Effect Of Different Doses Of Cadmium Chloride On Interleukin-2, Lymphocyte Transformation And DNA Fragmentation In Adult Male Albino rats

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ABSTRACT

Forty adult male albino rats were divided randomly into 4 equal groups (one control and three treated groups). The control group received only dis. water, while the treated groups received 5 ppm, 50 ppm and 100 ppm of cadmium chloride (CdCl2) for consecutive 90 days in drinking water. Blood samples were collected just before scarification of the rats and serum was separated. The level of interleukin-2 in the serum of control and treated male albino rats was measured by ELISA. Lymphocyte transformation and DNA fragmentation assay in blood lymphocyte of rats were evaluated. At Day 90 of the experiment, rats were sacrificed by cervical dislocation and thymus was collected, weighted and stored in 10% neutral buffered formalin for histopathology. Data were analyzed by one-way analysis of variance using GraphPad Prism software. The interleukin-2 level was declined in a dose-dependent manner in treated rats with different doses (5, 50 and 100 ppm) of CdCl2. Obviously the rats treated with 100 ppm of CdCl2 showed a significant decrease ($P<0.001$) in the level of interleukin-2 compared to control. Moreover, rats administrated 100 ppm of CdCl2 showed a significantly ($P<0.05$) high rate of lymphocyte transformation compared to control. Rats treated with 5, 50 and 100 ppm of CdCl2 showed certain levels of DNA strand breaks in blood lymphocyte. Male rats exposed to100 ppm ($P<0.001$) and 50 ppm ($P<0.05$) of CdCl2 showed a significant decrease in the relative thymus weight than that of control ones. The thymus of rats treated with 50 and 100 ppm of CdCl2 showed a reduction in the medulla's diameter and interlobular fat globules compared to control. In conclusion, CdCl2 decreased the production of interleukin-2 in dose-dependent pattern and triggered the lymphocyte transformation particularly in rats exposed to 100 ppm of CdCl2. While, CdCl2 at different doses might induce DNA fragmentation in blood lymphocyte and might lead to thymic degeneration.

INTRODUCTION

There have been some conflicting results in the effect of cadmium (Cd) exposure on various immune components. Despite, the well-documented effects of Cd ***(1-2)*** some studies demonstrated only negligible influences of Cd on the immune response. Chronic Cd feeding of 5 mg/kg body wt up to 7 weeks in rats ***(3)*** and for 2–6 months in the primate Rhesus monkeys ***(4)*** did not result in any signs of immunomodulation. Furthermore, proliferation was insignificantly increased in these monkeys. Similarly, chronic exposure of B6C3F1 mice to 10–250μg/ml of CdCl2 for 90 days, revealed no changes in humoral immunity even though Cd had accumulated in significant quantities in the tissues ***(5)***. Cd at a dose of 20 μg/ml preferentially enhances the proliferation of murine TH2 cells activated *in vitro* by concanavalin A ***(6)***.

Interleukin-2 is a cytokine released from T-helper lymphocytes plays a central role in the activation and proliferation of lymphocytes. IL-2 plays a pivotal role in the expansion of most T-cells, natural killer cells and B-cells during certain phases of their response. Through these mechanisms, it plays an important role in antitumor immune responses ***(7)***.

There are different research results on the effect of cadmium on lymphocyte transformation. In some reports, cadmium did not significantly affect the response of lymphocyte proliferation by concanavalin A (Con A) ***(8)***, while in other reports Spleen cells from mice given 200 ppm Cd for 3 to 4 weeks showed increased proliferative responses to Con A ***(9)***. The stimulation index of spleen lymphocytes to Con A tended to be higher in cadmium-treated animals than control mice ***(10)***.

Apoptosis, or physiological cell death, can be distinguished from necrosis on the basis of a series of morphological and biochemical parameters. Among these parameters, DNA fragmentation is very typical of the apoptotic process. *In vitro*, thymocytes exposure to Cd resulted in a DNA ladder confirming the induction of apoptosis by the heavy metal. The same result has been observed for mouse thymocytes ***(11)***, human T cell line ***(12)***, human promonocytic cells ***(13)*** and normal human mononuclear cells ***(14)***.

