Determination of the lethal dose 50% (LD_{50}) of cadmium chloride and the histopathological changes in male mice liver and kidneys

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The lethal dose 50% (LD_{50}) of cadmium chloride (CdCl_{2}) was determined for a group of mice by administering graded doses to determine the dose that killed 50% of the mice within a certain period (24-48 hours). The body weight of cadmium in the liver and the number of mice in the experimental group were measured using a spectrometer, and the activity of the target enzyme was measured in the target tissue. The mice were then injected with a subcutaneous dose of 0.15 ml of cadmium chloride (equivalent to the LD_{50} value determined in the current study and equal to 5.98 mg/kg of body weight) for short periods (24, 48, and 72 hours) and long periods (1, 4, and 8 weeks) and then killed. The results showed the effects of short-term and long-term exposure to cadmium in the liver and the number of mice, depending on the exposure period. The results of the spectrometric analysis of cadmium in the liver and the number of mice showed a significant increase depending on the exposure period, ranging from 98% to 145% in the liver and 100% to 300% in the number of mice, respectively. The same trend was observed in the histopathological changes of the liver tissue, showing necrosis, degeneration, and other pathological changes. The results of the histochemical analysis of the effects of cadmium showed that the effects of long-term exposure were more pronounced than those of short-term exposure.

الخلاصة
تحديد الجرعة القاتلة (LD_{50}) للكلوريد الكادميوم لمجموعة من الحيوانات وجرعات متدرجة لتحديد الجرعة التي تقتل 50% من فئران التجربة ضمن فترة زمنية محددة (24-48 ساعة). وحدد محتوى الكلوريد الكادميوم في كبد و клمية الفئران المعاملة وحيوانات السطيرة بواسطة جهاز الامتصاص الصحي وتم قياس مستوى أنزيم الفسفاتاز الفاعلي في أنسجة الهدف. ثم جرعت ذكور الفئران بجرعة مساوية لـ LD_{50} وتحت المراقبة في الدراسة الحالية والمساوية لـ 5.98 ملغ/كم من وزن الجسم وفترات قصيرة الأمد (24 و48 و72 ساعة) وفترات طويلة الأمد (1 و4 و8 أسابيع) ثم شرحت الفئران بعد ذلك.

أظهرت النتائج تأثير تجريع الكادميوم القصير وطويل الأمد في كبد وكليه الفئران والتي اعتمد على فترة التجريع. وأظهر فحص جهاز الامتصاص الصحي لحجم الكلوريد الكادميوم في كل من الكبد والكلية زيادة ملحوظة واعتمادا على فترة التعرض وتراوحت بين 98% - 145% في الكبد و 100% - 300% في الكلية عند فترات التجريع قصيرة الأمد أما فترات التجريع طويل الأمد فكانت الزيادة من 143% - 173% في الكبد و 270% - 370% في الكلية. كذلك أظهرت هذه الدراسة تأثيرات نسجية ألمراضية في أنسجة الكبد والكلية. تسبب الكادميوم عند فترات التجريع طويل الأمد تلف نسيجي وشملت اضطرابات الأوعية الكلوية وبور الكليا. المبطنة لها والتهاب الأنسجة البنية وانتفاخ الكبيبة في الكلية. كذلك لوحظ تلف أنسجة الكبد والمتصلة في الالتهابات غير المخصصة ومتلازمًا فيما كانت هذه التأثيرات أقل وضوحًا في فترات التجريع قصير الأمد. تشير نتائج فحوصات الكيميائيات لتأثير الكادميوم السمى.
ABSTRACT

The lethal dose 50 (LD<sub>50</sub>) test involves the administration of cadmium in the form of CdCl<sub>2</sub> to group of animals at an increasing dose in order to determine the dose that kills 50 percent of the test mice within a set of time frame (24 and 48h). The cadmium contents of liver and kidneys from dosed and control animals were determined by atomic absorption spectroscopy moreover, levels of alkaline phosphatase (ALP), in the target tissue were measured. Male mice were dosed by 0.15ml cadmium (equivalent to the LD<sub>50</sub> 5.98 mg/kg body weight) in the form of CdCl<sub>2</sub> via intraperitoneal injection for short-term treatment once per 24, 48 and 72h and, for long-term treatment once per week for 1, 4and 8 weeks then, sacrificed.

