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Histological Changes of Mesenteric Lymph Nodes Affected by Cadmium Chloride in Male Albino Rat: Experimental Study

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Abstract

Background: Cadmium (Cd) is a toxic heavy metal can be found in many sources, including cigarette smoke, e-cigarettes, batteries, contaminated water, plant-based foods, air, fertilizers, plastic toys, ceramics, soil and paints. the exposure to environmental factors may affect different body systems including lymphatic system.

Objectives: to investigate the effect of ingested cadmium chloride on mesenteric lymph nodes in rat.

Materials and Methods: twenty adult male albino rats were used in this study, which divided equally into two groups, on group served as control group administrated of tap water while the second group used as experimental group exposed to daily dose of cadmium chloride (CdCl_2) at a dose 100 mg/kg/day for two weeks via gastric gavage. The weight measured before and after administration and also blood samples were collected for hematological examination by using blood film before and after administration in experimental and control group, then the animals were sacrificed for routine histological examination under light microscope using (H&E) stain.

Results: there are significant differences showed decrease in the weight of experimental group ($186.8750 \pm 6.20034\text{g}$) after administration in compares to control group ($253.7500 \pm 1.56696\text{g}$). Histological examination of the mesenteric lymph nodes in the experimental group showed many defects were seen reactive lymphadenitis including paracortical and follicular hyperplasia (lymphoid hyperplasia), subcapsular sinus expansion, sinus histiocytosis and enlargement of mesenteric lymph node. While the hematological changes in blood smears showed that WBC differential count in the experimental group after two week was increase in the ratio of neutrophil count (29%) and monocyte (4%) while in control group (18%) and (2%).

Conclusions: The present study appeared that exposure to oral Cadmium chloride after two weeks caused decrease in the weight with hematological changes that affect increase in neutrophil count mainly while histopathological changes include lymphadenitis that progressed to lymphoid hyperplasia, sinus histiocytosis and enlargement of mesenteric lymph node.

Keywords: Cadmium chloride, Mesenteric lymph node, Lymphoid hyperplasia, Sinus histiocytosis

1. Introduction

There are many extrinsic factors that affect the vascular and immune systems. The effects varied from single exposure to multiple, also for short period or long periods. Cadmium represents one of the toxic agents and prolonged exposure through different sources may lead to cancer including blood dyscrasia.

Ingestion of cadmium one of these sources by food, which may require emergency treatment. Food contamination represents one of the major sources of cadmium toxicity through the gastrointestinal tract and would accumulate in different organs depending on its dose (Rahimzadeh *et al.*, 2017). It may affect cell proliferation, differentiation and apoptosis. Cadmium binds to the mitochondria and can inhibit

cellular respiration and oxidation phosphorylation at low concentrations (Peana *et al.*, 2022).

The ingestion of toxic material generally though GIT affects mainly the mesenteric lymph nodes, which is the first pass organ for nutrients and microbial substances entering the lymph fluid in the intestinal lamina propria. It is located along the superior and inferior mesenteric arteries and drain lymph from the duodenojejunal flexure until the anal canal (Gelberg, 2018). The presence of cadmium in the blood and body of smokers may be twice of non-smokers due to the content of cadmium in cigarettes (Wiener & Bhandari, 2020). Cd concentrations in the body fluids indicate tobacco addiction more than other types of smoking and could be used as a biomarker for tobacco smoking (Mannino *et al.*, 2004). The switch of smokers from conventional cigarettes to e-cigarettes may have similar Cd urinary levels (Park & Choi, 2019). While other studies indicated the reduction of exposure to Cd in smokers who completely switched from conventional cigarettes to e-cigarettes (Prokopowicz *et al.*, n.d). There were many studies concerned with the carcinogenic effect of cadmium on these lymph nodes. Our study took a new approach to investigate the histological effect of certain toxic doses of cadmium on rat mesenteric lymph nodes within certain period.