The objective of this study is to evaluate the effect of different doses of cadmium chloride in drinking water for 90 days on interleukin-2, lymphocyte transformation, DNA fragmentation and thymus histopathology in adult male albino rats.

MATERIALS AND METHODS

Experimental animals

Forty male albino rats weighted (100-140g) were assigned randomly and divided into 4 equal groups, one control and three treated groups. All groups received distilled water for one week. From the second week of the experiment, the control group received only dis. water, while the treated groups received 5 ppm, 50 ppm and 100 ppm of CdCl2 for 90 days.

Interleukin assay

Blood samples were collected from control and CdCl2 treated rats at the end of experimental period and serum was separated. Serum Interleukin-2 assay was measured using rat IL-2 ELISA kit (Cat # KA0276, Abnova, USA).

Lymphocyte transformation test

Blood samples were collected from control and treated rats in heparinized tubes just before slaughter of the rats. The sample immediately put on ice bags and transferred immediately to the laboratory for lymphocyte transformation assay by MTT reduction assay **(15-19)**.

DNA fragmentation assay

DNA extraction

Blood samples were collected from control and treated rats in heparinized tubes just before slaughter of the rats. The sample immediately put on ice bags and transferred immediately to the laboratory for DNA fragmentation assay. Lymphocyte were pelleted by centrifugation at 200 *xg* for 10 min and the pellet was lysed with 0.5 ml lysis buffer (10mM Tris–HCl, pH7.5, 20mMEDTAand 0.5%Triton X-100) on ice for 30 min. The DNA in lysed solution was extracted with phenol/chloroform and precipitated with 3M sodium acetate (pH 5.2) and cold ethanol. After repeated washings, the DNA was dissolved in TE buffer (10mM Tris–HCl, pH 8.0, and 1mM EDTA). The purity of DNA at 260 and 280 nm absorbance ratio was between 1.7 and 1.9.

DNA fragmentation test

The internucleosomal fragmentation pattern (DNA ladder) was carried out by Agarose gel electrophoresis as DNA (2µg) was loaded on 0.7% agarose gel and electrophoresis carried out then the bands were visualized by ethidium bromide staining under UV light **(20-21)**.

Organ relative weights

At the end of the study period, rats were sacrificed by cervical dislocation and thymus was dissected. Thymus were removed and weighed. The organ relative weight (organ weight / body weight X 100) was measured for each treated and control groups.

Histopathological examination

Immediately after scarification of the rats, the rats were necropsied and all organs and tissue were subjected to gross examination. Whole thymus was fixed in 10% neutral buffered formalin. They were gradually dehydrated and embedded in paraffin; 5-µm sections were stained with hematoxylin and eosin (H&E) for histopathological examination ***(22)***.

Statistical analysis

Data were analyzed by one-way analysis of variance followed by Tukey's posttest using GraphPad Prism version 3 for Windows (GraphPad Software, San Diego California, USA). A p-value below 0.05 was considered statistically significant.

RESULTS

Interleukin-2

The interleukin-2 level was declined in a dose-dependent manner in treated rats with different doses (5, 50 and 100 ppm) of CdCl2. Obviously the rats treated with 100 ppm of CdCl2 showed a significant decrease ($P<0.001$) in the level of interleukin-2 compared to control and rats treated with 5 ppm of CdCl2 (Table 1 and Fig. 1).

Lymphocyte transformation assay

Rats received 100 ppm CdCl2 in drinking water for 90 days showed a significant increase in the lymphocyte transformation level. However, rats treated with 5 and 50 ppm of CdCl2 showed a relatively decline in the level of lymphocyte proliferation than that of control rats (Table 1 and Fig. 2).

Thymus weight

The relative weight of the thymus was decreased in a dose dependent pattern as male rats exposed to100 ppm ($P<0.001$) and 50 ppm ($P<0.05$) of CdCl2 showed a significant decreased in the relative thymus weight than the control ones (Table 1).

