EFFECT OF ZINC ON THE CADMIUM ACUTE INTOXICATION IN THE GASTRIC INJURY INDUCED IN RATS

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ABSTRACT

Cadmium is a toxic heavy metal that has been implicated in dysfunction of several tissues. However, its effect in gastric injury is not completely understood. The aim of this study was to investigate the consequence of the acute intoxication with $CdCl_2$ in gastric ulcer induced by several experimental models. $CdCl_2$ administration (1.2, 2.4, 4.8 and 9.6 mg/kg) increased the ulcer index in a dose-dependent fashion through the induction by absolute ethanol (230%), indomethacin plus histamine (280%), acidified acetylsalicylic acid (330%) and 0.6 N HCl (350%). Absolute ethanol induced the minor severity of gastric injury; therefore it was selected to compare the $ZnCl_2$ antiulcer effect in presence of cadmium. Administration of $ZnCl_2$ (5, 10, 20, 40, 80 mg/kg, s.c.) decreased in a dose-dependent manner the ethanol-induced ulcer index, whereas $CdCl_2$ (1.2, 2.4 and 4.8 mg/kg, s.c.) increased the ethanol-induced gastric damage. Pretreatment with $ZnCl_2$ at 20 mg/kg completely prevented the ethanol-induced damage increase produced by $CdCl_2$. These results suggest the important participation of an environmental contaminant like cadmium in the development of the gastric ulcer, but also, the mechanism of action of this methal might be associated with the zinc targets. *www.relaquim.com*

Key words: cadmium chloride, zinc chloride, ulcer, ulcer rat models, toxicity,

RESUMEN

El cadmio es un metal pesado tóxico que ha sido implicado en la disfunción de varios tejidos. Sin embargo, su efecto en la lesión gástrica no es del todo entendido. El objetivo de este estudio fue investigar la consecuencia de la intoxicación aguda con $CdCl_2$ en úlcera gástrica inducida por varios modelos experimentales. La administración de $CdCl_2$ (1.2, 2.4, 4,8 and 9.6 mg/kg s.c.) aumentó de manera dosis-dependiente el índice de úlcera producido por etanol absoluto (230%), indometacina más histamina (280%), el ácido acetilsalicílico acidificado (330%) y HCl

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0.6 N (350%). Debido a que una menor gravedad de la lesión gástrica fue inducida en el modelo de etanol absoluto, éste fue seleccionado para comparar el efecto antiulceroso de $ZnCl_2$ en presencia de cadmio. La administración de $ZnCl_2$ (5, 10, 20, 40, 80 mg/kg, s.c.) disminuyó de manera dependiente de la dosis el índice úlcera inducido por el etanol. Además, el daño gástrico provocado por el etanol en presencia de $CdCl_2$ (1.2, 2.4 y 4.8 mg/kg, s.c.) fue prevenido totalmente cuando se administró en combinación con $ZnCl_2$ a una dosis de 20 mg/kg. Estos resultados dan evidencia de la participación del cadmio como contaminante en el desarrollo de úlcera gástrica y la importancia del zinc para contrarrestar dicho efecto. *www.relaquim.com*

Palabras clave: cloruro de cadmio, cloruro de zinc, ulcera, modelos de ulcera en rata, toxicidad,