2. Material and methods

2.1. Animal care

The study was performed on (20) adult male Albino rats as mammalian models Which was done in the animal house of Iraqi Center for Cancer Research/ Al-Mustansiriyah University. The adult males were used during the experimental study with an average body weight of 230–250g and aged between 6–8 weeks. Rats were housed in plastic cages with metallic mesh covers and kept under controlled conditions at room temperature $21 \pm 4^{\circ}\text{C}$ and photoperiods at 12:12 hrs. Light/dark; the dry food pellets and water were provided *ad libitum* (Chelab *et al.*, 2009).

2.2. Experimental design and treatment

The total rats were divided equally into two groups, one group served as control group which administered tap water and second group as an experimental group which administered oral cadmium chloride (CdCl_2) at a dose of 100 mg/kg/day for two weeks via gastric gavage. in rats and mice, the acute oral LD50 of cadmium was estimated to be 100–300 mg/kg/bw (Substances & Registry, 2008).

2.3. Histological preparations

Some of the experiment animals were dead during the experiment and other was sacrificed by decapitation under ether anesthesia and the mesenteric lymph nodes surrounding the intestinal wall were excised for histological examination. The samples were immediately washed and placed in 10% formaldehyde for 24 hr. for routine histological preparation and (H&E) staining (Suvana *et al.*, 2013). The sections were examined under a light microscope at magnification $\times (10,40,1000)$. The target tissue were the germinal centers and follicular area of the mesenteric lymph nodes, while the target cells were mainly B and T lymphocytes.

2.4. Hematological analysis

Blood film enables us to evaluate differential white blood cell (WBC) count (neutrophils, eosinophils, lymphocytes, basophils and monocytes), furthermore estimation of WBC counts. Blood films are made on glass microscopic slide.

2.5. Statistical analysis

Statistical analysis results were performed using an independent t-test by SPSS V. 23 software. Values are presented as the (mean \pm S.E.) and significant is considered at $P \leq 0.001$.

3. Results

The present study found several findings that related to cadmium chloride toxicity at a dose 100 mg/kg/day on mesenteric lymph nodes in male albino rats for two weeks including external feature like laziness, loss of appetite and weight.

3.1. Changes in the weight of the animals

At the beginning of experiment, we noted that the mean weight of all animals in the two groups which was $(243.7301 \pm 1.432\text{g})$. while the statistical analysis results showed that there were significant differences in the weight of animals between the experimental and control groups ($p \leq 0.001$). The mean weight of animals in the experimental group after two weeks was $(186.8750 \pm 6.20034\text{g})$ compared with the control group (Table 1) and (Fig. 1).

3.2. Hematological analysis

Blood smears showed that WBC differential count in the experimental group after cadmium chloride