The results showed that the effect of short-term and long-term Cd-administration on hepatic and renal Cd accumulation in the Swiss mice was found to be time dependent fashion. Atomic absorption examination showed that the Cd-content in both liver and kidneys increased significantly time dependent by 98-145% in liver and 100-300% in kidney at short-term treatment whereas for long-term treatment this increasing were 143-173% in liver and 200-370% in kidney respectively. This study also presents the histopathological effects in mice liver and kidney. Long-term Cd exposure produced damage to the entire kidney, including tubular degeneration, tubular cell apoptosis, interstitial inflammation and glomerular swelling and also resulted in liver injury including non-specific chronic inflammation and apoptosis, while it was less pronounced in short-term exposure. Results of the biochemical profile indicated marked hepatic and renal toxic effect of cadmium reflected by the dramatic change in alkaline phosphatase (ALP) activity level, in the liver and kidney tissue. a significant increase (from 100 to 130%) in hepatic ALP activity, while the renal ALP activity level decreased by (from 95% to 65 %) as compared with those levels of the control in all relative periods of exposure.

KEY WORDS: Cadmium, Histopathological, LD<sub>50</sub>, ALP.
INTRODUCTION

Cadmium (Cd) is an environmental pollutant commonly considered to be one of the most important toxic for human and animals, due to increasing level in the environment as a result of industrial and agricultural practices (1). Cadmium is extremely hazardous to life and has been involved in historic poisoning episodes of human and animal population. It is a serious lethal occupational and environmental toxic, known for its high toxicity, which may affect living systems in various ways (2). The effective dose values (LD$_{50}$) of cadmium as documented in environmental health criteria for a single intraperitoneal dose of cadmium in the form of CdCl$_2$ for the control mice was ranging between 5.6, 7.5 and 7mg/kg b.w (3,4,5).

Cadmium is deposited in various organs and tissues, cadmium toxicity has been known to result via ingestion of cadmium containing food (6). During chronic exposure, Cd induced toxicity in the liver and kidneys is dependent on renal and hepatic Cd concentration (7,8,). There were differences in animal susceptibility to Cd toxicity regarding concentration level and the reason for this differences is not known and remain to be elucidated (9). Cadmium has been shown evidence to cause histopathological changes in liver and kidney in small rodent (10). Most of the Cd that is absorbed after oral exposure accumulates in the liver and kidneys, where it induces injury including protein denaturation and formation of reactive oxygen species (ROS) and lipid peroxidation (11), which in turn depress hepatic and renal functions (12,13). Acute exposure to Cd produces hepatic injury, whereas chronic exposure results in renal injury (1).

Chronic Cd exposure produces damage to the entire kidney, including tubular degeneration, tubular cell apoptosis, interstitial inflammation and glomerular swelling (14). Chronic exposure to Cd results also in liver injury including non-specific chronic inflammation and apoptosis (8).

Enzyme are necessary for normal cellular metabolism including that of liver and the degenerative changes due to metal toxicity exhibit in the liver alter the level of number of enzymes. The morphological changes are associated with biochemical evidence including the change of alkaline phosphatase activity. ALP is a membrane bound enzyme found in liver and it is present in all membranes where active transport occurs. This enzyme is biomarker of hepatic damage(15).

Most studies investigate the heavy metals accumulation in plasma and soft tissues (brain) in mice and rats. The aim of the present to evaluate liver and kidney tissues cadmium (Cd) content in mice liver and kidneys after different treatment periods. In addition, to determine the toxicity of cadmium metal on liver and kidney architecture and the activity alkaline phosphatase (ALP), the marker enzyme of the degree of organ damage.
MATERIALS AND METHODS

Male Swiss mice (aged at least 2 months) not less than average initial body weight of about 25g were used in this study. The animals were housed at laboratory conditions (22±1 °C) in plastic cages (one per cage) and allowed free access to drinking water and commercial rodent chow.

Determination of cadmium LD$_{50}$

The lethal dose of Cadmium50(LD$_{50}$) test involves the administration of cadmium via Intraperitoneal injection in the form of hydrous CdCl$_2$ and was determined using 30 male mice divided randomly into 5 groups (n =5), intraperitoneal injected (i.p) at increasing dose (1mg/kg, 3mg/kg, 5mg/kg, 7mg/kg and 9mg/kg respectively), in order to determine the dose that kills 50 percent of the tested animals within a set time frame (24h and 48h LD$_{50}$). Several dose levels of cadmium were given until the lethal dose kills half of the test mice population. A sixth group of mice used as the control, received no treatment. This test also was allowed to proceed for 24 and 48hrs, then the lethal dose for 50% of the population (LD$_{50}$) was obtained using the computer software EPA probit analysis program version 1.5 of Finney$^{(16)}$. The 24h and 48h LD$_{50}$ were graphed by plotting the log concentration against percentage mortality occurred at 24 and 48hrs respectively, using Microsoft Excel program.