Table 1. Effect of CdCl2 (5, 50 and 100 ppm in drinking water) on interleukin-2, lymphocyte transformation and thymus relative weight (mean ± SE) in treated rats for 90 days

	Control	5 ppm	50 ppm	100 ppm
Interleukin-2 (pg/ml)	30.6 ± 1.9^{a}	14.9 ± 1.3^{b}	7.4 ± 0.4^{cd}	2.7 ± 0.3^{d}
Lymphocyte transformation (OD)	0.6 ± 0.05^{a}	0.4 ± 0.04^{a}	0.4 ± 0.02^{a}	0.9 ± 0.01^{b}
Thymus relative weight (gm)	0.05 ± 0.01^{a}	0.04 ± 0.003^{ab}	0.03 ± 0.01^{b}	0.02 ± 0.004^{b}

Superscripts with dissimilar values are significantly different within the same line

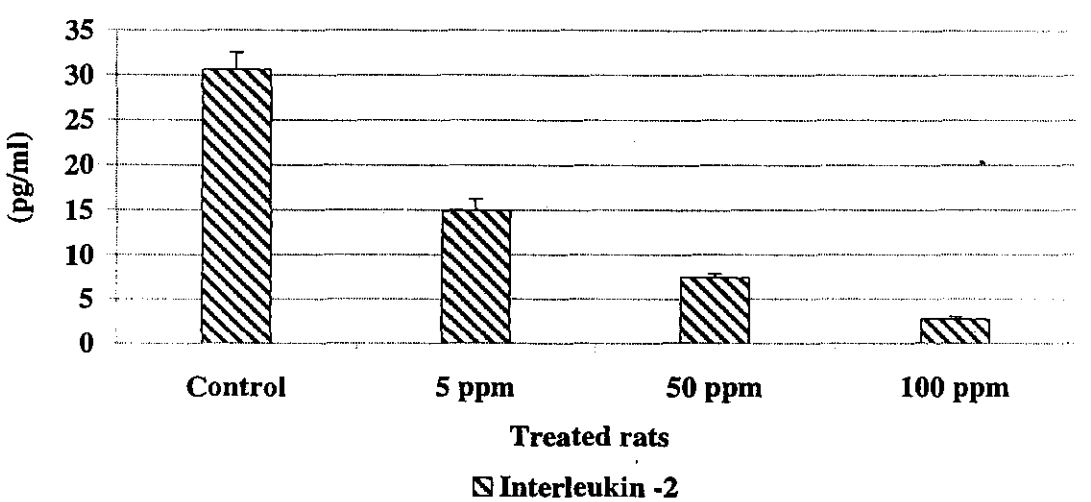

Figure 1. Effect of $CdCl_2$ (5, 50 and 100 ppm in drinking water) on interleukin-2 (mean ± SE) in treated male albino rats at Day 90

