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RESEARCH ARTICLE

Toxicopaphological Effects of Intravenous Injection of Layer Double Hydroxide (LDH) Nanoparticles in Male Rats

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Abstract

The rapid growth of using nanoparticles in different field of science, lead to the urgent necessity to study the toxicpathology of this submicron particle. Layer Double Hydroxide NPs have qualified features such as bio-compatibility, ion exchange and pH effect which make LDH NPs potential candidate for various biological applications such as drug delivery. Hence, in this study Mg-Al LDH NPs have been prepared by co precipitation method and injected intravenously. Twenty rats were divided into two groups as follows: Group I: Rats served as control and injected 1ml of distilled water intravenously for one month. Group II: Rats served as experimental and received by I/V injected in the tail vein 1ml of 0.00276 of Mg-Al LDH NPs g/ml. daily for one month. The sizes of LDH NPs have been measured by SEM which resulted size ranging between 50- 80nm in diameter. The liver, kidney, heart, lung, spleen and testes were examined histologically. The toxicopathological results were very severe in kidneys, lung, spleen and heart, while being moderate in liver and testes. In general, all organs showed necrotic changes with inflammatory cell infiltration, congestion and thrombosis in blood vessels.

Keywords: *LDH, Toxicopathological study, Nanoparticles, Rat.*

Introduction

In line with the scientific and commercial needs of synthesis new materials that have the ability to alter the notorious properties of the conventional materials. Many types of nanoparticles have been employed in various field of science including Biology, chemistry, medicine, agriculture, etc. Along with the increase of using nanoparticles globally; the fear of their side effects has spontaneously increased and that is due to their small size, which enabled them to penetrate through the tissue and organelles and cause unwanted side effects [1,2], which that have increased the real need of study the toxicity of this material.

Layer double hydroxide nanoparticles LDH NPs among those materials have been extensively used in biology and medicine because its distinguished properties such as low toxicity [3], easy to synthesis at the Nano size, the good ability to maintain and control the release of the biomolecule that intercalated into it at different pH [4], ion exchange property which can keep the carrier molecule according to its charge [5]. LDH can be synthesized chemically either by ion exchange or by co-precipitation methods [6]. Many types of drugs have been successfully encapsulated in to LDH NPs such as levodopa, which gain high stability due to the anion and cation of the inter layer sheet of LDH NPs [7]. Dong *et al* [8].Have developed a new method to transfer DNA using LDH NPs as a carrier. The distinguished result of using LDH in bio-medicine has stimulated the researchers to apply it on animals, which required the real need to study the toxicity of LDH NPs toxicity *in-vivo*.

However, the toxicity of NPs depends on the size of nanoparticles, dose and time of exposure [9]. The present investigation was designed to synthesis LDH NPs and to study its toxicity on a male rat model where the influence of the LDH NPs have been histopathology examined of spleen, liver,

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kidney, tests, heart and lung after intravenous injection.

Materials and Methods

Experimental Animals

Twenty male rats were used in the present study. The animals were housed in metal cages in the animal house of the Veterinary Medicine College- University of Alqasim green and were fed with standard rat pellets, with water provided *ad libitum*, they were allowed to acclimatize for 10-14 days at room temperature.

Experimental Design

Twenty rats were divided into two groups as follows: Group I: Rats served as control and injected 1ml of distilled water in tail vein for one month. Group II: Rats served as experimental and received by I/V injected in the tail vein 1ml of 0.00276 of Mg-Al LDH NPs g/ml. daily for one month.

Preparation of LDH Nanoparticles:

LDH nanoparicles were prepared by co-precipitation where magnesium nitrate and aluminum nitrate mixed in an ammonia solution followed by washing with hot water. 0.3 M of Mg $(NO_3)_2$ and 0.6 M of Al $(NO_3)_3$. Were thoroughly mixed and solution of 2M of NaOH was added drop wise to the mixture under vigorous stirring at 80 ^oC. The pH value of the mixture was maintained at 10 ± 0.5 during the reaction processes. The precipitate was heated at 80^{o}C for 30 min and then washed several times with distilled water and ethanol. After centrifugation at 5000rpm for 10 min. And removal of the supernatant the precipitate washed with with distilled water three times and finally dried in a vacuum oven at 70^{o}C [26].