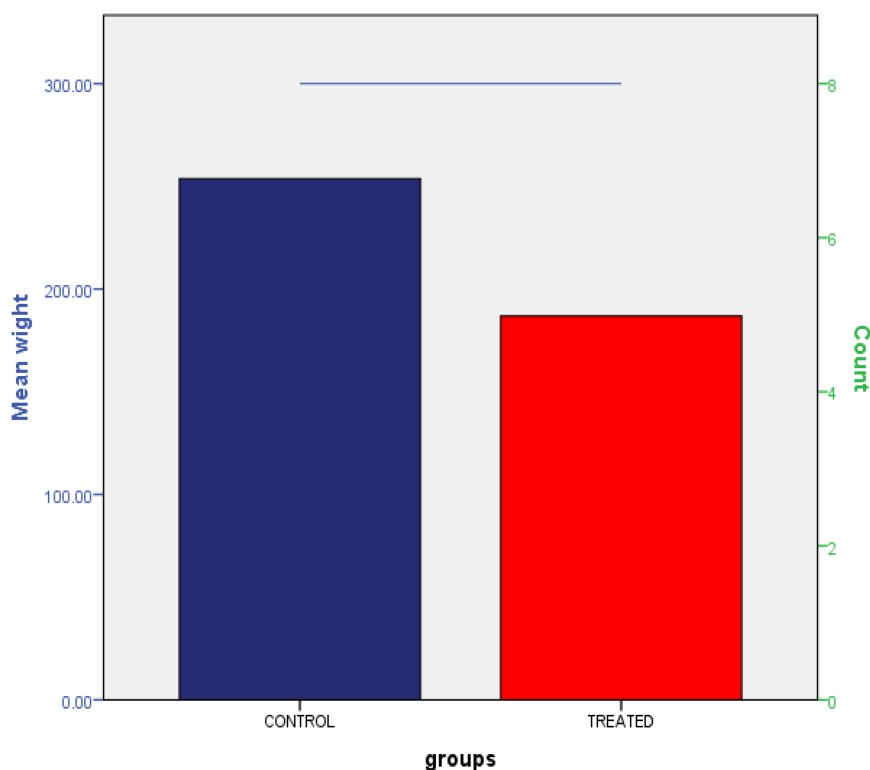


Fig. 1. The toxic effect of cadmium chloride on the weight of the experimental group.

Table 1. The toxic effect of cadmium chloride on the weight of male albino rat after two weeks of administration.

weight of male's albino rat (gm) (Mean ± S.E.)	
Control group	Experimental group
(253.7500 ± 1.56696g).	186.8750 ± 6.20034* g

* Significant differences ($p \leq 0.001$).

administration was increase in the ratio of neutrophil count (29%) and monocyte (4%) compare to the ratio in both group before administration (18%) and (2%) (Fig. 2).

3.3. Histopathological changes

In the present study, the results obtained by using (H&E) stains showed normal mesenteric lymph nodes in the control group (Fig. 3). While the experimental group showed many defects were seen reactive lymphadenitis including paracortical and follicular hyperplasia (lymphoid hyperplasia), sub-capsular sinus expansion, sinus histiocytosis and enlargement of mesenteric lymph node (Figs. 4 and 5).

4. Discussion

In the present study, we found that cadmium caused effects on the experimental animals' group

morphological features and mesenteric lymph node tissue. The previous research confirmed that; cadmium is a heavy toxic metal; it considers primary sources of exposure in the general population are tobacco and food (Faroon *et al.*, 2012). The most sensitive part of the body that is affected by cadmium is the gastrointestinal tract (Lech & Sadlik, 2017), in addition to that, cadmium in rats leads to intestinal damage and inflammation. It has attacked the Immune primary response of major gut-associated mesenteric lymph nodes (Zhao *et al.*, 2006).

4.1. The effect of cadmium on morphological features of rats

We found decrease in the weight of the experimental group, death of some animals, loss the hair of animal and loss of appetite compared with the control group. This agreed with study that showed CdCl_2 caused loss in appetite, poor vitality, significantly reduced activity, weight loss and dark hair color in mice (Chenet *et al.*, 2023; Adegoke *et al.*, Adegoke *et al.*).

4.2. The effect of cadmium on blood

In this research the CdCl_2 appears to increase in the ratio of neutrophils 29% in treated group compared with control group 18% as in (Fig. 2) it agreed with the