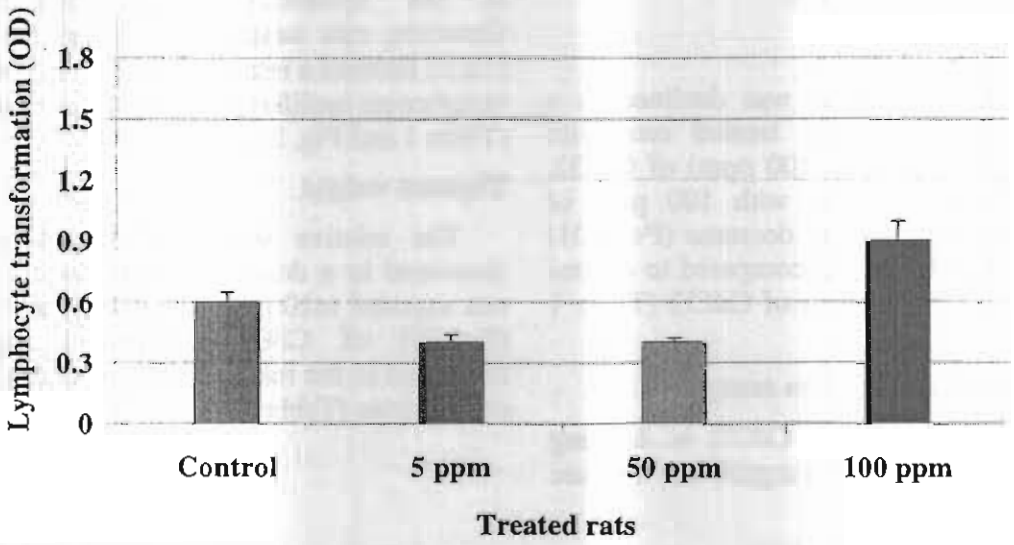

Figure 2. Effect of CdCl2 (5, 50 and 100 ppm in drinking water) on lymphocyte transformation test (mean ± SE) in treated rats at Day 90

DNA fragmentation test

The DNA fragmentation assay indicated the level of DNA strand break due to the CdCl2 exposure. Moreover, it indicated the level of apoptosis in the lymphocyte of cadmium chloride treated rats. The incidence of DNA break and apoptosis increased where the dose of CdCl2 increased. The high level of DNA fragmentation (23.7%) is mostly observed in rats exposed to 100 ppm CdCl2 for 90 days (Table 2 and Fig. 3).

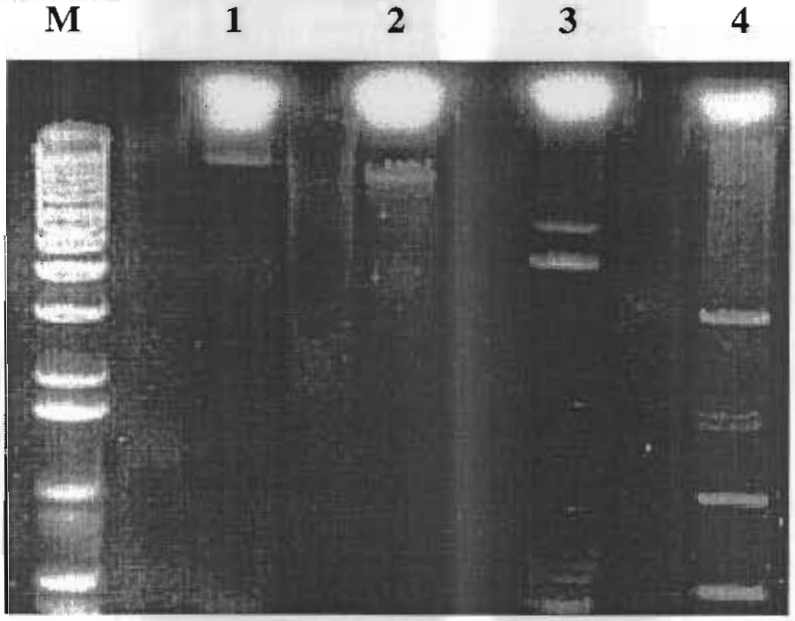

Figure 3. DNA fragmentation test of blood lymphocyte of control and treated male albino rats collected just before scarification. Ladder marker 100- 3000 bp (M), control (Lane no. 1), rats treated with 5 ppm of CdCl2 (Lane no. 2), rats treated with 50 ppm of CdCl2 (Lane no. 3) and rats treated with 100 ppm of CdCl2 (Lane no. 4)

Table 2. DNA fragmentation test of blood lymphocyte collected just before scarification of treated adult male albino rats treated with different doses of CdCl2

	Ladder		Control		5 ppm		50ppm		100 ppm	
	bp	%	bp	%	bp	%	bp	%	bp	%
1	3000	6.6	3820.3	100	3861.1	90.3	3957.2	80.3	3809.3	76.3
2	2000	5.3			2975.3	6.4	1021.4	6.2	971.1	1.4
3	1500	6.3			1942.4	3.3	813.1	3.2	790.4	4.2
4	1200	7.8					360.1	3.0	720.4	1.2
5	1000	7.3					180.4	7.3	540.3	4.8
6	900	7.3							360.2	3.2
7	800	5.9							210.4	2.8
8	700	7.2							180.4	2.8
9	600	9.7							125.5	3.3
10	500	5.1								
11	400	8.4								
12	300	8.9								
13	200	8.3								
14	100	5.9								
Sum		100		100		100		100		100

Thymus histopathology

The thymus of rats treated with 50 ppm of CdCl2 showed a reduction in the medulla's diameter compared to control (Fig. 4 & 5). Furthermore, the thymus of rats treated with 100 ppm of CdCl2 showed fat globules between the lobes of the thymus. The cortex showed lymphocyte depletion. The medulla replaced with C.T with a reduction in its diameter with low number of lymphocytes. This might indicate degenerative changes in the thymus of rats particularly treated with 100 ppm of CdCl2 (Fig. 6 & 7).