Histopathology Study

At the end of experiment period, the animals of each group were sacrificed by intramuscular injection of a high dose of ketamin hydrochloride. Specimens were taken from, liver, kidneys, lung, spleen, heart and testes, the tissues were fixed in 10% formaldehyde solution then processed routinely by using the histokinette. Tissue sections were embedded in paraffin, sectioned by microtome and stained with hematoxylin and eosin [11].

Results

LDH NPs Dose Determination

One ml of LDH NPs suspension has been placed in a foil boat (Schumacher *et al.,* 1994) and let to dry up at 80^{o}C the differentiation of the foil boat before and after placing LDH NPs suspension was 0.00276 g which represents the concentration of LDH NPs in 1 ml.

Characterization of LDH Nanoparticles

Scanning Electron Microscope (SEM) has been conducted to characterize the size and shape of LDH NPs, which showed size ranging between 50- 80 nm and amorphous shape Figure 1.

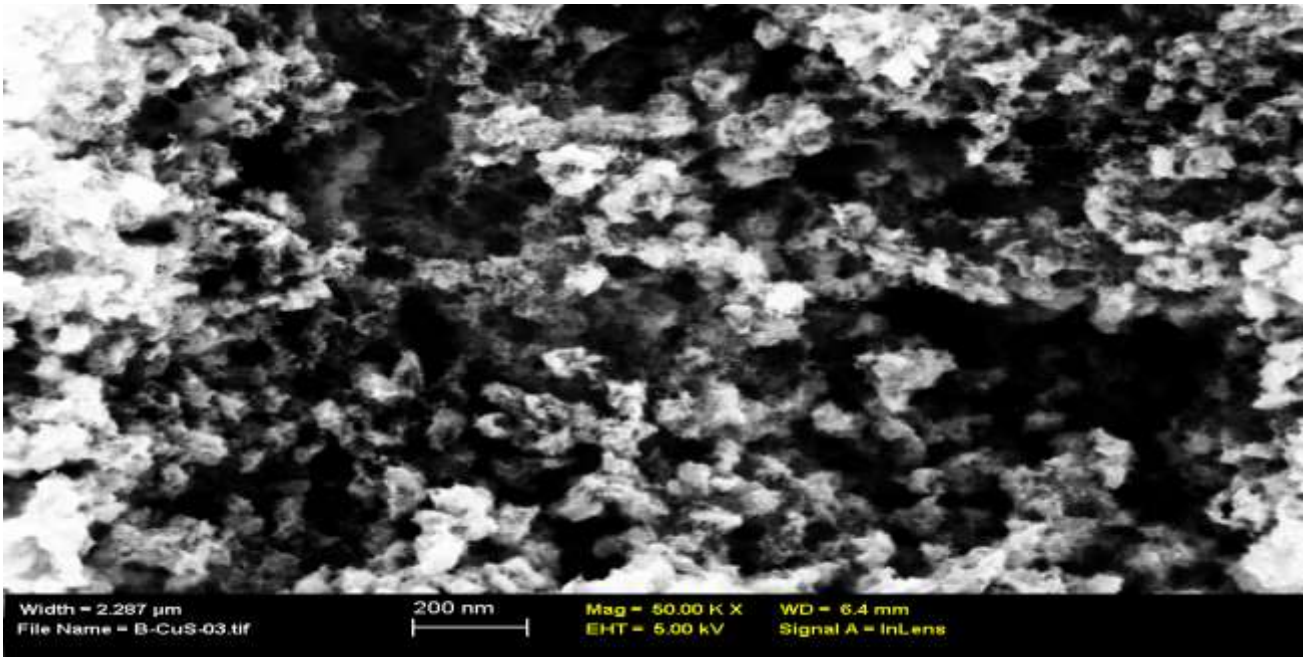

Figure 1: SEM image of LDH NPs which shows the size and shape of the NPs

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Histopathology Study

Group I: control group showed normal histological changes for all organs Fig (2, 3, 4, 5, 6).