INTRODUCTION

Peptic ulcer is a condition showing an increment in the recent years in the entire world (Sostres and Lanas, 2011). The kind of environmental and biological factors participating in its manifestation and protection are a target to establish new treatment therapies or strategies. Due to their extensive use in a vast number of industrial processes and their wide natural distribution, metals are ubiquitous in both natural and working place environments (Waalkes *et al.*, 1992). Among them, cadmium is a very potent metallic toxicant in both environmental and occupational aspects. Air, water, food and smoking represent significant sources of cadmium exposure (Friberg *et al.*, 1986) as well as a contaminant in shellfish (Rom, 1988). Cadmium is an element of the group IIB in the periodic table, together with zinc and mercury. But in contrast to zinc, cadmium is a non-essential element that presents high rate of soil to plant transference compared with other non-essential soil constituents (Satarug *et al.*, 2002). Cadmium has a variety of industrial uses resulting in an increase of cadmium abundance in the immediate human environment worldwide for the last decades (Waalkes *et al.*, 1992). In relation to a general population exposure, much of the information on the effects of cadmium in the human being has come from occupational exposure and excessive intake in the diet has been limited to only a few localities. When a person is exposed to cadmium inhalation for periods comprising several years, the kidney is most frequently the critical organ affected; although, under some conditions, the target organ may be the lung (Sisman *et al.*, 2003). Fully developed intoxication among industrial workers may present the major features of emphysema and renal dysfunction similar to those described in animals (Sisman *et al.*, 2003). People can be exposed to cadmium at work or when doing hobbies, including metal plating, semiconductor manufacture, wire, plastic, or battery manufacture, welding, soldering, ceramics, or painting (Bonnel *et al.*, 1959). Other important source of cadmium is cigarette smoking; cadmium blood levels is approximately twice in smokers in comparison to nonsmokers (Satarug *et al.*, 2002).

Cadmium has an extremely long half-life in man. Even low exposure levels may, in time, cause considerable accumulation and toxic effects (Friberg *et al.*, 1986; Morselt, 1991). In fact, metals are considered an emerging class of carcinogens. Carcinogenic and teratogenic effects of cadmium have been studied in a variety of animal species (Parzyck *et al.*, 1978; Waalkes *et al.*, 1992). Studies have demonstrated the noxious effect of cadmium on kidney, lung, liver, cardiovascular system, bone, testes and ovaries (Parizek *et al*, 1968; Gabbiani

et al., 1974; Tarasenko *et al.*, 1974; Friberg *et al.*, 1986; FAO/WHO, 1986).

Epidemiological studies from occupationally exposed workers to cadmium have identified tumors of the lungs and prostate and, to a lesser extent, kidney and stomach (Klaassen and Liu, 1998; Satarug *et al.*, 2003). Also, it is known that exogenous administration of zinc possesses anti-ulcer activity (Troskot *et al.*, 1997). In this study, the effect of acute intoxication of cadmium alone was evaluated in gastric lesions induced by several experimental models of ulcer. This toxic effect was also tested after pretreatment with zinc in the ethanol-induced gastric injury.

MATERIALS AND METHODS

Animals

Female Wistar rats weighing 180-220g were used. The animals were housed under standard laboratory conditions and maintained on standard pellet diet (Lab diet) and water *ad libitum*. Subsequently, animals were starved by placing them in single cages with wire-net floors and deprived of food for 24 h before producing a gastric injury, but free access to tap water was allowed throughout. All experiments were carried out using 10 animals per group. Procedures involving rats and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-Z00-1999) and in compliance with the NIH Guide for Care and Use of Laboratory Animals.

Drugs

Histamine dihydrochloride, indomethacin, acetylsalicylic acid, paraformaldehyde, cadmium chloride hemipentahydrate ($CdCl_2$-$2.5H_2O$) and zinc chloride ($ZnCl_2$) were purchased from SIGMA (St Louis, MO). Absolute ethanol, ether and hydrochloric acid (HCl) were purchased from J.T. Baker.

Experimental gastric ulcer

Simultaneously to rat fasting; $CdCl_2$ was subcutaneously (s.c.) administered at doses 1.2, 2.4, 4.8 and 9.6 mg/kg in a volume of 2 ml/kg body weight. Control rats received vehicle (0.9% saline solution) in the same volume and route of administration. After 24 h, gastric ulcer was induced by one of the following experimental models: absolute ethanol, 0.6 N HCl, acidified acetylsalicylic acid and, indomethacin plus histamine and pylorus ligature (Shay´s ligature). To measure gastric lesions, the animals were sacrificed in a CO_2 chamber and each stomach was dissected and examined.