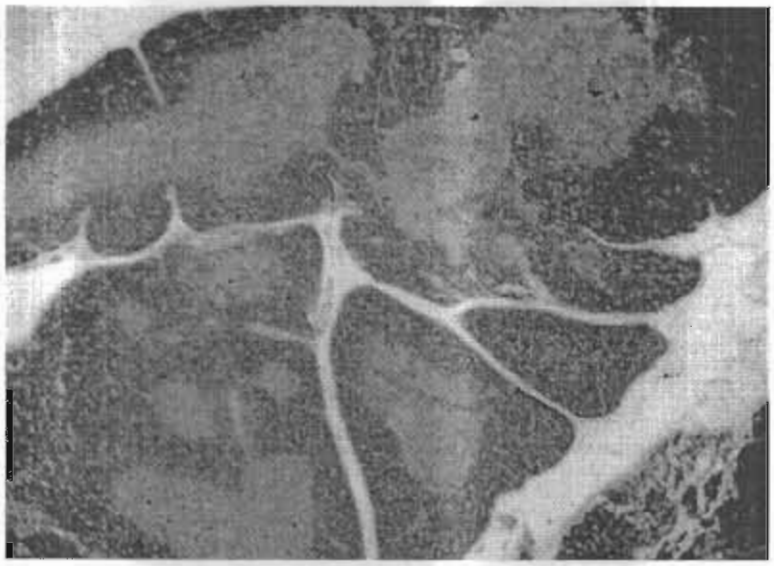

Figure 4. Section in thymus of control rats. The medulla showed normal diameter. H&E 40 X

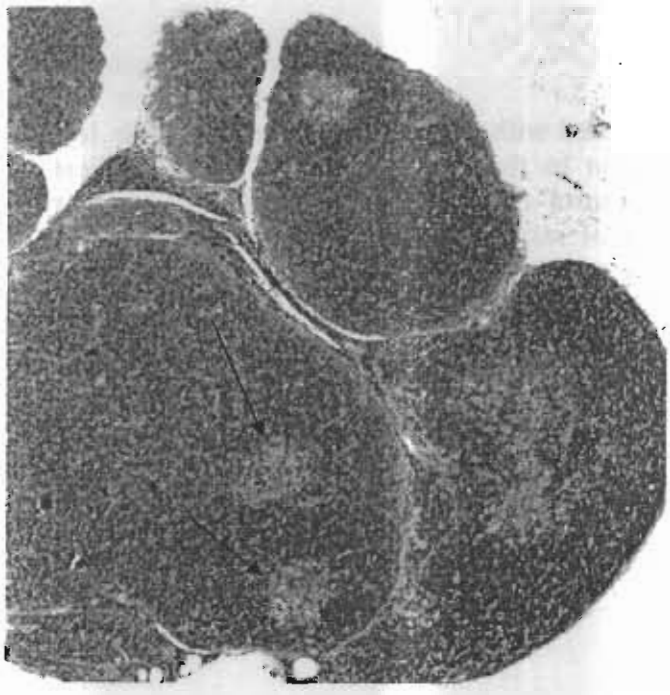

Figure 5. Section in thymus of rat treated with 50 ppm of CdCl2. The medulla showed a reduction in its diameter compared with control rats. H&E. 40 X