Groups II: The hisopathological changes in examining organs were as follows:

Kidney:

The main histopathological lesion was severe tubular necrosis with atrophy or shrinkage of glomerular tuft Fig (7), also capsular thickening due to sub capsular inflammatory cells infiltration Fig (8).Hyalinization and inflammatory cells around blood vessels Fig (9).

Liver

The histopathological changes were less severe and characterized by mild infiltration of inflammatory cells in the lumen of central vein with dilation of sinusoids Fig (10). Fatty changes and vacuolation were also seen Fig (11).

Lung

The Histological Results Showed Sever Inflammatory Cells Infiltration in The Lung Parenchyma and thinking of alveolar wall Fig (12) Mainly Alveolar Macrophages FIG (13).With thrombosis of pulmonary blood vessels and hyperplasia of bronchiolar epithelia Fig (14).

Hearts

Main changes were necrosis, hemorrhage and atrophy of cardiac muscles Fig (15).With thrombosis in the lumen Fig (16).

Spleen

The histopathological results showed increases in the red pulp with depletion in white pulp Fig (17).

Testes

The testes were showed mild histological changes characterized by mild interstialedema with testicular blood vessels congestion Fig (18).

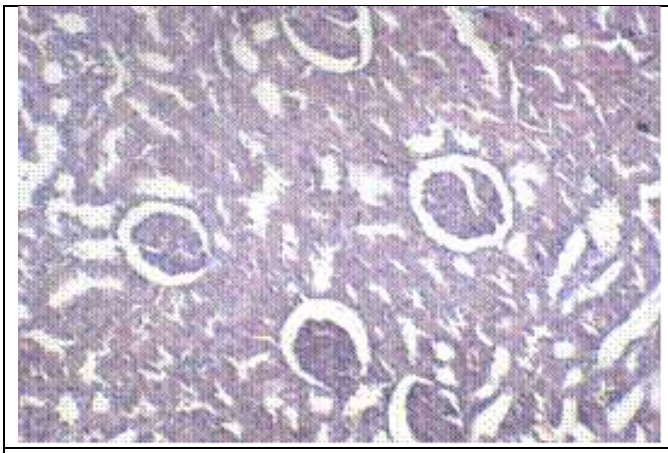

Fig. 1: Histopathological section of kidney showing normal histological strictures. H&E (100X)

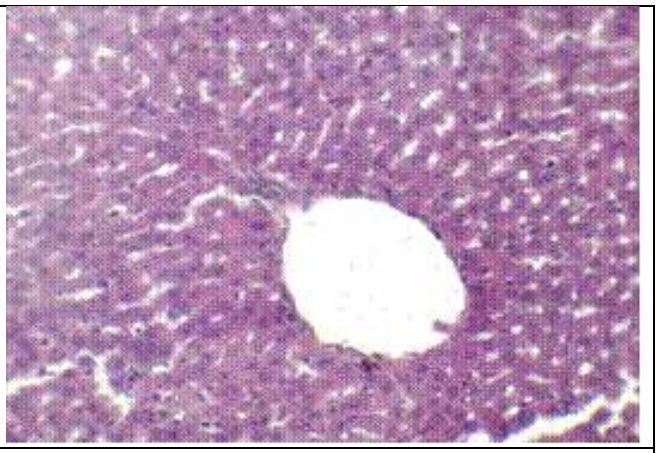

Fig. 2: Histopathologicalsection of liver showing normal histological strictures. H&E (100X).

