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The Protective Effects of DMSA and Some Vitamins against Toxicity Induced By Lead in Male Albino Rats. "I"

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Abstract: The present study was carried out to evaluate the protective effects of vitamins C, E and DMSA against lead acetate-induced histopathological changes in the livers, kidneys and spleen of albino rats. Exposed rats to Lead acetate (100 ppm) exhibited degeneration and necrosis of hepatocytes associated with hemorrhage. The Kidneys showed severe tubular nephrosis and necrosis and periglomerular lymphoid cell reaction and dilatation of renal tubule. Hyaline tubular cast associated with hemorrhage was also observed. Exhaustion of lymphoid elements in the splenic white and red pulps was noticed. Moreover, necrosis of lymphocyte in the white and red pulps associated with hemorrhage had occurred. Co-administration of DMSA with lead acetate resulted in reducing the severity of pathological changes induced by lead on the affected organs compared to the lead acetate-intoxicated group. There was a significant improvement in lead acetate-induced the hepatic, renal and splenic histopathological changes by the administration of vitamins C and E as compared with DMSA treated rats. In contrast, significant recovery of the histological picture of liver, kidney and spleen in the lead acetate-induced hepatotoxicity, nephrotoxicity and splenic toxicity occurred after treatment with both DMSA and vitamin C combined with vitamin E. These results indicated that combination of DMSA, as a chelating agent for lead, with both vitamins C and E, as antioxidants; provide complete protection against lead-induced hepatotoxicity, nephrotoxicity and splenic toxicity in male albino rats.

Keywords: Vitamin C; Vitamin E; DMSA; Lead toxicity; Liver; Kidney; Spleen.

1 Introduction

It is well known that lead has been used since ancient times. However the quantity of lead using nowadays is far greater than that used in the previous periods. Although lead is one of the most toxic metals, it produces severe organ damages in animals and humans (Spivey, 2007; Haleagranara *et.al*, 2010; Dewanjee *et.al*, 2013). Lead exposure may induce hepatic, renal and splenic disorders in animals and human (Kasten-Jolly *et.al*, 2010; El-Neweshy and El-Sayed, 2011; Dewanjee *et.al*, 2013). The absorbed lead is conjugated in the liver and transported to kidney, where some of it excreted in the urine and the rest accumulated in different body organs and induce many biological activities at molecular, cellular and intra-cellular levels which may led to morphological and histological alternations that can remain even the lead levels have reduced (Flora *et.al*., 2006; Ajayi, *et.al*., 2009; Abdel-moneim *et.al*., 2011). Although, the precise mechanism of lead toxicity is not clear, there is evidence that lead can cause generation of reactive oxygen species (ROS) and this leads to the inhibition of antioxidants activity which may cause pathological incidences in the body organs (Jurzuk *et.al*., 2007; Franco *et.al*, 2009). It has been stated that lead increases the lipid peroxidation (Upasani *et.al*, 2001).

Several chelating agents have been used to reduce the toxic effects of lead on animal body organs. Meso-2,3-dimercaptosuccinic acid (DMSA) is a chemical derivative of dimercaptol and has been shown to be an effective chelator of toxic metals mainly lead and arsenic. It is safe, for oral administration and there is no redistribution of metal from one organ to another. DMSA is an effective chelator of lead in soft tissue (Flora, *et.al*., 1997; Zhang, *et.al*., 2004).

Vitamin C (ascorbic acid), a water soluble vitamin, is known as chelating agent with non-enzymatic antioxidant properties. It has been reported that vitamin C has the ability to protect cells from oxidative stress (Patra and Swarup, 2004; Palaniappan *et.al*, 2005; Rai *et.al*, 2009). In lead

exposed rats, vitamin C has protective role against toxic effects of lead on liver tissue (Shalan *et.al*, 2005; El-Neweshy and El-Sayed, 2011). It may increase urinary elimination of lead and reduce its hepatic and renal disorders (Wang *et.al*, 2007; jabeen *et.al*, 2011).

Vitamin E (α-tocopherol), a fat soluble vitamin is known to be one of the most potent endogenous antioxidants (Ramanathan *et.al*, 2005). Vitamin E protects liver in albino mice against lead induced hepatotoxicity (Al-Attar, 2011). Also, vitamin E protects against lead-induced renal injury (Al-Attar, 2011). Several studies have indicated that vitamin E is a powerful biological antioxidants and inhibits free radical formation (Cemek *et.al*, 2010; Kalender *et.al*, 2004; 2005). It may effectively minimize lipid peroxidation in biological system (Kalender *et.al*, 2002).