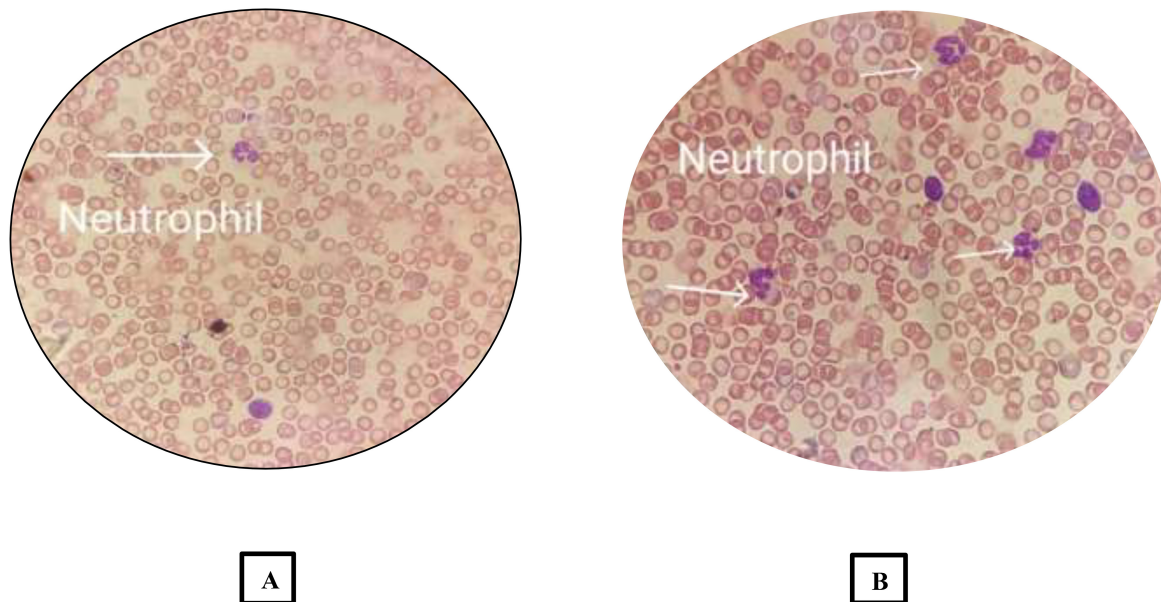


Fig. 2. Blood smear showed increase in neutrophil count in the experimental group (B) compare to control group (A).

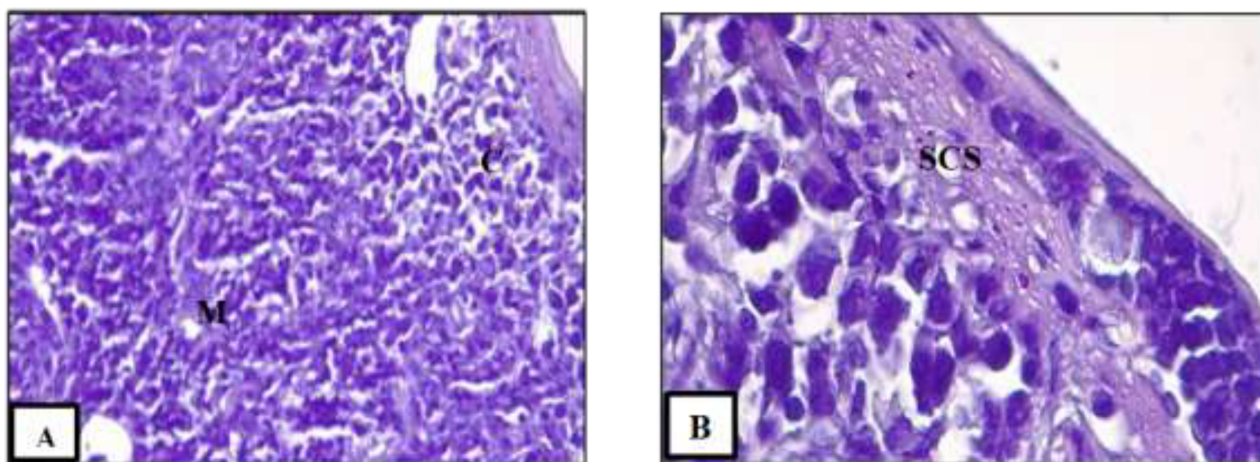


Fig. 3. Cross section of mesenteric lymph node in the control group showed cortex (C), medulla (M) and sub capsular sinus (SCS), (H&E) A-40X, B-1000X.