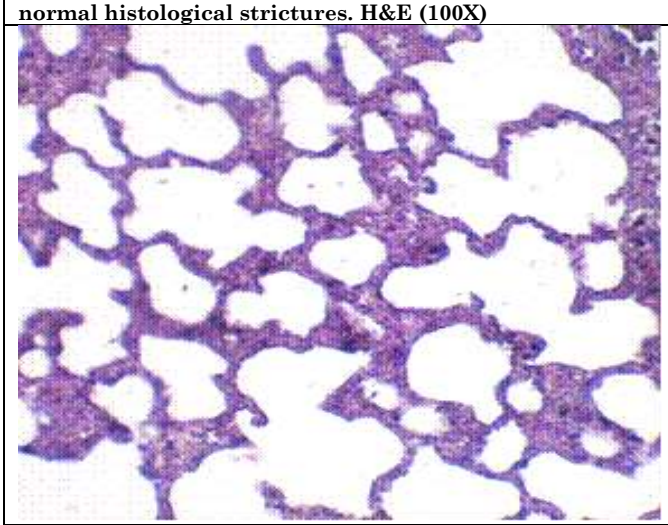

Fig.v3: Histopathological section of lung showing normal histological strictures. H&E (100X)

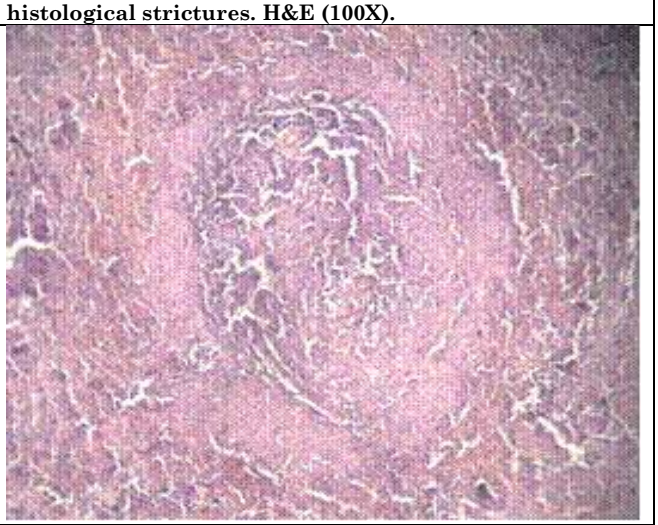

Fig. 4: Histopathological section of spleen showing normal histological strictures. H&E (100X)

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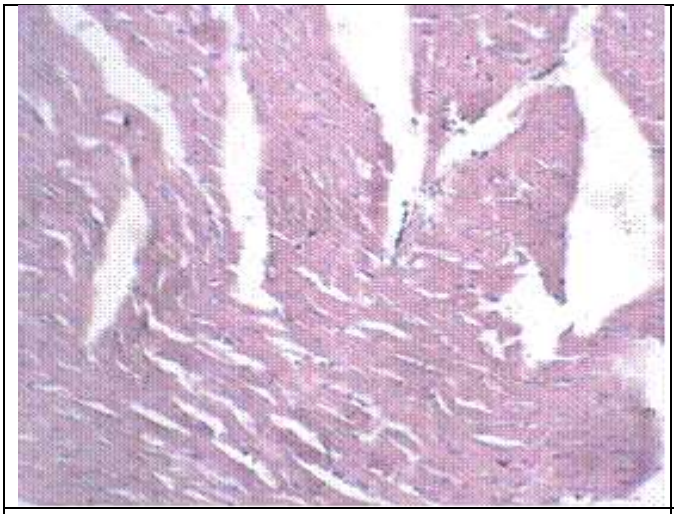

Fig. 5: Histopathological section of heart showing normal histological strictures. H&E (100X)

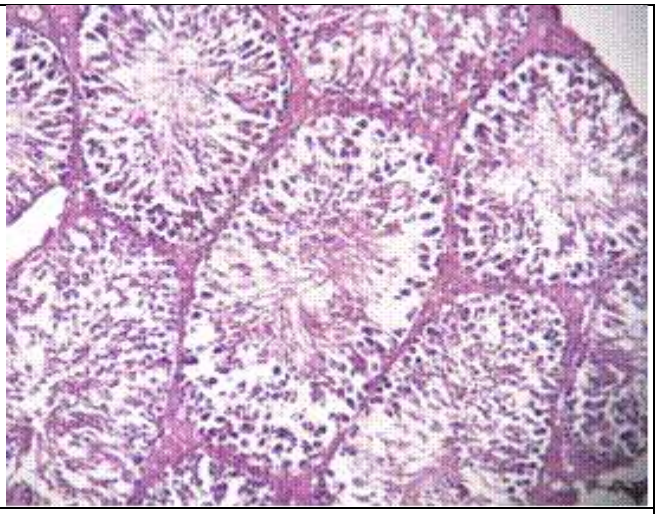

Fig. 6: Histopathological section of tests showing normal histological strictures. H&E (100X)