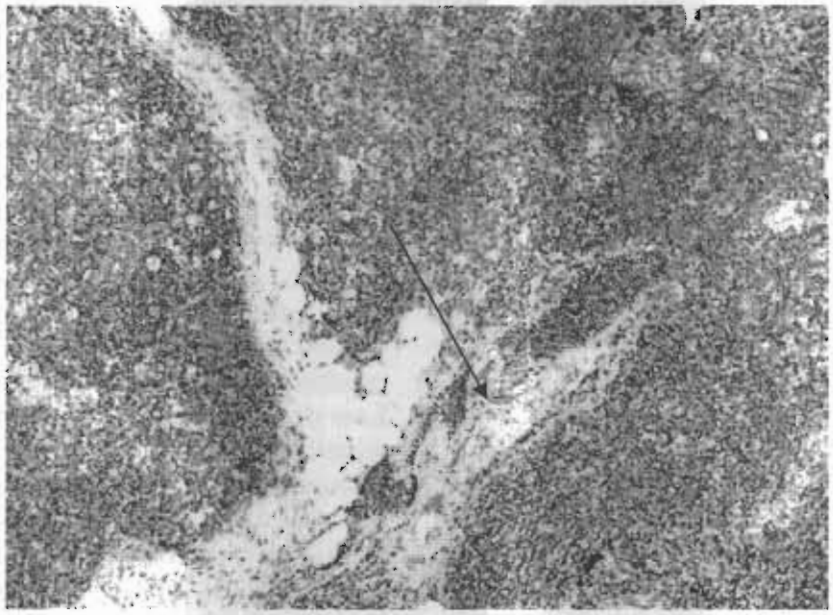

Figure 6. Section in thymus of rat treated with 100 ppm of $CdCl_2$ showed lymphocyte depletion in the cortex and the medulla replaced with connective tissue (black arrow). H&E 100 X

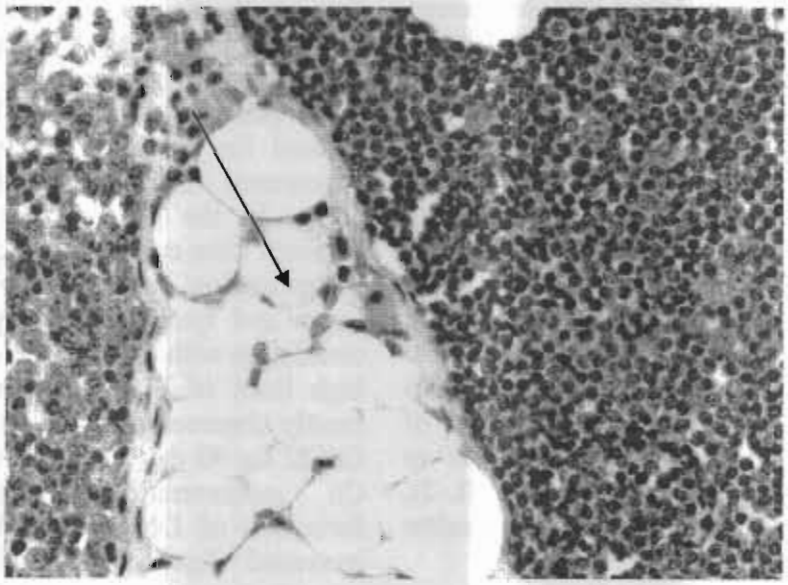

Figure 7. Section in thymus of treated rats with 100 ppm of CdCl2 showed fat globules in the interlobular space. H&E 400 X

DISCUSSION

The immunotoxic effects of cadmium remain controversial. Exposure of humans or animals to cadmium is still of major toxicological importance. One site of attack of cadmium in the body is considered to be the immune system *(23)*. Although immunomodulatory activities have been investigated in several *in vivo* and *in vitro* model systems, conflicting results of Cd immunotoxicity have emerged. Data about the immunotoxicity of cadmium are contradictory; in some cases it stimulates the immune response, in others Cd acts as an immunosuppressant, while yet other studies find no effect, even if seemingly identical aspects of immunity were investigated *(24)*.