It has been indicated that DMSA combined with vitamin C succeeded to improve the histological picture of the liver and kidney towards the normal view of the control in lead-exposed rabbits (Raafat *et.al*, 2011). The combined supplementation with vitamin C and vitamin E reduces and protects the liver of rats from lead toxicity through their action as antioxidants which stop the generation of reactive species and lipid peroxidation (Bashandy, 2006). It has been reported that co-administration of naturally occurring vitamins like vitamin C or vitamin E during administration of thiol chelator like DMSA may be more beneficial in restoration of altered biochemical variables, particularly the effects on heme biosynthesis and oxidative injury (Flora *et.al*., 2008).

The objectives of the present study are to evaluate the possible protective effects of DMSA, vitamin C combined with vitamin E and the combination of DMSA with both vitamins C and E against lead- toxicity in liver, kidney and spleen in male albino rats.

2 Material and Methods

A total thirty male albino rats (Rattus rattus) approximately 8-10 weeks old, weighing 160-180 grams obtained from nation experimental house in Helwan, Egypt were used. Animals were housed in stainless cages at room temperature, six rats each and acclimized to laboratory condition two weeks before the experiments and feed commercial pellet feed food and water were available ad libitum.

2.1 Chemicals:

Lead acetate was purchased from Sigma chemical Co. (St. Louis, Mo). DMSA (meso-2, 3-dimercaptosuccenic acid), vitamin C (ascorbic acid) and vitamin E (α-tocopherol) were obtained from Loba chemicals, India. All chemical used in the present study were of analytical grade.

2.2 Experimental treatments:

Rats were randomized and divided into five groups (6 rats each). Rats of group one (G1) served as control and received normal drinking water without lead acetate. Rats of group two (G2) were exposed to 100 ppm of lead acetate for six weeks in their drinking water. Animals of group three (G3) were injected interperitonially with DMSA 50 mg/Kg/body wt. two times/week and received 100 ppm lead acetate in their drinking water for six weeks. Rats of the group four (G4) received 100 ppm of lead acetate in their drinking water and 160 mg/Kg/body wt. of vitamin C combined with 50 mg/Kg/body wt. of vitamin E two times/week orally for six weeks. The animals of the fifth group (G5) were exposed to the same dose of lead acetate of the previous groups and received the same doses of vitamin C and vitamin E orally as group four in addition; they were injected with 50 mg/Kg/body wt. of DMSA two times/week for six weeks.

2.3 Histopathological Examinations:

Samples of the livers, kidneys and spleen tissues were taken from each rat and fixed in neutral buffer formalin solution, dehydrated and embedded in paraffin wax and sectioned at 4 µm-thick sections were stained with haematoxyline and eosin.

3 Results

3.1 The Livers

The livers of the control group (group 1) showed the normal hepatic organization (Fig. 1A). In lead acetate-intoxicated rats (group 2), the livers had diffuse vacuolar degeneration of hepatocytes (Fig. 1B); diffuse necrosis of hepatocytes associated with hemorrhage (Fig. 1C) and large focal area of necrosis associated with hemorrhage (Fig. 1D).

The liver of rats treated with lead acetate combined with DMSA (group 3) showed marked congestion of blood vessels and edema in Disse spaces (Fig. 1E), and mild vacuolar degeneration of hepatocytes (Fig. 1F).

Rats treated with lead acetate combined with both vitamin C and vitamin E (group 4) showed mononuclear cell infiltration in the portal area with slight congestion of blood vessels was also observed (Fig. 1G). Rats treated with lead acetate combined with DMSA and vitamin C plus vitamin E (group 5) showed slight mononuclear cell reaction in the portal area (Fig. 1H).

3.2 The Kidneys

The kidneys of control rats (group 1) exhibited the normal structure of glomerulus and renal tubules (Fig. 2A). Rats treated with lead acetate (group 2) had severe tubular nephrosis and necrosis in the glomeruli (Fig. 2B); marked interstitial hemorrhage (Fig. 2B1) and renal nephrosis with periglomerular lymphoid cell reaction (Fig. 2B2) were observed.

Lead acetate induced dilation of renal tubules with presence of hyaline tubular cast in the renal medulla (Fig. 3B) and acidophilic hyaline tubular cast associated with hemorrhage were also observed in the renal medulla (Fig. 3B1).