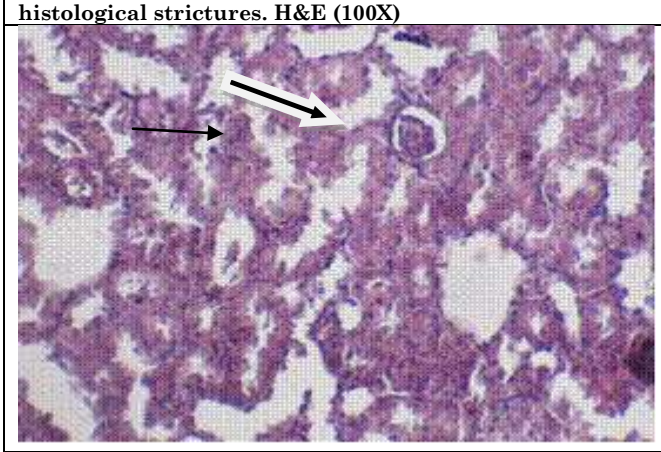

Fig. 7: Histopathological section of kidney showing severe tubular necrosis (⟷) with atrophy or shrinkage of glomerular tuft(⟶) . H&E (100X).

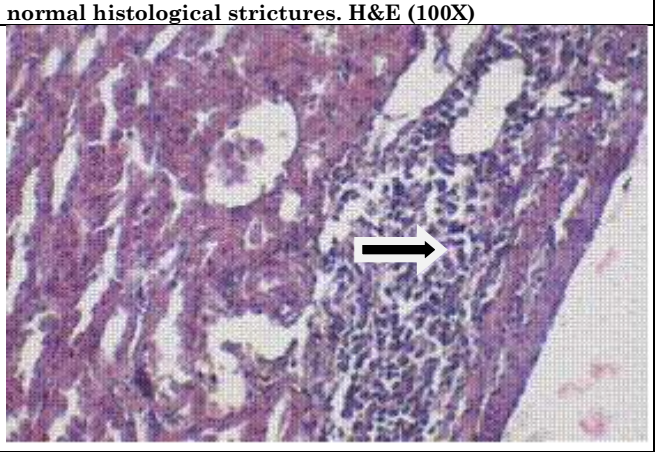

Fig. 8: Histopathological section of kidney showing capsular thickening due to subcapsular inflammatory cell infiltration ➡ H&E (100X).

Fig. 9: Histopathological section of kidney showing hyalinization (➡) and inflammatory cells around blood vessels (⟶). H&E (100X).

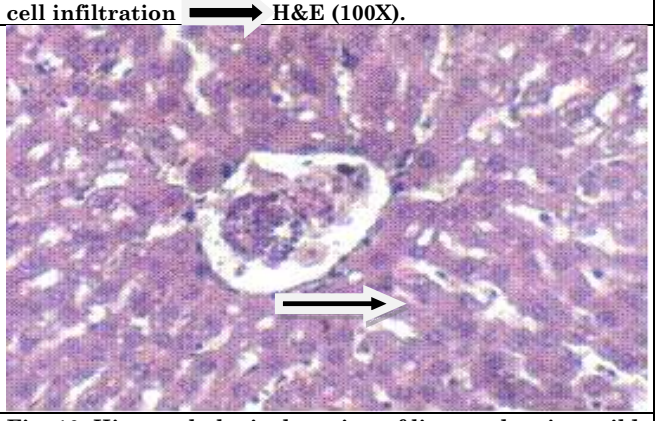

Fig. 10: Histopathological section of liver showing mild infiltration of inflammatory cells in the lumen of central vein with dilation of sinusoids (⟶) H&E (100X).