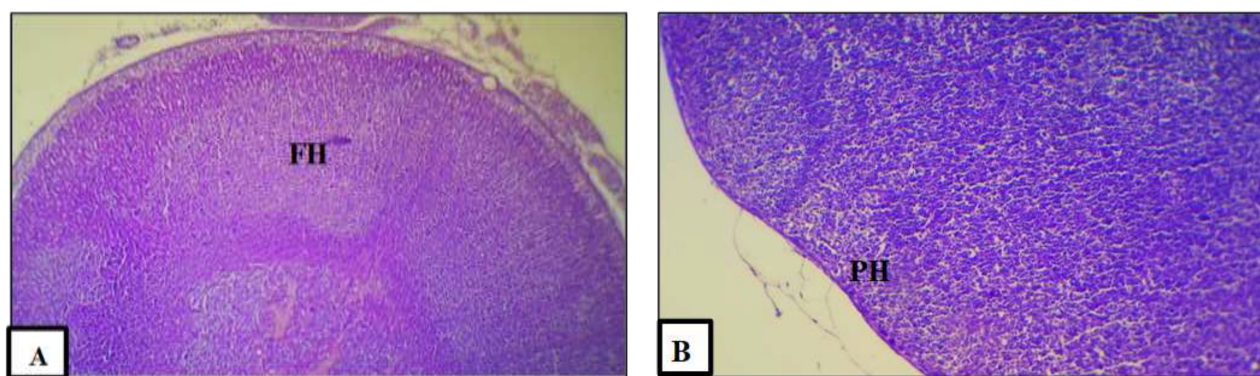


Fig. 4. Cross section of mesenteric lymph node in experimental group showed follicular hyperplasia (FH) and paracortical hyperplasia (PH) (H&E) A&B-X10.

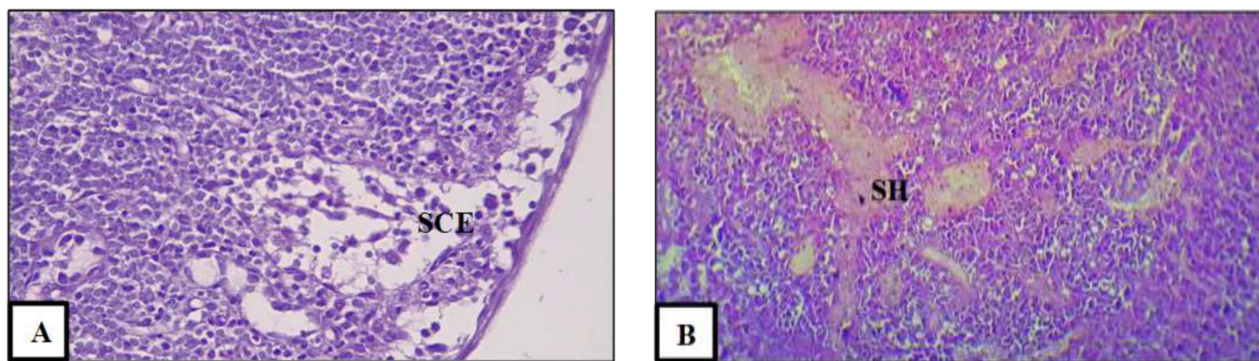


Fig. 5. Cross section of mesenteric lymph node in the experimental group showed subcapsular sinus expansion (SCE) and sinus histiocytosis (SH) (H&E A&B-40X).

authors found CdCl_2 stimulates neutrophil inflammatory activities are responsible for primary tissue injury followed by secondary tissue injury via infiltrated innate immune cells (neutrophils, monocytes and macrophages) (Hosseini-Khannazer *et al.*, 2020; Mirkov *et al.*, 2021).

4.3. Histopathological changes of cadmium chloride

Histological assessment showed obvious enlargement in the size of the mesenteric lymph nodes tissue in the experimental group compare with the control group, as a result of CdCl_2 toxicity. is agreed with researchers who found that the enlargement of mesenteric lymph nodes can occur when the immune system recognizes foreign proteins to mount an attack; then the responding cell lines become duplicated, and therefore increase in size (Freeman & Matto, 2023). In addition, our study showed expansion in subcapsular mesenteric lymph node, this result was in accordance to Which found that experimental group dosed with CdCl_2 ; degeneration of the plasma cell and cellular infiltration were observed in the rise and expansion of the sinuses (Luo *et al.*, 2023).