Rats treated with lead acetate combined with DMSA (group 3) showed congestion, mild nephrosis with presence of eosinophilic debris in the renal tubules (Fig. 2C) and dilation of renal tubule with presence of tubular cast associated with interstitial lymphoid cell reaction in the renal medulla (Fig. 3C) were also observed.

Rats treated with lead acetate combined with vitamin C plus vitamin E (group 4) showed congestion and mild tubular nephrosis in the renal cortex (Fig. 2D). Dilation of renal tubule with presence of tubular cast in the renal medulla were also seen (Fig. 3D). In contrast, rats treated with lead acetate combined with DMSA and vitamin C plus vitamin E (group 5) showed only congestion of the interstitial blood vessels in the renal cortex (Fig. 2E). The renal medulla showed more or less the normal histological structure (Fig 3E).

3.3 The Spleen

The non-intoxicated control animals (group 1) showed normal splenic tissue appearance including lymphatic nodules of white pulp and splenic cords of red pulp (Fig. 4A). The spleen of rats treated with lead acetate (group 2) showed exhaustion of lymphoid elements in the splenic pulp (Fig. 4B1); necrosis of lymphocyte in the white pulp and vacuolar degeneration in the tunica media of the central artery (Fig. 4B2). Moreover, exhaustion of lymphoid elements and hemorrhage in the red pulp (Fig 4B3) and necrosis and hemorrhage of the red pulp (Fig. 4B4) were also observed.

Rats treated with lead acetate combined with DMSA (group 3) showed only exhaustion of lymphoid elements in the white pulp (Fig. 4C). While, those treated with lead acetate combined with vitamin C plus vitamin E (group 4) had mild vacuolar degeneration in the vessel wall of the central artery (Fig. 4D), whereas rats treated with lead acetate combined with DMSA and vitamin C plus vitamin E (group 5) showed improvement, but with blastogenic activity surrounding the white pulp (Fig. 4E).

4 Discussion

It is known that lead is a ubiquitous environmental and industrial pollutant that induces abroad range of toxic effects within biological systems. Although the exact mechanism of lead toxicity is still remains unclear but the cumulative data showed that lead exposure induces over-production of reactive oxygen species (ROS) and depletes cellular antioxidant capacity (Dewanjee *et.al*., 2013). It has been postulated that generation of reactive oxygen species, stimulation of lipid peroxidation and depletion of antioxidants are the major contributors to lead exposure related biochemical and histopathological changes in the animal body organs (Silbergeld *et.al*, 2000; Patrick, 2006; Jabeen, *et.al*, 2011).