Cadmium tissue content

For cadmium accumulation and Alkaline phosphatase activity experiments, thirty five mice, were randomly divided into the following experimental groups (5 animals each):

a) Control receiving only distilled water.
b) 0.15mg cadmium chloride CdCl$_2$, the intraperitoneal Cd-injection dose of 0.15 mg was derived from the LD$_{50}$ calculated in this study, for a single intraperitoneal dose of cadmium in the form of CdCl$_2$ in mice was 5.98 mg/body weight). Mice before scarification were kept at the following designed experimental periods: 1- For short-term treatments, mice exposed to intraperitoneal Cd-injection once at 24, 48 and 72 hours. 2- For long –term treatment mice exposed to intraperitoneal Cd-injection once at week 1, 4 and week 8.

Atomic absorption spectroscopy (AAS) was selected as the analytical technique to determine elemental cadmium content in liver and kidney from sacrificed animal, using the method described by Chapman$^{(17)}$ method. Briefly, 25 mg of liver, kidney were fixed for two hours in formaldehyde, and then heated in quarts beakers at 150 °C to evaporate the excess formaldehyde. Samples were digested overnight in a fume hood with mixture of 3:1 pure nitric and sulpheric acids. The mixture was heated with perchloric acid for 2 hrs until the color became whitish, then
cooled for 10 minutes and diluted with deionized distilled water. The colorless solution was filtered before it is read by Pye Unicam SP9 atomic absorption spectrometer (Philips). The same deionizing water was used as a blank.

**Light microscope preparation**

For light microscopic preparation, small tissue samples from liver and kidney were dissected out and fixed in Bouin’s solution for 16h and subsequently placed in 70% ethanol, dehydrated in a graded ethanol series, cleared with xylene, infiltrated and embedded in paraffin wax. Thin section (5 µ) were cut and stained with hematoxylin - eosin for histological examination.

**Level of Alkaline phosphatase (ALP)**

Mice were sacrificed, liver and kidney specimens from each experimental group (short and long-term treatments) were homogenized. The homogenates were centrifuged at 300rmp for 10 minutes. The clear supernatants were used for the estimation of ALP activity. The colometric method using commercial Kit (Biomieux Ltd) was used. For ALP activity assay, the procedures outline in the respective Kit manuals was adopted. The color formation at the end was read at 510 nm using visible spectrophotometer. The enzyme activity was expressed in Unit / 100mg/ protein.

**Statically analysis : using one-way analyses of variance.**

**RESULTS**

The LD$_{50}$ values of both 24 and 48hours of cadmium were 5.98 mg/kg and 3.59mg/kg respectively, no death was recorded among the control group (Fig1).

![Fig(1): The lethal dose of Cadmium50 (LD$_{50}$)](image-url)
Determination of the lethal dose 50% (LD50) of cadmium chloride and the …

Atomic absorption spectroscopy revealed that cadmium accumulated in liver and kidneys as a consequence of Cd toxicity. Tissue injury was indicated by the histopathological changes and change in alkaline phosphatase activity in both liver and kidneys were found to be positively correlated with exposure time.

Table 1 and Table 1.2 shows the results from AAS analyses of the liver and kidney of mice exposed to both short-term and long-term Cd exposure, the concentration of Cd for short-term Cd exposure, significantly increased (p<0.05) in both liver and kidney, and the percentage of the increase ranged from 98-145% in liver 100-300% in kidney and by 143-175 in liver and by 200-370% in kidney at long-term treatment in comparison to untreated control group. The results highlights that Cd precipitated in kidney more than that of liver. Moreover, Cd content in both liver and kidney increased gradually as period of exposure prolonged (72 hrs, 1,4 and 8 weeks) respectively.