Absolute ethanol or 0.6 N HCl-induced ulcers

Gastric ulcers were induced with absolute ethanol (Robert *et al.*, 1979) or HCl 0.6 N administered p.o. in a 1 ml volume for each rat. Two and a half hours after the administration, the animals were sacrificed to get the stomachs.

Acidified acetylsalicylic acid-induced ulcer

Animals were dosed p.o. with 200 mg/kg of acetylsalicylic acid in 150 mM HCl and suspended in 0.5% carboxymethylcellulose (Wang *et al.*, 2011). After 4 hours, rats were sacrificed and gastric lesions were quantified.

Indomethacin plus histamine-induced gastric ulcers

Ulcers were induced by a modified method of Takeuchi *et al.* (1990). Gastric lesions were induced by administration of three injections of indomethacin (10 mg/kg suspended in 0.5% carboxymethylcellulose p.o.) at 0, 12 and 24 h, followed by three injections of histamine dichloride (40 mg/kg dissolved in saline solution, s.c.) at 1.5, 4 and 6.5 h from the last indomethacin dose. Animals were sacrificed 2 h after the last administration of histamine.

Shay´s ligature

The pylorus-ligature was done as described

by Shay *et al.* (1954). The pylorus of each rat was tied under light ether anesthesia and the abdominal incisions were closed. The animals were deprived of water during the post-operative period. Sixteen hours later, rats were sacrificed and each stomach removed and examined to register gastric lesions.

Protective effect of zinc in ethanol-induced ulcer

At the beginning of the fasting period, rats received $ZnCl_2$ (5, 10, 20, 40 and 80 mg/kg, s.c.). Twenty-four hours later, animals received 1 ml of absolute ethanol p.o. and after 2.5 h they were sacrificed.

Zinc protection in ethanol-induced ulcer plus cadmium

At the beginning of the fasting period, a group of rats were administrated with $ZnCl_2$ (20 mg/kg, s.c.) and 30 min later they received $CdCl_2$ at 1.2, 2.4 and 4.8 mg/kg, s.c. Twenty-four hours later, animals received 1 ml of absolute ethanol p.o. and after 2.5 h they were sacrificed.

Gastric lesions measure

Each stomach was dissected out from esophagi to pyloric portion and inflated with 5 ml of formalin 2% and placed in formalin 2% for at least 15 min to fix both the inner and outer gastric layers. Stomachs were incised along the greater curvature and examined for ulcers. The hemorrhagic lesions were measured in millimeters under a dissection microscope (10X) with an ocular micrometer (Zeiss 475029902). The ulcer index was defined as the product of length and width of the ulcers present in the corpus of the stomach (mm^2) for each animal.

Statistical analysis

Data are presented as the mean ± SEM of the percentage of ulcer index from 10 rats per group and compared with control group. Significant difference was tested by Mann-Whitney test or Kruskal-Wallis ranks test followed by Dunn's test. Statistical significance was considered at $P<0.05$.

RESULTS

No gastric damage was observed with $CdCl_2$ alone and all animals survived at all doses tested. In contrast, $CdCl_2$ increased gastric lesions induced by several experimental models in a dose-dependent fashion in comparison with control groups (Fig. 1).

The ulcer index obtained in animals treated with ethanol combined with several doses of $CdCl_2$ was from 60 to 210% higher than control group; in gastric lesions induced by HCl, the injury was increased between 80 and 250% at 2.4 and 4.8 mg/kg of $CdCl_2$; whereas in the indomethacin plus histamine-induced gastric lesions, it was observed an increase between 220 and 280% more. Finally, the ulcer index produced by acetylsalicylic acid in presence of this metal was augmented until 190% at 6.0 mg/kg dose (Fig. 1).