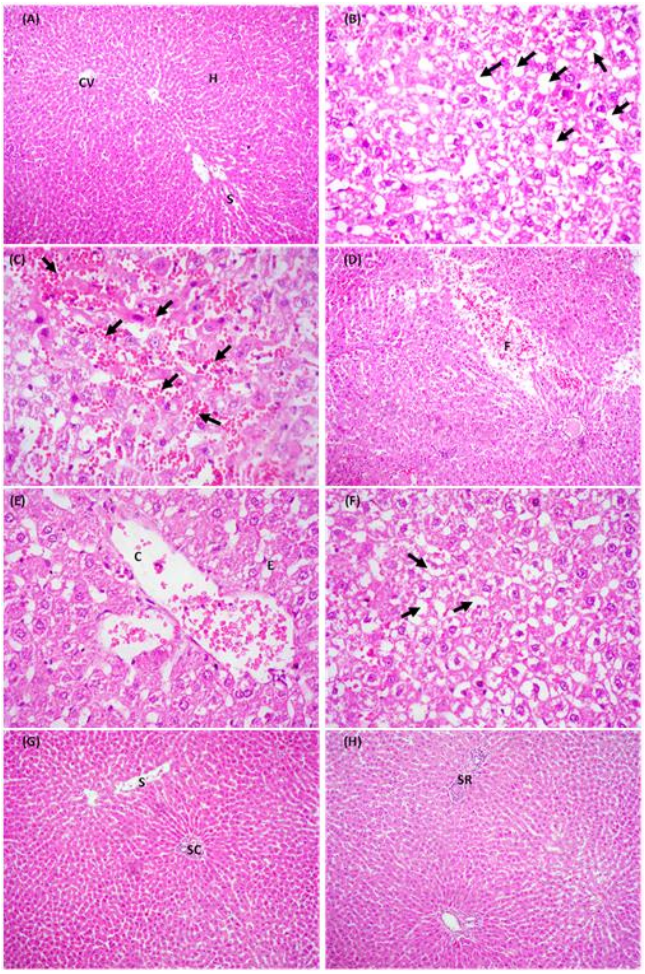

Fig. 1: Photomicrograph of rat liver section stained with haematoxyline and eosin. **(A)** Showing hepatocytes [H] arranged into hepatic cord around central vein [CV] separated by adjacent sinusoids [S]. Hepatocytes are cuboidal cells with one or two large euchromatic nuclei with abundant grainy cytoplasm. (10X). **(B)** Rats treated with lead acetate (100 ppm) show diffuse vacuolar degeneration of hepatocytes (arrows). (40X), **(C)** diffuse necrosis of hepatocytes associated with hemorrhage (arrows). (40X) and **(D)** large focal area of necrosis [F] associated with hemorrhage [H]. (10X). **(E)** Rats treated with lead acetate (100 ppm) and DMSA (50 mg/Kg/body wt.) show marked congestion of blood vessels [C] and edema [E] in Disse space. (40X) and mild vacuolar degeneration of hepatocytes (arrows) **(F)**. (40X). **(G)** Showing mononuclear cell infiltration in the portal area [CI], with slight congestion of blood vessels [SC] in rats treated with lead acetate (100 ppm) combined with vitamin C (160 mg/Kg/body wt.) plus vitamin E (50 mg/Kg/body wt.). (10X). **(H)** Rats received lead acetate (100 ppm) and DMSA (50 mg/Kg/body wt.) combined with vitamin C (160 mg/Kg/body wt.) plus vitamin E (50 mg/Kg/body wt.) show only slight mononuclear cell reaction [CR] in the portal area. (10X).