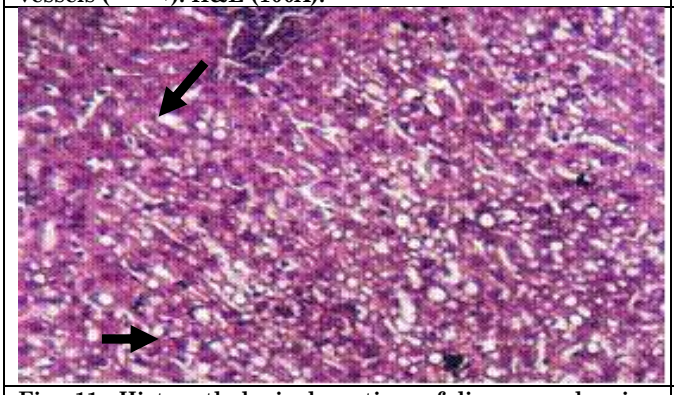

Fig. 11: Histopathological section of liver showing vaculation and fatty changes (➡) H&E (100X).

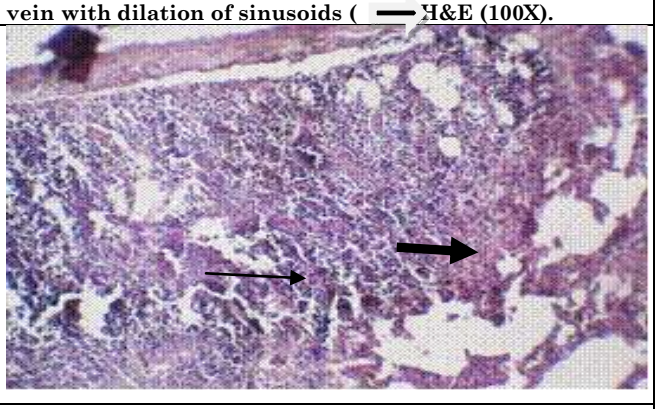

Fig. 12: Histopathological section of lung showingsever inflammatory Cells Infiltration Mainly Alveolar Macrophages, (➡ with thickening in alveolar wall (⟶) H&E (100X).

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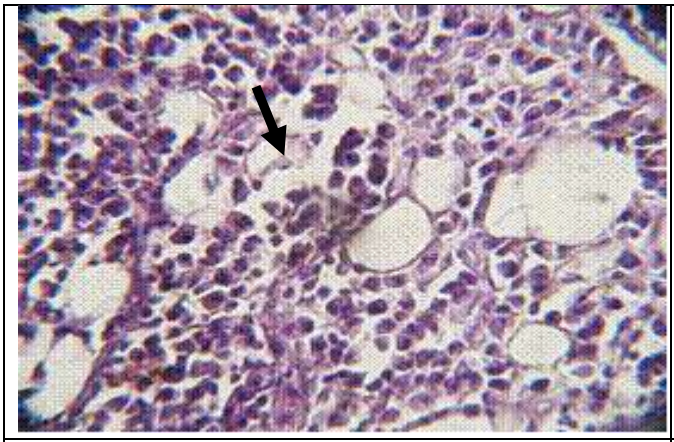

Fig. 13: Histopathological section of lung showing alveolar macrophages (➡) H&E (400X)

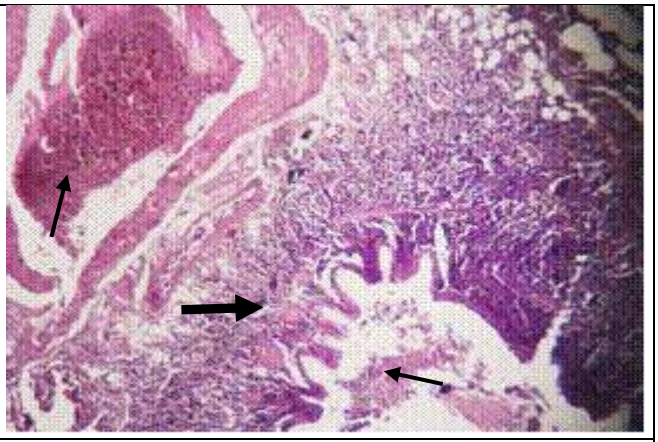

Fig. 14: Histopathological section of lung showing thrombosis of pulmonary blood vessels (→) and hyperplasia of bronchiolar epithelia (➡) H&E (400X)

