Comparison of Protective Effect of *Apium graveolens* and *Aloe vera* Supplemented with Zinc on Cadmium Induced Hepato and Nephro-Toxicity in Wistar Rats

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ABSTRACT

BACKGROUND

Cadmium (Cd) is an environmental pollutant that accumulates in various organs such as liver, kidney, and other organs. It generates reactive oxygen species, thereby resulting in pathological changes in the organs it accumulates in by depleting antioxidants. *Apium graveolens* (AG) and *Aloe vera* (AV) are rich sources of antioxidants. Zinc (Zn) is an important antioxidant trace element present in various tissues and this protects the organs from the toxic effects of cadmium. We wanted to compare the protective effect of AG and AV with and without Zn supplementation in Cd exposed liver and kidneys of Wistar rats.

METHODS

Male Wistar albino rats were divided into 11 groups. The control group received only vehicle, the experimental groups were administered with 10 mg / Kg bw of $CdCl_2$, 40mg / Kg bw of $ZnCl_2$, 200 mg / Kg bw of AG and AV, 400mg / K bw AG and AV separately and in combination. After 56 days, the animals were sacrificed and histopathology was done.

RESULTS

Cd resulted in significant tissue damage of liver and kidney. AG, AV and Zn were able to offer protection to these tissues.

CONCLUSIONS

AG, AV and Zn by virtue of their antioxidant properties, protect the liver and kidney from damages due to Cd more effectively in rats.

KEY WORDS

Cadmium, Zinc, Kidney, Liver, *Apium graveolens, Aloe vera*

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DOI: 10.14260/jemds/2021/426

How to Cite This Article:
Sushanth NK, Vijayaraghavan R, Vijayalakshmi S, et al. Comparison of protective effect of apium graveolens and aloe vera supplemented with zinc on cadmium induced hepato- and nephro-toxicity in wistar rats. J Evolution Med Dent Sci 2021;10(28):2083-2088, DOI: 10.14260/jemds/2021/426

Submission 29-01-2021,
Peer Review 12-05-2021,
Acceptance 18-05-2021,
Published 12-07-2021.

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BACKGROUND

Cadmium (Cd) is a heavy metal and a well-known environmental toxicant. The major source of exposure is drinking water and food contaminated with Cd.[1] It could not be shown that Cd has any physiological role in human body. There are basically three ways of Cd resorption into the body: pulmonary, gastrointestinal and dermal. Majority of Cd that reaches blood, is transported bound to proteins. The first organ the Cd reaches is the liver, where it induces metallothionein production forming Cd-Metallothionein (Cd - MT) complex. This causes hepatocyte necrosis and apoptosis. The Cd-MT complex reaches sinusoids, from where by absorption Cd enters enterohepatic cycle in the form of Cd-glutathione conjugate. Cd then enters small intestine as Cd-cysteine complexes following enzymatic degradation.[2]

For long term accumulation, Cd chooses kidney as main organ.[3] Long term accumulation results in tubule cell necrosis. Cd reaches the kidney as Cd-MT, filtrated in the glomerulus and in the proximal convoluted tubules it is reabsorbed. Cd^{2+} ion has similarities, due to which it is likely to substitute Ca^{2+} and Zn^{2+} in various physiological processes resulting in activation and or inhibition of a number of important signaling pathways. Cd increases oxidative stress by binding to sulfhydryl groups of proteins and depleting glutathione, an antioxidant.[4] Additionally, Cd exposure gives rise to generation of reactive oxygen species (ROS) and enhances lipid peroxidation leading to tissue damage.[5]

All organs contain an essential antioxidant trace element zinc (Zn) that is essential for cell proliferation, differentiation, and normal growth. It offers protection to tissues against free radicals and oxidative stress.[6] It prevents cell damage by activation of antioxidant system.

The present study was to analyse and compare the protective effect of AG, AV with and without Zn in Liver and Kidney of rats exposed to Cd. Use of antioxidant properties of AG (commonly known as celery) against Cd induced pathology is not available in the literature.

METHODS

66 healthy adult male Wistar rats were obtained from Biogen, Bangalore. They were housed in cages at animal facility, Department of Research and Development, SIMATS, with natural light. These conditions were maintained throughout the experimental period. The animals weighing 200 – 250 g were randomly divided into the following 11 groups (N = 6) after 4 days of acclimatisation.