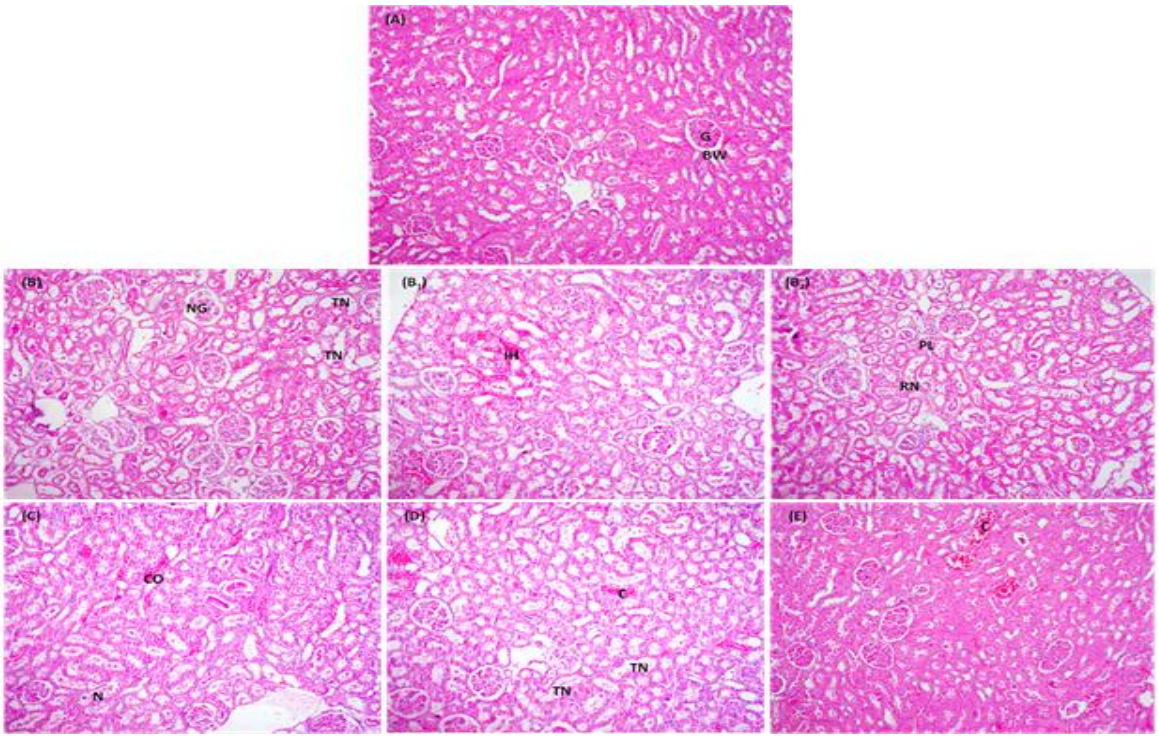

Fig. 2: Photomicrograph of rat kidney section stained with haematoxyline and eosin (10X): **(A)** The histological structure of the control rat kidney show Bowman's capsule [BC] containing glomeruli [G] as well as the renal tubules. **(B):** Renal cortex section from rats which was treated with lead acetate (100 ppm) show severe tubular nephrosis [TN] and necrosis in the glomeruli [NG] (10X), marked interstitial hemorrhage [IH]. **(B_1)** and renal nephrosis [RN] with periglomerular lymphoid cell reaction [PL]. **(B_2)**. Congestion [CO] and mild nephrosis [N] with presence of depries in the renal tubule is seen in the renal cortex **(C)** of rats treated with lead acetate (100 ppm) combined with DMSA (50 mg/Kg/body wt.). Congestion [C] and mild tubular nephrosis [TN] are conspicuous **(D)** in the renal cortex of rats treated with lead acetate (100 ppm) combined with vitamin C (160 mg/Kg/body wt.) plus vitamin E (50 mg/Kg/body wt.). Renal cortex of rats treated with lead acetate (100 ppm) and DMSA (50 mg/Kg/body wt.) combined with vitamin C (160 mg/Kg/body wt.) plus vitamin E (50 mg/Kg/body wt.) show only congestion [C] of the interstitial blood vessels.

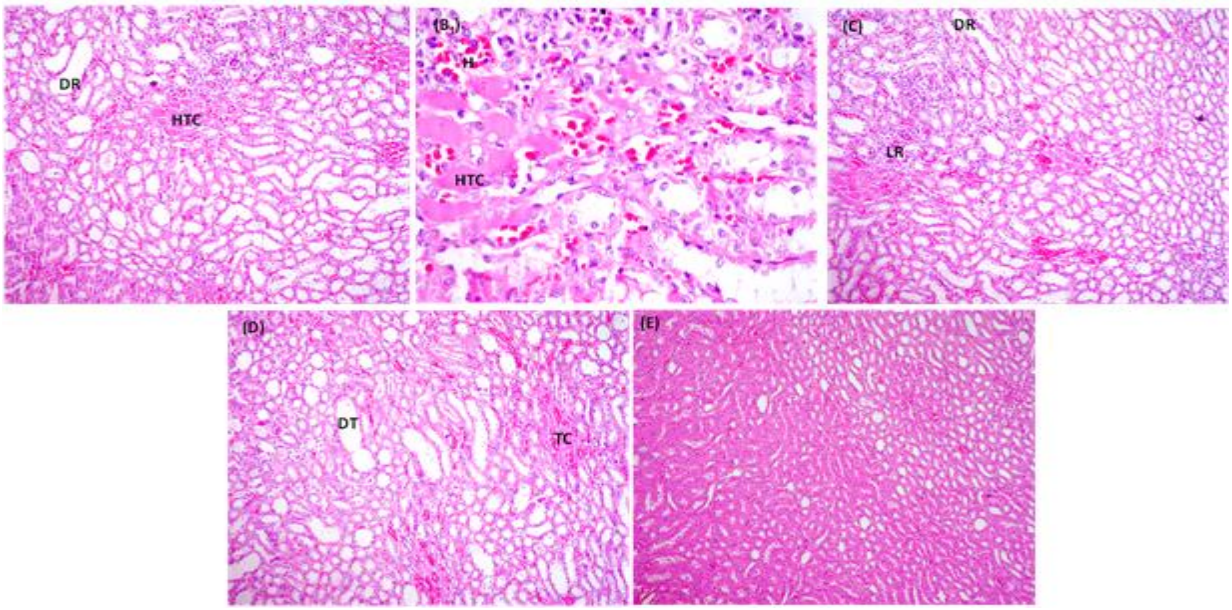

Fig. 3: Photomicrograph of renal medulla stained with haematoxyline and eosin (10 X). **(B)** Rats intoxicated with lead acetate (100 ppm) show dilation of renal tubule [DR] with presence of hyaline tubular cast [HTC] and acidophilic hyaline tubular cast [HTC] associated with hemorrhage [H]. **(B_1)** (40X). Dilation of renal tubule [DR] with presence of tubular cast associated with interstitial lymphoid cell reaction [LR] is seen in rats treated with lead acetate (100 ppm) combined with DMSA (50 mg/Kg/body wt.) **(C)**. **(D)** Rats treated with lead acetate (100 ppm) combined with vitamin C (160 mg/Kg/body wt.) and vitamin E (50 mg/Kg/body wt.) show dilation of renal tubule [DT] with presence of tubular cast [TC]. **(E)** More or less normal histological structure is seen in renal medulla of rats intoxicated with lead acetate (100 ppm) and supplemented with DMSA (50 mg/Kg/body wt.) and vitamin C (160 mg/Kg/body wt.) plus vitamin E (50 mg/Kg/body wt.).