In the present study, the interleukin-2 (IL-2) level was declined in a dose-dependent manner in treated rats with different doses (5, 50 and 100 ppm) of CdCl2. In agreement to our result, a decrease in IL-2 production in the presence of 10^{-4} M Cd^{2+} by human peripheral blood lymphocytes *in vitro* was found *(25)*. Moreover, it has been estimated that the IL-2 staining in cadmium-treated spleen lymphocyte cells removed from male mice was markedly reduced. Generally, a cadmium-induced decrease in intracellular IL-2 content *(26)*. Failure of IL-2 secretion and reduction of IL-2R expression in the *in vitro* Cd-treated cells were reported for human lymphocytes *(27)*. Cadmium led to a significant increase in the level of some cytokines such as IL-1a and IL-10, however, the production of IL-2, on the other hand, was significantly depressed *(6)*. Exposure of activated T-cells to low Cd doses leads to suppression of early T_H1 cytokine events (IL-2), and stimulation of the T_H2 cytokines IL-4 and/or IL-10 *(28)*.

In the present study, the lymphocyte transformation is significantly increased in rats treated with 100 ppm of cadmium chloride. In agreement to our result, Spleen cells from mice given 200 ppm Cd for 3 to 4 weeks showed increased proliferative responses to Con A. The authors conclude that Cd enhances the proliferative response to T-cell mitogens *(9)*. Furthermore, an increased proliferative response of T-cells to PHA mitogens after 4 weeks of oral cadmium treatment was observed *(9)*. Moreover, in other study, cadmium at the dose of 50 ppm in drinking water tended to increase the proliferative response of spleen cells to T-cell mitogen-PHA, but with no significant difference from the non-treated controls *(29)*. CdCl2 at very low concentrations were able to activate murine lymphocytes activated *in vitro* by Con A resulting in increased rate of DNA synthesis *(6)*. Low doses of cadmium treatment, at 1 μM level, stimulated sheep lymphocytes proliferation *(30)*. In contrary, cadmium did not significantly affect the response of lymphocyte proliferation by Con A *(8)*. Mouse strain variations in Cd-mediated suppression of lymphocyte proliferation are not based on intrinsic lymphocyte sensitivities, but likely are due to differences in the metabolism of Cd, which is under genetic control *(31)*.

DNA fragmentation is one of the hallmarks of apoptosis in Cd-exposed rats. Cadmium triggers an apoptosis like form of cell death in many cell types including T lymphocytes *(12)*. Extensively fragmented double-stranded DNA can be separated from chromosomic DNA upon centrifugal sedimentation. Agarose gel electrophoresis of cellular DNA is the usual method for demonstrating apoptosis, with the appearance of a characteristic ladder pattern *(32)*. In the present study, the lymphocyte obtained from control rats didn't show any DNA fragmentation. However, the incidence of DNA break and lymphocyte apoptosis increased in correlation with the increase of CdCl2 dose. The high level of DNA fragmentation (23.7%) is mostly observed in rats exposed to 100 ppm of CdCl2 for 90 days. In the presence of the lower Cd concentrations, the dose-dependent formation of DNA strand breaks in the cells increased significantly *(33)*. However, high concentration of Cd destabilizes DNA causing DNA strand breaks and other DNA damage. It was found that internucleosomal cleavage of DNA from apoptotic cells gave products showing a 'ladder' pattern on agarose gel electrophoresis *(34)*. These morphological and biochemical features are accepted to be important criteria for confirmation of apoptosis *(35)*. Activation of endogenous endonuclease,

resulting in DNA degradation, is thought to be responsible for the morphological and biochemical alterations seen in apoptosis *(36)*. Furthermore, a significant increase in DNA fragmentation in the presence of 10 mM Cd in a Ca^{+2} free system was observed *(37)*. *In vitro* Cd exposure induced apoptotic features in mouse thymocytes *(11)*.

In the present study, The adult male albino rats exposed to 50 and 100 ppm of CdCl2 showed a significant decline in the relative thymus weight than that of control. Thymus, an important primary lymphoid organ, the place where successive stages of cell development and selection generate functionally competent T cells from immature precursor cells is a target organ of Cd-induced immunotoxicity *(38)*. For instance, cadmium exposure resulted in thymic damage and modified the proliferation rate of thymocytes in rats *(39)*. In agreement to our result, a number of *in vivo* experiments have demonstrated that cadmium is able to cause marked weight decrease of thymus or thymus atrophy *(40-41)*.