1. Group 1: 1 ml of 0.5 % CMC in distilled water.
2. Group 2: 10 mg / kg bw of $CdCl_2$ in 0.5 % CMC
3. Group 3: 10 mg / kg bw of $CdCl_2$ + 40 mg / kg bw of $ZnCl_2$ in 0.5 % CMC
4. Group 4: 10 mg / kg bw of $CdCl_2$ + 200 mg / kg bw of *Aloe vera* in 0.5 % CMC
5. Group 5: 10 mg / kg bw of $CdCl_2$ + 400 mg / kg bw of *Aloe vera* in 0.5 % CMC
6. Group 6: 10 mg / kg bw of $CdCl_2$ +40 mg / kg bw of $ZnCl_2$ + 200 mg / kg bw of *Aloe vera* in 0.5 % CMC
7. Group 7: 10 mg / kg bw of $CdCl_2$ + 40 mg / kg bw of $ZnCl_2$ +400 mg / kg bw of *Aloe vera* in 0.5 % CMC
8. Group 8: 10 mg / kg bw of $CdCl_2$ 200 mg / kg bw of *Apium graveolens* in 0.5 % CMC
9. Group 9: 10 mg / kg bw of $CdCl_2$ + 400 mg / kg bw of *Apium graveolens* in 0.5 % CMC
10. Group 10: 10 mg / kg bw of $CdCl_2$ +40 mg / kg bw of $ZnCl_2$ +200 mg / kg bw of *Apium graveolens* in 0.5 % CMC
11. Group 11: 10 mg / kg bw of $CdCl_2$ +40 mg / kg bw of $ZnCl_2$ +400 mg / kg bw of *Apium graveolens* in 0.5 % CMC

The concentration and quantity of the dose / rat was calculated according to body weight and were administered orally using a gavage for 56 days. At the end of the treatment, animals were sacrificed, and liver and kidney were collected and preserved in 10 % formalin for histopathological analysis.

Histopathological Study

The vital organs (Liver and Kidney) after isolation were fixed in 10 % formalin and embedded in paraffin wax. Paraffin sections were made at 5 µm and stained with haematoxylin and eosin (H&E). The slides were studied under a light microscope.

Statistical Analysis

Sigma Plot 13.0 Systat software, USA was used for statistical analysis.

RESULTS

Histopathology of Liver

The liver of control group showed normal lobules, parenchyma, hepatic cords and sinusoids. The liver of Cd treated rats on light microscopic examination showed distorted architecture with shrunken and discontinuity of central vein along with degeneration of cells in the radiating cord of hepatocytes.

The portal triad accumulated collagen tissue around it and sinusoidal space was enlarged. Small nuclei with vacuolization of hepatocyte cytoplasm, rupture of cell membrane, infiltration of mononuclear cells in to the interstitium were observed. Intercellular necrotic gaps in the hepatic lobules, hepatocellular swelling and atrophy of hepatic portals were other noticeable features. The Cd + Zn treated rats, showed near normal architecture with central vein and radiating hepatocytes with intact portal triad and normal sinusoidal space.

Similarly, the rats treated with Cd + AV200 and Cd + AG200 showed that the extracts were able to protect the tissue from Cd insult since the cellular architecture was preserved to a significant extent in them. However, Cd + AV400 and Cd + AG400 was not effective in maintaining the normal histology by protecting against Cd.

Among the rats treated with 200 mg / Kg and 400 mg / Kg of AV and AG extracts in combination with Zn, the 200 mg / Kg of both AV and AG showed significant protective properties against Cd unlike the 400 mg / Kg b.w. which were not equally effective. This protective nature of the extracts could be attributed to their antioxidant properties (Figure 1).