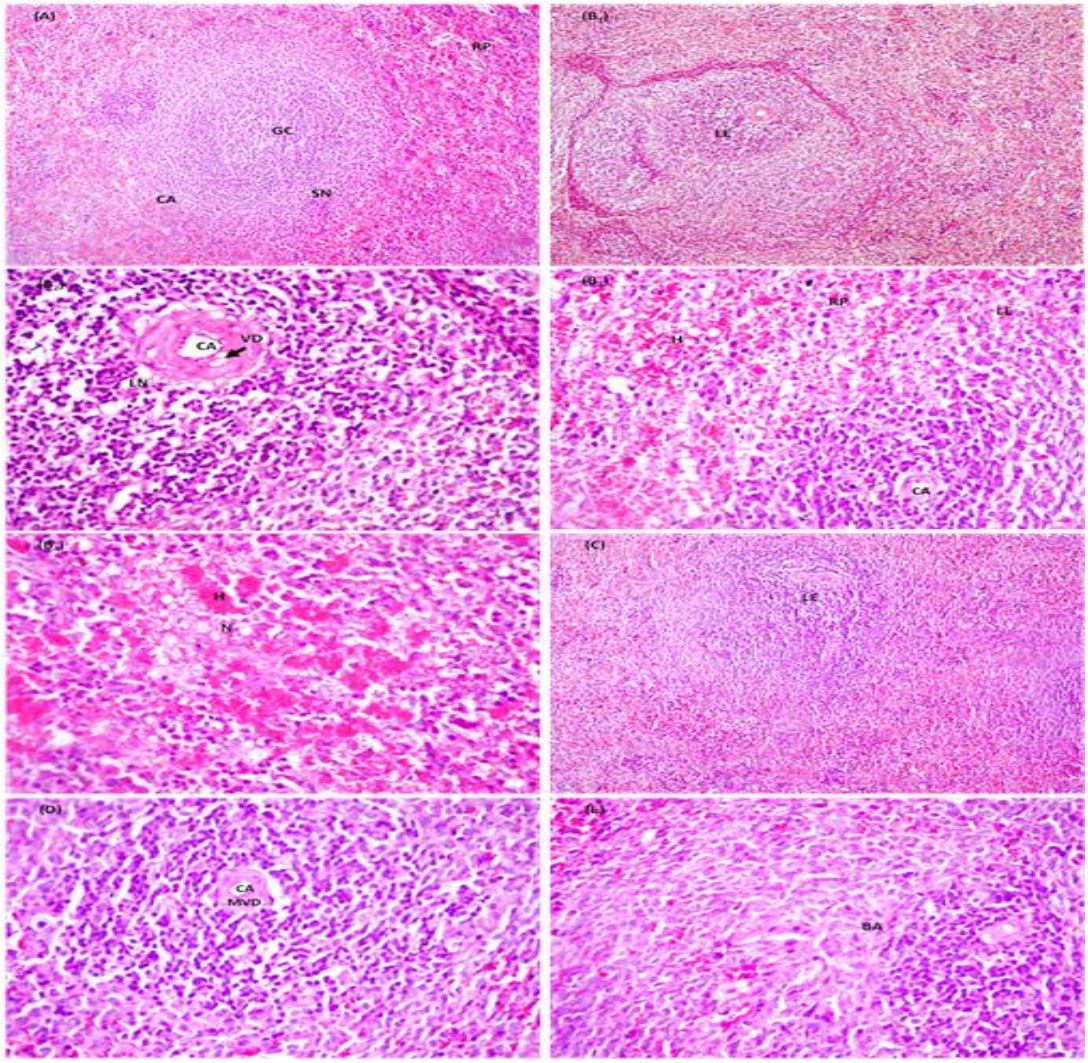

Fig. 4: Photomicrograph of the rat spleen section stained with haematoxyline and eosin: **(A)** the histological structure of the control rats spleen show the red pulp [RP] and white pulp (splenic nodule) [SN], central artery [CA] and the germinal center [GC]. (10X). **(B_1)** Rats intoxicated with lead acetate (100 ppm) show exhaustion of lymphoid elements [EL] in the splenic pulp. (10X), necrosis of the lymphocyte [LN] in the white pulp and vacuolar degeneration [VD] in the tunica media of the central artery [CA] (**B2,** 40X), exhaustion of lymphoid elements [LE] and hemorrhage [H] in the red pulp [RP] (**B_3**, 10X), and necrosis [N] and hemorrhage [H] in the red pulp (**B_4**,40X). **(C)** Exhaustion of lymphoid elements in the white pulp is seen in rats intoxicated with lead acetate (100 ppm) and supplemented with DMSA (50mg/Kg/body wt.). (40X). **(D)** Rats received lead acetate (100 ppm) and supplemented with vitamin C (160 mg/Kg/body wt.) plus vitamin E (50 mg/Kg/body wt.) show mild vacuolar degeneration [MVD] in the vessel wall of the central artery [CA]. (40X). **(E)** Blastogenic activity [BA] surrounding white pulp is seen in rats treated with lead acetate (100 ppm) and DMSA (50mg/Kg/body wt.) combined with vitamin C (160 mg/Kg/body wt.) plus vitamin E (50 mg/Kg/body wt.). (40X).

Recent studies on dead exposed animals showed that a therapeutic strategy to increase antioxidant defense system of the body may be of help for long-term effective treatment of lead poisoning. It can cause hepatic, renal and splenic changes (Patrick, 2006). Recently, several chelating agents approaches have been proposed therapeutically, including supplementation with antioxidants and up-regulation of endogenous anti-oxidative defense system for lead induced oxidative stress in various body organs (Raafat *et.al*, 2011; Hamadouche *et.al*, 2012; Dewanjee, *et.al*, 2013). However, the mechanism of actions of these chelating agents and antioxidants is still indistinct. It is believed that chelating agents reduce the lead toxicity in soft organs through its chelating activity, whereas antioxidants protect the cells from influence of oxidative damage by scavenging the free radical generation and inhibiting of lipid peroxidation (Bashandy, 2006; Halergraphara, *et.al*., 2010; El-Neweshy and El-Sayed, 2011; Jabeen, *et.al*., 2011). It has been indicated