Administration of $ZnCl_2$ alone produced a dose-dependent gastric-protection on the ethanol-induced gastric lesions (Fig. 2A). Given that ethanol-induced gastric lesions were not as severe as those observed in the others models; we decided to use this model to test the effect of $CdCl_2$ on gastric injury induced by ethanol in presence of $ZnCl_2$. Cadmium chloride at 1.2, 2.4 and 4.8 mg/kg (s.c.) increased in a dose-dependent manner the ethanol-induced gastric lesions (Fig. 2B), but the pretreatment with 20 mg/kg of $ZnCl_2$ completely inhibited the effect of $CdCl_2$ on the ethanol-induced lesions (Fig. 2B).

The figure 3 shows representative pictures of the absolute ethanol-induced gastric injury in presence of different doses of $CdCl_2$ alone and in presence of 20 mg/kg of $ZnCl_2$.

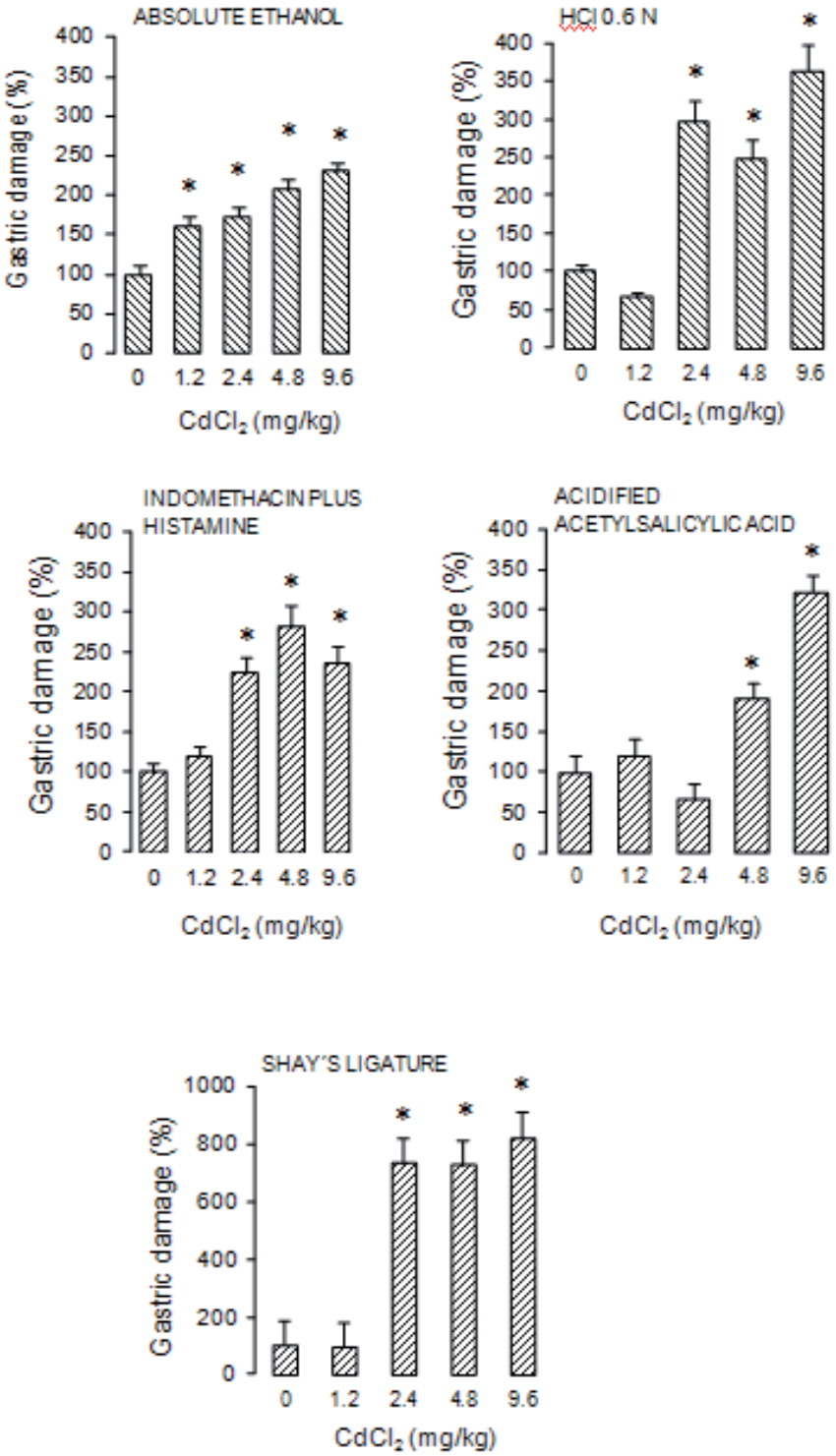