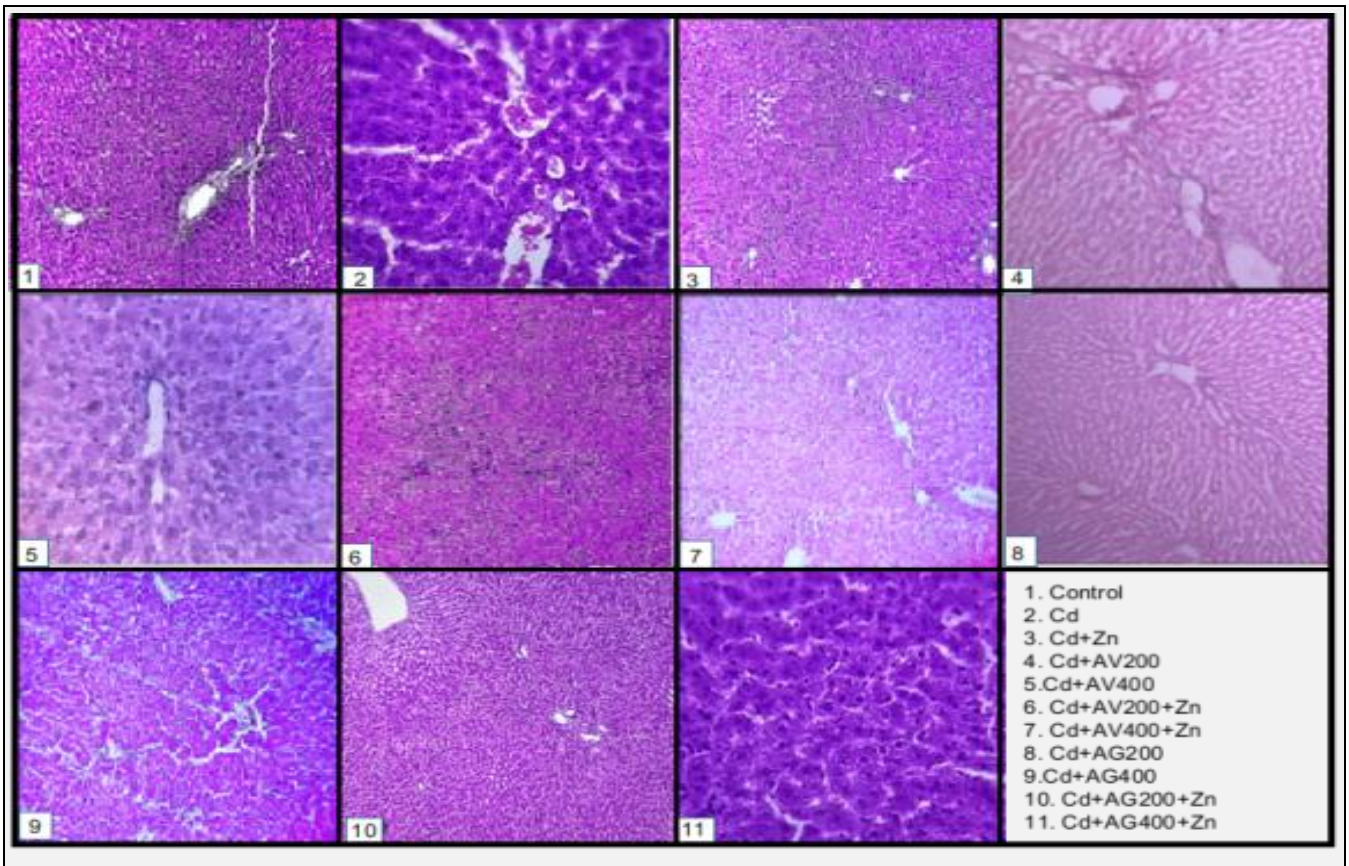

Figure 1. Photomicrograph of H & E Stained Section of Liver (10X Magnification) in Cadmium (Cd, 10 mg / kg, p.o.) Toxicity and Its Protection by Zinc (Zn, 40 mg / kg, p.o.), Aloe Vera Extract (AV, 200 and 400 mg / kg, p.o.), Apium Graveolens Extract (AG, 200 and 400 mg / kg, p.o.) and Their Combinations (N = 6 Each)