The occurrence of clear defects in the mesenteric lymph nodes tissue was seen because of cadmium dosing, the examination showed signs of inflammation presented as the occurrence of reactive lymphadenitis, lymphoid hyperplasia and sinus histiocytosis and this accordance with the visible signs and symptoms that occurred in the experimental animals, including loss of appetite, feeling ill, fatigue or lack of energy, raised of neutrophils count and death of some animals. This would agree with the researchers found reactive lymphadenitis infections condition usually associated with wide different of exposures such as chemicals, environmental pollutants, infection, drugs, and even malignancy (Chemonges *et al.*, 2021). Furthermore, the condition

of mesenteric lymphadenitis (Lymphadenopathies) such as follicular hyperplasia can show diversified symptoms such as chills, fever, unexplained weight loss night sweats and prominent localizing symptoms Freeman & Matto (2023) consequence of the occurrence of reactive lymphadenitis, sinus histiocytosis appeared clearly as in (Fig. 5) this corresponds with the authors found the phenomenon of sinus histiocytosis is a relatively common associated with a wide variety of inflammatory conditions and infectious. It is characterized by the expansion of sinuses due to the increased number of histiocytes and the lesions of histiocytes admixed with small plasma cells, lymphocytes and neutrophils (Tzankov & Dirnhofer, 2018; Naeim *et al.*, 2018) moreover, the authors showed reactive lymphadenitis associated sinus histiocytosis (Iannitti *et al.*, 2010).

The present study reveals the CdCl_2 caused the lymphoid hyperplasia as in (Fig. 4) this is compatible with the researchers found an enlargement of a tissue (hyperplasia) it resulted from cell proliferation which leading to an increase in the amount of organic tissue (Sembulingam & Sembulingam, 2012). However, the hyperplasia occurs as a pathological response, if an excess of growth factor or hormone is responsible for the stimuli (Kumar *et al.*, 2013). As a result, the authors found that CdCl_2 which are positive for epidermal growth factor receptor and its promoted cell growth (Wei *et al.*, 2015), in addition; CdCl_2 even at low concentrations has a potential to promote cell proliferation and reported that Cd significantly promoted the proliferation by elevated cell viability (Zhang *et al.*, 2021; Wang *et al.*, 2021) furthermore, (L. M. Weiss, and D. O'Malley) revealed this hyperplasia: is the most common sort of reactive lymphadenitis (Weiss & O'Malley, 2013). Also, we noticed that hyperplasia stroked the B-lymphocytes and T lymphocytes regions this agree with authors Stand firm the Lymphoid hyperplasia can involve both the B-cell-rich follicles and T-cell-rich paracortex can be

indicative of humeral response or a cell-mediated, due to stimulation by antigens in the gastrointestinal tract (Yan *et al.*, 2022).

5. Conclusions

The present study appeared that exposure to oral Cadmium chloride after two weeks caused decrease in the weight with hematological changes that affect increase in neutrophil count mainly while histopathological changes include lymphadenitis that progressed to lymphoid hyperplasia, sinus histiocytosis and enlargement of mesenteric lymph node. In future researches, we recommend prolonging the dosing period for a month or two due to the possibility of lymphoma occurring. And using other tests such as IHC and SEM.

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Ethics approval and consent to participate

Written approval was obtained from the Ethical Approval Committee of the University of Mustansiriyah, Iraq. Study data/information was used for research purposes only.

Consent for publication

No personal data are included.

Availability of data and material

The datasets analyzed and produced during this study can be acquired from the corresponding author on reasonable request.

Competing interests

The authors profess that there is no conflict of interest.

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Authors' contributions

All authors have made significant, direct, and mental contributions to the work and have confirmed it for publication.

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