that chelating agent, like DMSA, reduces the toxic effect of heavy metals on the histological sections of soft tissues (Raafat *et.al*, 2011). It is believed that vitamins with antioxidant activity protect soft tissues against damaging effect of free radicals which produced as a result of heavy metals toxicity (Hamadoche *et.al*, 2012). Moreover, it has been reported that the co-administration of vitamins combined with chelating agents may have better beneficial role and protective effects against lead intoxication (Flora *et.al*, 2008). The role played by DMSA alone or in combination with vitamins in the modulation of the toxic

effects of lead acetate on the histological section of the target organs, like liver, kidney and spleen, is little known. Although the investigations about prevention of lead toxicity utilizing the combination of vitamin C and vitamin E in the hepatic, renal and splenic tissues are previously documented, studies on the influences of administration with the combination of DMSA with both vitamin C and vitamin E on lead exposure may be novel. Also, the information in the literature about the impacts of vitamins C and E, and DMSA in preventing the lead toxicity in splenic tissues are scarce. Furthermore, it has been reported that the combination therapy is a new and better approach to treat cases of metal poisoning (Flora *et.al*, 2008). So, the present study demonstrates the efficacy of DMSA alone, the combination of vitamin C and vitamin E and the co-administration of DMSA with vitamin C plus vitamin E in treating lead acetate toxicity on the rat liver, kidney and spleen, and are expressed as histopathological scores.

In the present results lead exposure produced pronounced hepatic, renal and splenic histopathologies as indicated by vacuolar degeneration, hepatic necrosis and focal necrosis associated with hemorrhage. These findings were similar to those reported by El-Neweshy and El-Sayed, (2011). In accordance with the present results, El-Sokary (2005) showed that liver of lead-treated rats indicated remarkable degenerative alternations. In accordance with the present results, lead toxicity resulted in focal necrosis with hepatocyte vacuolization and swelling, pyknotic nuclei and dilation of central vein (Hamadouch *et.al*, 2012). Dewanjee *et.al*. (2013) showed that liver of rats treated with lead acetate exhibited hepatocytes focal necrosis.

