layer. **B.** Postnatal day 7, shows increases in length and size of villi (V) covered with thickened epithelium (E), developing lacteals (L) in upper villi and shrinkage in crypts (C). **C.** Postnatal day 14, shows thickened villi (V) with complete development of lacteals in the centre of villi, swollen crypts (C), thin layer of submucosa (S M) and connective tissue proliferation in circular layer of muscularis externa (M E). **D.** Postnatal day 21, shows indistinguishable epithelium (E) which covered the villi and intermingled epithelial cells (E C), highly damaged crypts(C) , thickened submucosa (S M) and components of both layers of muscularis externa (M E) can not be identified.

Lead treated: Histopathological analysis of duodenum showed severe damage when compared to normal. At the time of birth, villi were scattered with vacuolated epithelial cells. Submucosa was undifferentiated. Both layers of muscularis externa and outer serosa layer were not clearly seen. At 7th day after birth, length and size of villi were markedly increased with thickness in villi covering epithelium. Columner epithelial cells were undifferentiated and developing lacteals were observed. Shrinkage was also observed in crypt cells.

At 14th day of postnatal period, villi were thickened and blunt compared with control and developed lacteals in villi were clearly visible. Submucosa layer was thin. Connective tissue proliferation was observed in the circular layer of muscularis externa. At 21st day of birth, 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.

Discussion: In general, the duodenum of the newborn mice shows a pattern of development similar to that reported in the new born of other mammalian forms. The duodenum shows a well developed lumen with projection of villi. Additional villi apparently form as a result of evagination of the epithelium, together with the underlying mesenchyme and vasculature, into the intestinal lumen.

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 birth, the small intestine of the rat is structurally and functionally immature[13, 14]. Postnatal maturation involves replacing the immature epithelium with the mature epithelium[13]. In the rat, maturation of the small intestine involves changes in the cell population covering the villi[13].

During the postnatal development, rapid proliferation occurs in the intestinal crypts, the length of the crypts increase along with the length of the villi. Different types of cells also increases in number with advancing age. Conteas et al[15] also reported that rapid proliferation occurs in the intestinal crypts, the length of the crypts increase along with the length of the villi, and an increase in cell population occurs, along with an increase in the number of mature mucosal cells during postnatal development.

The thickness of the wall of small duodenum also increases at the end of lactation. Thomas and Keelan[16] also observed the wall thickness of the small intestine doubles at weaning. The change in diet that occurs with weaning (day 17 to 26) requires the successive changes in digestive function[14]. In rodents, crypts are formed after birth[17]. During weaning (postnatal day 15 to 30) a marked increase in cell division and migration occurs in intestinal crypts. This results in increased epithelial cells and thus an increase in the absorptive ability of the small intestine[18].

Villi were observed at various stages of maturation until just prior to weaning. The cellular changes in the villi were observed during birth up to the lactation and there was cellular growth in the developing mice. A similar pattern of late villus formation and growth has been reported in other vertebrates, including man[19, 20]. In the rat during suckling, the number of villi present in the small intestine does not change with age, but villus height can change (increase and/or decrease) [21, 16]. After administration of lead the villi became scattered at the time of birth and there was change in length and size of developing villi during postnatal development. Fusion of intestinal microvilli and wide intercellular spaces among epithelial cells and microvilli, an increased rate of cell death, nacrosis and irregularities of the microvilli in lead exposed mollies were recorded by Yomn Mohamed et al[22].

Intestinal glands (crypts) are not present in the newborn mice. As in other species, the intestinal glands develop as outgrowths between adjacent villi[20] but are shallow in appearance, even in the adult. In present investigation the crypts were not visible at the time of birth but with advancing age at 14 day of postnatal period, plenty of crypts were present beneath the villi. The crypts-villus complex is considered the functional unit of the small intestine and plays an important role in the continual maintenance of

mucosa[16]. Crypts were largely destroyed at the end of lactation after administration of lead. Dietary factors increase the height of intestinal villi as well as the number of depth of crypts[23].

There are four major types of cells that make up the mucosa of the duodenum; these include absorptive cells (enterocytes), goblet cells, granular cells (paneth cells) and endocrine cells[24]. We have also observed these cells at different stages of development. In the neonatal intestinal epithelium, enterocytes have a longer life span than adult enterocytes. Goblet cells were found less in number at 21st day of postnatal period after lead treatment. Tomczok[25] observed presence of lead in conjunction with the goblet cell membrane.

Modification of the small intestine for absorption in the early stages apparently results delay in the appearance of intestinal glands and cell types. From the above discussion we can conclude that the lead exposure during gestation and lactation did not alter the normal development of the duodenum but the constant exposure during pre and postnatal development have its impact on the basic precursors of the gastrointestinal tract.

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