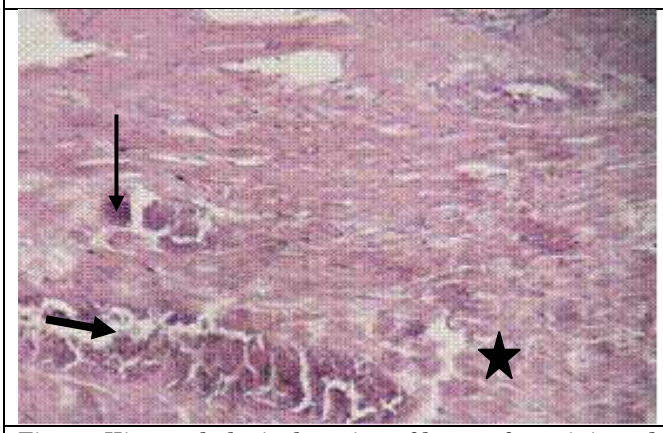

Fig. 15: Histopathological section of heart of rats injected with 1 ml LDH NPs showing necrosis (➡), hemorrhage (→) and degeneration of myocardial muscles (★) H&E (400X).

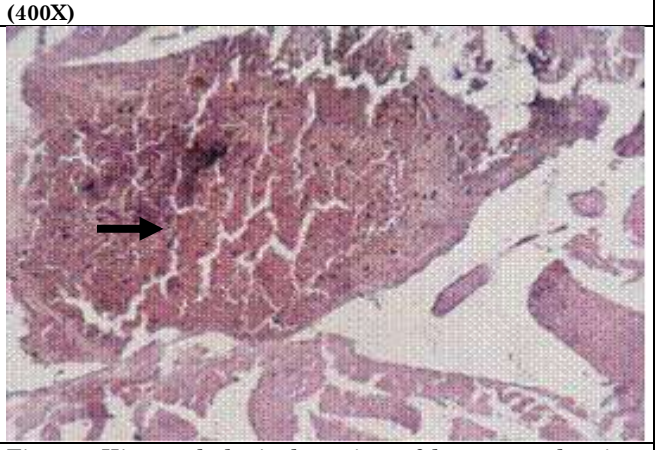

Fig. 16: Histopathological section of heart showing thrombosis in the lumen (➡) H&E (400X).

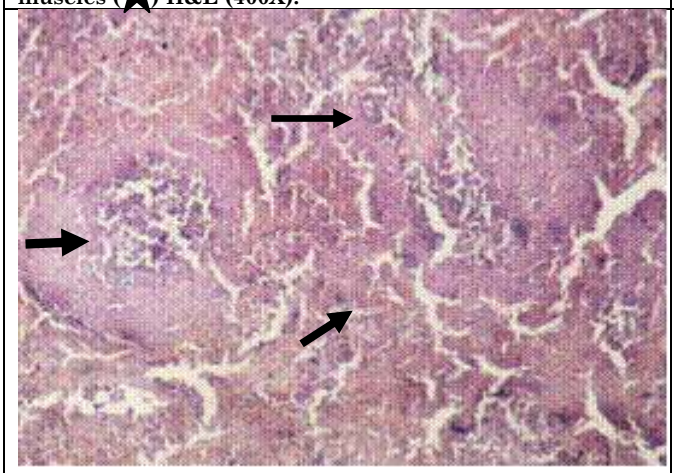

Fig17: Histopathological section of spleen showing increases in the red pulp (→) with depletion in white pulp (➡) H&E (100X).