Figure 1. Effect of the acute intoxication with $CdCl_2$ (1.2, 2.4, 4.8 and 9.6 mg/kg) in several experimental models of gastric ulcer like absolute ethanol, HCl 0.6 N, indomethacin plus histamine, acidified acetylsalicylic acid and Shay´s ligature. Gastric damage in percentage from the average of the control values are shown in the figure as mean ± S.E.M. of 10 rats. *P< 0.05, Kruskal-Wallis ranks test followed by Dunn's test.

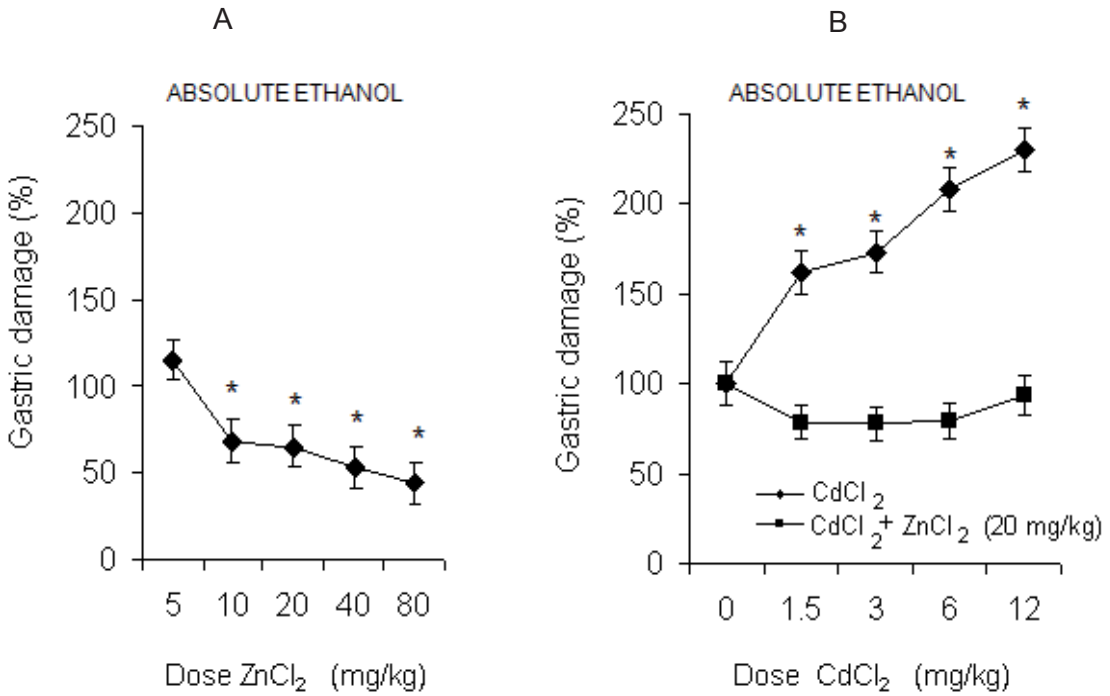

Figure 2. Dose-dependent protection by subcutaneously injection of 5, 10, 20, 40, 80 mg/kg, s.c. doses of $ZnCl_2$ alone (A) and combined with 1.2, 2.4 and 4.8 mg/kg of $CdCl_2$ (B) in the absolute ethanol-induced gastric lesions. Gastric damage in percentage from the average of the control values are shown in the figure as mean ± S.E.M. of 10 rats. *P< 0.05, Kruskal-Wallis ranks test followed by Dunn's test or Mann-Whitney test respectively.