Table 1: Concentration (µg/g tissue) of cadmium in liver and kidney following short-term CdCl₂ exposure and associated controls.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Organ</th>
<th>Liver (µg/g tissue)</th>
<th>Kidney (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.5177±0.0392</td>
<td>0.379±0.0.021</td>
</tr>
<tr>
<td>24h Cd</td>
<td></td>
<td>1.045 ±0.288*</td>
<td>0.79 ±0.053*</td>
</tr>
<tr>
<td>48h Cd</td>
<td></td>
<td>1.101 ±0.112*</td>
<td>1.315±0.0403*</td>
</tr>
<tr>
<td>72h Cd</td>
<td></td>
<td>1.27 ±0.0.12*</td>
<td>1.59 ±0.0.159*</td>
</tr>
</tbody>
</table>

*Significant at (P ≤ 0.05)

Table 2: Concentration (µg/g tissue) of cadmium in liver and kidney following long-term CdCl₂ exposure and associated controls.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Organ</th>
<th>Liver (µg/g tissue)</th>
<th>Kidney (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.5177±0.0392</td>
<td>0.379±0.0.021</td>
</tr>
<tr>
<td>1 Week Cd</td>
<td></td>
<td>1.26 ±0.17*</td>
<td>1.21 ±0.063*</td>
</tr>
<tr>
<td>4 Week Cd</td>
<td></td>
<td>1.35 ±0.078*</td>
<td>1.58 ±0.097*</td>
</tr>
<tr>
<td>8 Week Cd</td>
<td></td>
<td>1.412±0.045*</td>
<td>1.87±0.036*</td>
</tr>
</tbody>
</table>

*Significant at (P ≤ 0.05)

The impact of long-term Cd exposure on mice, reflected a higher rate of deterioration in the liver and kidneys histoarchitecture. Light microscope observation revealed that treatment with 5.98mg kg/B.W Cd induced morphological changes in the liver. These changes included
nuclear damage, a marked cytoplasmic vacuolization, liver cell necrosis and eventually followed by degenerative and cell death (fig.2b).

Fig (2). Transverse section of liver mice (a) control (b) treated with Cd (H – Hepatocyte, d – degeneration, arrows – Sinusoid)

Fig(2). (a) Shows the control mice kidney feature and (b) shows the various histological alterations in kidney architecture due to Cd treatment. The mice renal capsule is composed of glomerulus surrounded by Bowman's capsule (Fig.2a). The degenerative of tissues were observed after Cd treatment regardless of exposure period. The glomerulus started to shrink as a result, an increase in Bowman's capsule space and a decrease in both Bowman's capsule and glomerular size were noted. The glomerulus cells were found to go through atrophy, the renal tubules were found to lose their regular shape and the intertubular space decreased (Fig.3a). The above mentioned histopathological alteration were less pronounced in short-time exposure (figure not shown in this study).

Fig (3) Transverse section of kidney Mice (a) control (b) treated with Cd (BC, Bowman's capsule, G-glomerulus, R-intratubular cavity, N-necrosisand, A, Atrophy)
The morphological changes are associated with biochemical evidence indicated by a change in alkaline phosphatase activity. (Table 3). Level of alkaline phosphatase (ALP) activity, in normal untreated control mice liver tissue, showed 4.32± 0.36. At median-lethal dose of CdCl₂, 72hrs and 8weeks treated mice, the level of ALP activity in the liver tissue increased significantly up to 46.71 ± 2.11 and 57.13 ± 4 respectively. While level of alkaline phosphatase (ALP), in normal untreated control mice kidney tissue, the level of ALP activity showed 502.46 ± 11.7. At median-lethal dose of CdCl₂ treated mice for both periods of exposure, the level of ALP activity significantly decreased down to 173.24 ± 2.37 and 21.45 ± 3 respectively.

Table (3): Alkaline Phosphatase (ALP) level in Mice liver and kidney at short-term and long Cd Exposure.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Organ</th>
<th>Liver Alkaline phosphatase (U/100mg/protein)</th>
<th>Kidney Alkaline phosphatase (U/100mg/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>4.32± 0.36</td>
<td>502.46 ± 11.7*</td>
</tr>
<tr>
<td>72h Cd</td>
<td></td>
<td>46.71 ± 2.11*</td>
<td>173.24 ± 2.37*</td>
</tr>
<tr>
<td>8Week Cd</td>
<td></td>
<td>57.13 ± 4*</td>
<td>21.45 ± 3*</td>
</tr>
</tbody>
</table>

*Significant at (P ≤ 0.05)

**DISCUSSION**

Results of the current study (Fig.1) showed that the LD50 values for CdCl₂ via intraperitoneal injection was 5.98 and 3.9 mg/kg/ b.w for 24h exposure and 48h exposure respectively in male mice.