Thymus of rats treated with 50 and 100 ppm of CdCl2 showed fat globules between the lobes of the thymus. The medulla showed a reduction in diameter with low number of lymphocytes. This might indicate degenerative changes in the thymus of rats particularly treated with 100 ppm of cadmium chloride. Our result was supported with the finding in male Wister rats that exposed to sc injections of CdCl2 at doses of 0.5, 1, and 2 mg Cd/kg, 3 days/week, for 4 weeks and showed histological changes of fibrous tissue proliferation in the thymus *(42)*.

In conclusion, the results indicated that cadmium chloride affected the immune system in adult male albino rats through decreasing the production of interleukin-2 in dose-dependent pattern, triggering the lymphocyte transformation and inducing DNA fragmentation in blood lymphocyte particularly in rats exposed to 100 ppm of cadmium chloride for 90 days.

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الملخص العربى

تأثير جرعات مختلفة من كلوريد الكادميوم علي الانترلوكين-٢ ومعدل تكسير الحامض النووى الديوكسى lymphocyte transformation,

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أجريت هذه الدراسة على عدد (٤٠) من ذكور الفئران البالغة البيضاء يتراوح أوزانها بين ١٠٠جم و ١٤٠جم. تم استخدام كلوريد الكادميوم في معالجة ذكور الفئران البالغة بجرعات مختلفة, تعرضت الفئران لهذه الجرعات من خلال مياه الشرب و لمدة تسعون يوما مدة إجراء البحث. قسمت الفئران إلى ٤ مجاميع كل مجموعة عشرة فئران: المجموعة الأولى: هي المجموعة الضابطة و التي تجرعت الماء المقطر فقط. المجموعة الثانية : هي المجموعة التي تعرضت إلى جرعة من كلوريد الكادميوم تعادل (٥) أجزاء في المليون.المجموعة الثالثة: هي المجموعة التي تعرضت إلى جرعة من كلوريد الكادميوم تعادل (٥٠) جزء في المليون.المجموعة الرابعة: هي المجموعة التي تعرضت إلى جرعة من كلوريد الكادميوم تعادل (١٠٠) جزء في المليون. في اليوم الأخير من التجربة تم أخذ عينات من الدم جزء منها سحب علي الهيبارين لإجراء lymphocyte transformation assay and DNA fragmentation و الأخر استخدم لاستخلاص السيرم لقياس مستوى Interleukin-2 . تم ذبح الفئران في نهاية التجربة و أخذت الغدة الصعترية و تم وزنها و فحص أنسجتها لمعرفة مدى التأثير السمي لكلوريد الكادميوم بجرعاته المختلفة.

و قد أسفرت نتائج هذا البحث عن الأتي: كان هناك انخفاض معنوي في مستوى Interleukin-2 في المجموعات التي عولجت بكلوريد الكادميوم مقارنة بالمجموعة الضابطة. حدث ارتفاع معنوي في معدل تكسير الحامض النووى الديوكسى ريبوز في المجموعة التي اعطيت (١٠٠) جزء في المليون كلوريد الكادميوم كما انة حدث ارتفاع معنوي في معدل Lymphocyte transformation في المجموعة التي تلقت (١٠٠) جزء في المليون كلوريد الكادميوم. كان هناك نقص معنوي في وزن الغدة الصعترية في المجموعة التي تلقت (١٠٠) جزء في المليون كلوريد الكادميوم.

و في الختام فان استخدام كلوريد الكادميوم في مياه الشرب لمدة تسعون يوما في ذكور الفئران البيضاء البالغة بجرعات مختلفة (١٠٠،٥٠،٥) جزء في المليون يقلل من انتاج الانترلوكين-٢ ويساعد على ضمور الغدة الصعترية, و أيضا يمكن لكلوريد الكادميوم بجرعاته المختلفة الحث علي تفتيت الحمض النووي في الخلايا اللمفاوية وزيادة معدلLymphocyte transformation .