DISCUSSION

In the present investigation, it was observed that acute $CdCl_2$ intoxication promoted the severity of the gastric lesions induced by several experimental models of gastric ulcer. This gastric injury was prevented by pretreatment of $ZnCl_2$.

The mechanism of action of cadmium that increases the severity of gastric ulcer in experimental models, as well as the protective effect promoted by zinc, are unclear. However, it has been demonstrated that oral administration of single high doses of cadmium compounds causes desquamation of the gastric epithelium (Tarasenko *et al.*, 1974). In addition, a significant diminution has been observed in the mucin content and prostaglandin levels as components of the gastric mucous barrier due to cadmium exposition (Szabo *et al.*, 1985; Öner *et al.*, 1994; 1995). This evidence may account for the gastric mucous vulnerability to injury observed in our experiments and may partly explain the high incidence of gastric ulcer in the exposed population. An apparent controversy has been observed in biochemical studies at similar doses of cadmium tested in Spague-Dawley rats (Dupuy and Szabo, 1986). In that study, $CdCl_2$ administration decreased the severity of injury and it was related with a possible participation of endogenous sulfhydryl groups. It is important to mention that this effect was analyzed in different experimental conditions (Dupuy and Szabo, 1986). Discrepancy in the results with similar doses of cadmium but at different times suggests that its effect involves diverse mechanisms in the gastric lesion.