The histopathological alternations in the present study, caused by lead in the kidney revealed by severe tubular nephrosis, glomeruli necrosis, marked interstitial hemorrhage, renal nephrosis with periglomerular lymphoid cell reaction in the renal cortex. Whereas lead induces dilation of renal tubules with presence of hyaline tubular cast and acidophilic tubular cast associated with hemorrhage in the renal medulla. El-Nekeety *et.al*. (2009) reported that rats treated with lead acetate showed tubular dilation, vacuolar and cloudy in epithelial lining, interstitial inflammatory cells associated with hemorrhage and glomerulus's hypercellularity.

The histopathological changes induced by lead in the spleen indicated exhaustion of lymphoid elements in the splenic pulp, necrosis of lymphocyte in the white pulp, vacuolar degeneration in tunica media of central artery, exhaustion of lymphoid elements with hemorrhage in the red pulp and necrosis and hemorrhage at the red pulp. Teijon *et.al*. (2003) also showed that spleen of lead-treated rats revealed an increase in the number of lymphocytes as well as edema, an expansion of lymphatic sinuses of red and white pulps, cell aggregation constituted by lymphocytes and specific cells of the red pulp which form a lattice. Al-Naimi *et.al*. (2011) reported that lead acetate resulted in severe depletion of white pulp lymphoid tissue and extra-medullary hemopoiesis characterized by presence of large numbers of megakaryocytes.

Oxidative stress has been proposed to be a principle mechanism involved in lead toxicity. Lead exposure disturbs the prooxidant-antioxidant balance (Maiti *et.al*., 1995; Abdel-Wahab *et.al*., 2005). So, the histopathological changes observed in the current study as a result of lead treatment could be attributed to the formation of free radical damage through the generation of ROS and direct depletion of antioxidant reserves.

Animal studies suggest that DMSA is an effective chelator of lead in the soft tissue (Flora *et.al*., 1997). The present study proved that DMSA reduced the toxic effect of lead on the histological sections of liver, kidney and spleen.

It is believed that vitamins with antioxidant property can protect tissues against the damaging effect of free radicals (Osawa and Kato, 2005; Hamadouche *et.al*, 2012). Non-enzymatic oxidants like vitamin C and E mitigate oxidative-stress, being a part of total antioxidant system. Vitamin C could save to scavage free radicals within extracellular space, whereas vitamin E would effectively scavage free radicals within the cells, where reactive metabolites are produced. Moreover, vitamin C may remove free radicals bound to vitamin E, thereby aids regeneration of vitamin E (Senthil Kumar *et.al*, 2004).

In the present study, vitamin C plus vitamin E showed marked improvement, compared to lead acetate treated group and to DMSA-treated group. Hamadouche *et.al*. (2012) showed that vitamin C along with lead acetate was effective in bringing about histological improvement of rat hepatocytes. It was assumed that combined supplementation of vitamin C with lead acetate diminished the severity of histopathological changes induced by lead in the liver and kidney sections of rat (El-Neweshy and El-Sayed, 2011). Al-Attar (2011) showed that administration of vitamin E protects against lead-induced renal toxicity in male mice. The present results revealed that it is more efficient to use vitamin C plus vitamin E against lead toxicity than DMSA alone. Herein, the beneficial role of co-administration of DMSA combined with vitamin C plus vitamin E in elimination of lead toxicity is shown in hepatocyte, renal and splenic tissues of rat. It is clearly shown that this combination restored to higher extent more or less the normal histological structure of liver, kidney and spleen of rat. Combined administration of DMSA with vitamins C and E ameliorated the adverse histopathological impacts of lead acetate toxicity.

5 Conclusion

These results demonstrate that lead acetate induced alternations in histological structures of liver, kidney and splenic tissue of rat. Treatment with a combination of vitamin C plus vitamin E decreased lead toxicity more than

treatment of DMSA, but did not confer complete protection. Moreover, Treatment with DMSA combined with vitamin C plus vitamin E was effective in bringing about histological improvement of hepatocytes, renal and splenic tissue and restored more or less the normal histological structure of the organs used in the present study. Moreover, we can stated that the administration of DMSA combined with the naturally occurring vitamin C and vitamin E is a new and a better approach to treat cases of lead poisoning, and may be more beneficial in protection against lead toxicity. So, it can be concluded that the administration of DMSA combined with vitamin C plus vitamin E may be more beneficial in the restoration of altered histopathological variables induced by lead in the liver, kidney and splenic tissues, more than the administration of either DMSA alone and/or vitamin C combined with vitamin E.

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