The designer of LD₅₀ test in 1927 acknowledged its serious inadequacies intending it only for certain narrow medical purposes (18). Inadequacies was due to the continuous changes in different factors affected Cd toxicity so it has been well documented that species, age, weight, sex, temperature, pH, animal susceptibility, food in addition to method of by which chemical administrated have marked effect on LD₅₀ results (19). Nevertheless, use of the LD₅₀ test has become widespread as general measure of chemical toxicity and has been challenged for decades as both unreliable and informative criteria. Thereby it is useful to reconsider the repeat determination of the LD₅₀ before carrying out laboratory experiments.

Liver and kidney are critical organs used to describe and document the effect of pollutants, during exposure, Cd induced toxicity in the liver and kidneys is dependent on hepatic and renal Cd concentrations (7). Based on results of this study and recent experiments(5,20), the selected dose of Cd (5.98 mg/kg b.w) appeared to be toxic to liver and kidneys.
Cadmium is transported in the blood and widely distributed in the body but accumulates primarily in the liver and kidneys (1). Hepatic and renal toxicity may occur if toxic Cd level is attained in these organs regardless of exposure periods (21).

Results in Fig.(1) of the present study demonstrate that, mice treated with Cadmium developed progressive liver alteration characterized by cytoplasmic vacuolation, necrosis, regenerative and eventually followed by cell death. The liver damage has been reported in other mammalian and non mammalian species, in addition necrosis strongly associated with oxidative stress (22,23). Beside direct damage of proteins, another underlying mechanism for toxicant induce cell injury may have influential factors which may have direct or indirect effect by generation bioactive molecules intracellularly (e.g. free radicals), causing functional and structural alteration at tissue levels due to the ability of oxidant substances to cause oxidative stress in the liver (24).

Many studies in experimental animals have demonstrated an association between morphological and functional changes in the kidneys. Exposure to cadmium leads to pathological changes in the kidneys. The morphological changes are initially limited to tubular epithelial cell degeneration, but this is followed by cellular atrophy WHO(4). Roya and Bhattacharya(26) reported the accumulation metals in various organs and tissues and excreted through glomerular filtration, but they did not explain the consequences of pathway impact. The present investigation highlights the effect of 5.98 mg/kg b.w. dose of cadmium chloride (Cd Cl₂) on kidney histopathology. Fig..(3b) showed that was a shrinkage in Bowman’s capsule space followed by enlargement of glomerulus, which may be due an increase in the filtration rate as a mechanism to overcome the toxic effect (25,26).

The morphological changes are associated with biochemical evidence including the change of alkaline phosphatase activity. ALP is a membrane bound enzyme found in liver and it is present in all membranes where active transport occurs. This enzyme is biomarker of hepatic damage, thus its bioassay can serve as a diagnostic tool for necrosis of the liver (15). Rich source of ALP are the bile canaliculi of liver and proximal tubules in the kidney. This enzyme is currently being used as markers of Cd-induced tissue damage, damage to any of the organs or tissues would lead to elevated activity of ALP. For example alkaline phosphatase (ALP) is released in both acute and chronic liver disorders (27,28).

Change in ALP activity induced by variety cadmium exposure periods are presented in Table 1 and 2. Decrease or inhibition in ALP activity in kidneys, probably indicates effect on cell growth and proliferation and may be taken as index for cell damage and necrosis (29). In Cd treated mice liver, the activity of ALP was significantly increased.
compared to their normal level of control group. Therefore the increase of the activity of the enzyme in liver, many be due to a leakage out of the enzyme from tissue, due to liver necrosis in heavy metal exposition (30,31). Results in Table1 and 2 of both exposure periods, shows relative to the liver Cd-induced increases in the activity of ALP, it was considerably low and was almost inhibited in kidney. This contradictory possibly interpreted either that liver responds more readily to Cd-induce ALP biosynthesis and enhanced activity than the kidney or the kidney does not respond to Cd-induced ALP synthesis, rather it responds to change brought about by Cd with enhance the activity of kidney constitutive ALP. The later appears to be more plausible interpretation to Obi and Olabode (28) with similar comparison on rat bone and kidney study.

In conclusion, the present study showed that the exposure of mice to Cd caused significant physiological alteration reflected in elevation of liver ALP activity. Decrease ALP activity may be taken as index of hepatic parenchyma damage and hepatocytic necrosis (32). Inhibition of ALP reflects alteration in protein synthesis (33).

REFERENCES


7) Liu, J., Habeebu, S.S., Klaassen, CD. Acute Cd MT injection is not a good model to study chronic Cd nephropathy; comparison of