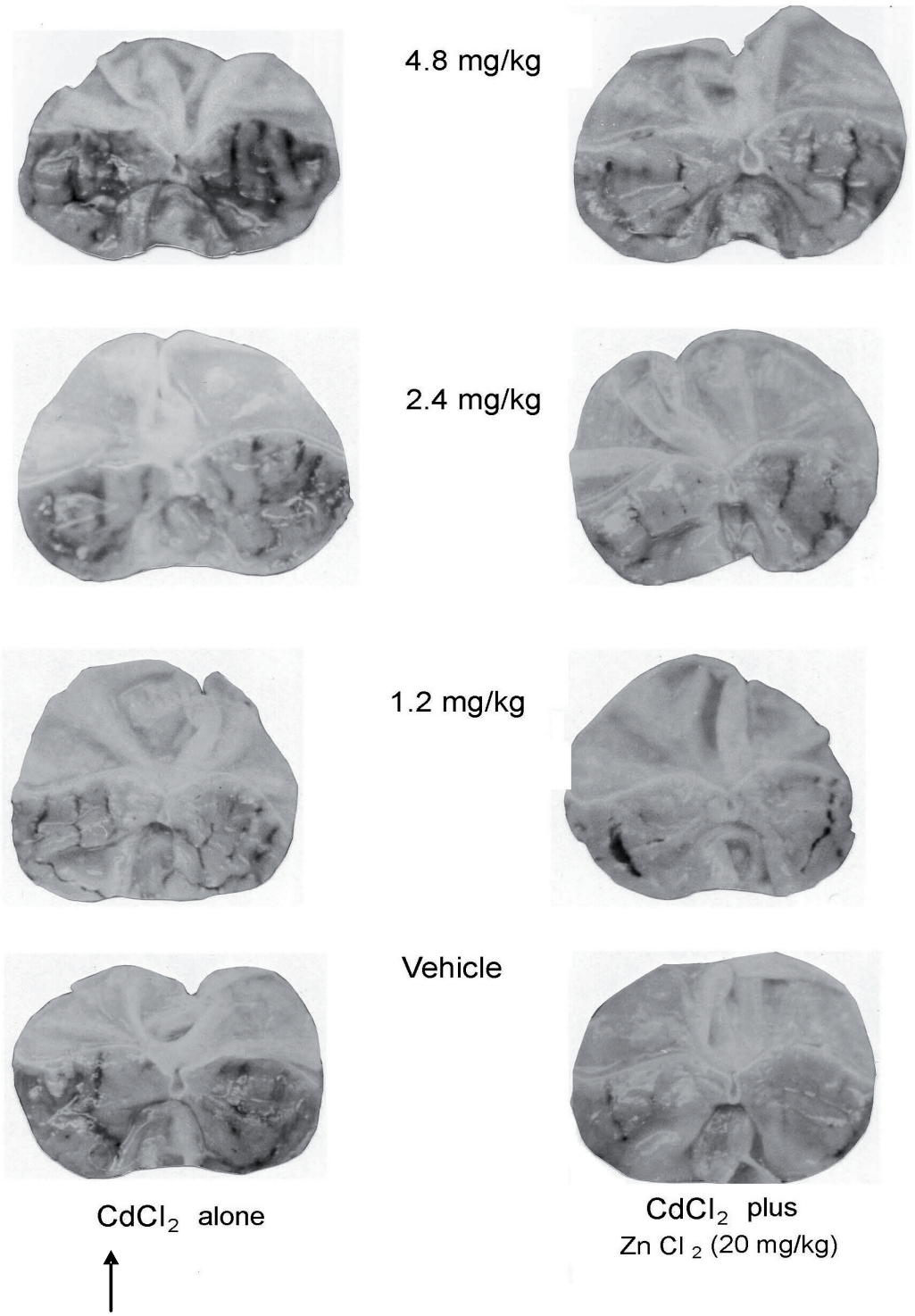

Figure 3. A representative-image of the absolute ethanol-induced gastric lesions in animals that received vehicle, $CdCl_2$ (1.2, 2.4 and 4.8 mg/kg) alone (stomachs from left side) or in presence of $ZnCl_2$ (20 mg/kg) (stomachs from right side).

On the other hand, the increased severity of gastric injury in the presence of $CdCl_2$ was prevented when combined with single or several doses of $ZnCl_2$ (Fig. 2B). From a biological standpoint, zinc plays an important role in the toxicity associated with cadmium exposure (Cotton and Wilkinson, 1988). In fact, zinc as a divalent metal, is considered to be bound to or oxidized by endogenous sulfhydryls mediating cellular responses to injury by producing gastric cytoprotection (Dupuy and Szabo, 1986). In this case, cytoprotection may be annulled by cadmium due to its similar chemical nature with zinc (Martell, 1981). Although both cadmium and zinc have affinity for sulfur ligands, the affinity of cadmium is greater than that of zinc (Jacobson and Turner, 1980). It has been considered that zinc is displaced by cadmium in a number of biological processes, altering the defensive protection of zinc by using its targets and producing toxicity (Wu and Wu, 1987). Our results, in agreement with other reports referring administration of different doses of $CdCl_2$ in presence of $ZnCl_2$, supports a competitive antagonism between these divalent metals (Merali and Singhal, 1975; Sorensen *et al.*, 1993; Chang and Huang, 1996) to maintain the gastric mucous in the ethanol-induced lesions.

Finally, the inhibition of gastric lesions in presence of $ZnCl_2$ alone indicates the participation of zinc in the prevention or relief of ulcers. Several authors have proposed a stabilization of the cellular membrane as a response to the effect of zinc (Kagi and Schaffer, 1988; Coogan *et al.*, 1992). It is important to notice that presence of environmental contaminants like cadmium not only can exacerbate gastric damage, but also adverse effects of drugs, such as non-steroidal anti-inflammatory drugs. In conclusion, current results show an agreement with related investigations (Öner *et al.*, 1994; 1995) that the stomach is another important target to the cadmium intoxication.

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