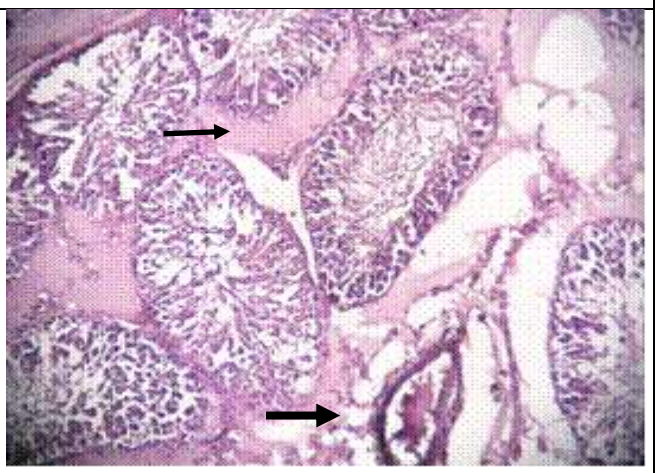

Fig18: Histopathological section of testes showing mild interstial edema (➡) with testicular blood vessels congestion (➡) H&E (400X).

Discussion

The toxicity of NPs depends on the size of nanoparticles, dose and time of exposure [9, 10] . However, in the present study, we have studied the toxicity of Mg-AL LDH nanoparticles after IV injection on the numerous body organs. While the size of LDH NPs has been confirmed by SEM which resulted 50-80nm with amorphous shape. After injection of Mg-AL LDH NPs in the tail vein, the nanoparticles rapidly disputed in the blood stream and reach to different tissue mainly in the kidneys, livers and captured in the alveolar macrophages. The excretion of nanoparticles occurs through the urine or biliary system [21], this explain why the lungs and kidneys are extensively affected. The histopathological changes were less severity of the liver and testes this may be due to ability of hypatocytes to neutralize the toxin or due to rapid excretion through the

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biliary system [12, 13]. The testes also have testicular Perrier that may give some protection against the toxicity [25]. Necrosis was the main changes in the renal tubules and myocardial muscles this may be due to the effect of oxidative stress associated with mitochondrial failure [22]. Also, this suggestion agreed with Fariss *et al.* [19], has reported that the nanoparticles have the ability to migrate into nucleus and cause DNA damage lead to several biological responses. Sadeghi *et al.* [23] and Somyaeh and Zahra [13] suggested that" mechanism that induce toxicity of iron oxide nanoparticles include: 1.role of ROS. Hemochromatosis in tissue and organs that cause damage to electron transport chain and subsequently cause apoptosis cascades [14].

The lung is extensively effected by Mg-AL LDH Nps showed massive infiltration of inflammatory cells mainly lymphocyte and alveolar macrophages in the lung parenchyma that cause thickening in the alveolar wall also hyperplasia in the bronchiolar epithelia these results agreed with previous studies that reported that toxicity of nanoparticles cause lung damages, hyperplasia and distraction in bronchial epithelia and increase in permeability of blood vessels [24]. However the thrombosis of blood vessels and inflammatory cells that infiltrates in different organs were due to the distraction of the cells and impairment in their function as result of Mg-AL LDH Nps toxicity cause activation of inflammatory cascades [15], the damaged cells cause expression pattern of membrane receptor and increase CD68,also active macrophages produce TNFα, IL6 and other chemokine that stimulate inflammatory response, this activate migration of lymphocytes, monocytes to site of injuries and cause endothelial cells damages [18].The liver showed fatty changes and vacuolation of hepatocytes these occur due to lipid peroxidation as a result of damage of endoplasmic reticulum this attributed to abnormal lipid metabolism [17,20].

The main histopathological changes in the spleen were increases in the red pulp with depletion in the white pulp compared with control groups, this may be due to increase in RBCs production this state occurs in many pathological conditions such as cardiac failure, portal hypertension or extra medullary hematopoiesis [16]. Many authors postulated the toxicity of nanoparticles in the testes, the histopathological changes in our result were less severe and characterized by mild interstial edema with congestion of testicular blood vessels this agree with [21].

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