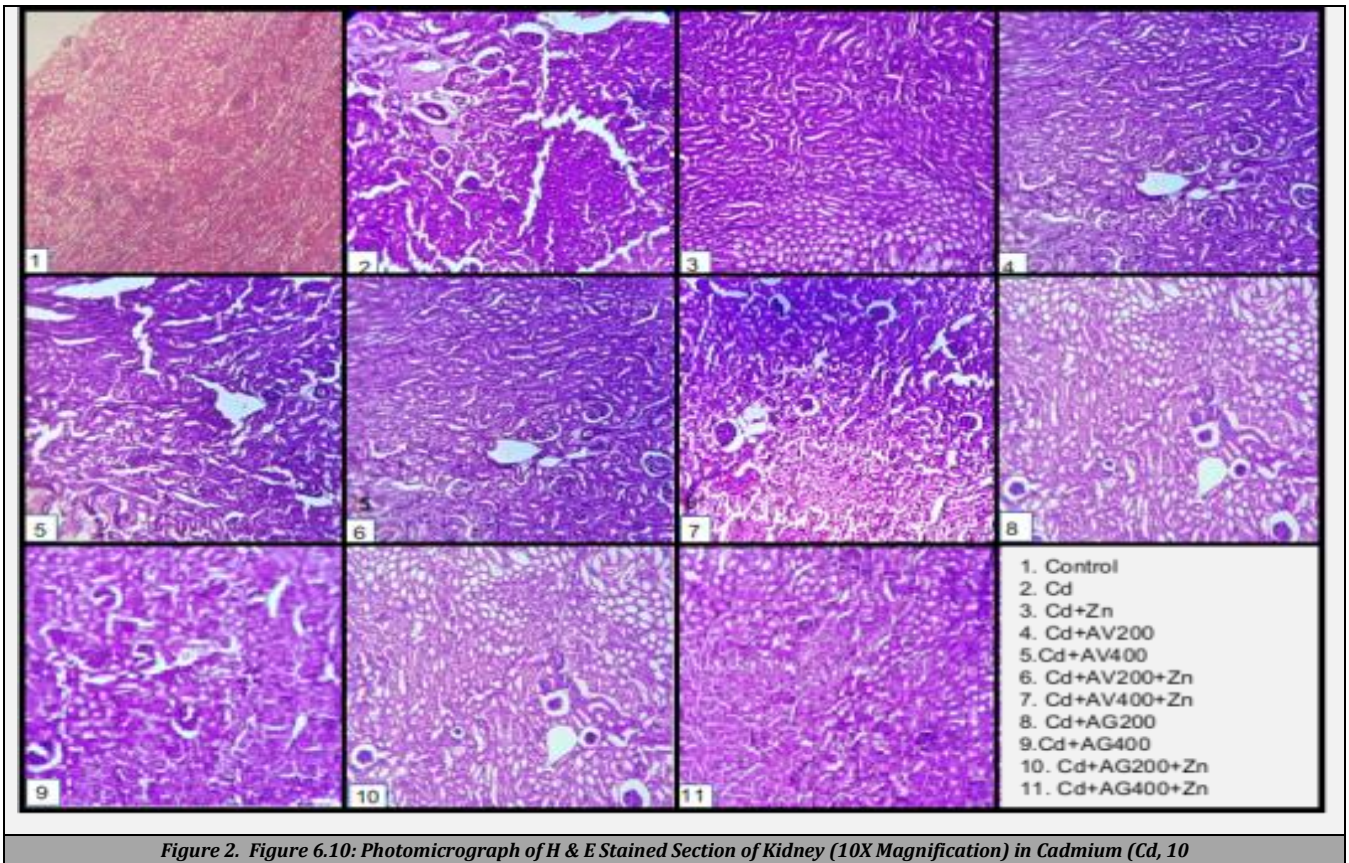

Figure 2. Figure 6.10: Photomicrograph of H & E Stained Section of Kidney (10X Magnification) in Cadmium (Cd, 10 mg / kg, p.o.) Toxicity and Its Protection by Zinc (Zn, 40 mg / kg, p.o.), Aloe Vera Extract (AV, 200 and 400 mg / kg, p.o.), Apium graveolens Extract (AG, 200 and 400 mg / kg, p.o.) and Their Combinations (N = 6 Each)

Photomicrograph of H&E stained section of liver in cadmium (Cd, 10 mg / kg, p.o.) toxicity and its protection by zinc (Zn, 40 mg / kg, p.o.), *Aloe vera* extract (AV, 200 and 400 mg / kg, p.o.), *Apium graveolens* extract (AG, 200 and 400 mg / kg, p.o.) and their combinations.

Histopathology of Kidney

On light microscopic examination, the kidney of control rats showed normal cortex and medulla regions with normal PCT, DCT, renal corpuscles, collecting duct and Henle's loop. Renal parenchyma, epithelium of the tubules, nuclei of the epithelial cells, glomeruli with the capillary, endothelial cells and mesangium all appeared normal. Cd treated rats showed abnormal architecture with the epithelial lining of proximal and distal convoluted tubules thinned out, shrunken and distorted lumen, degenerated glomeruli and reduced vascularity. The kidney tissue appeared pale, with tissue lesions in the cortex and medulla with visible necrosis. Massive local haemorrhage and destructed basement membrane were noted. Some abnormal renal structures, thin epithelial layer and irregular tubule lumen were observed. Severe necrosis and degeneration were noticed in some regions of the cortex associated with degenerated tubules, glomeruli and overall a compromised cellular integrity was observed. The Cd + Zn treated rats, showed near normal architecture with mostly intact glomeruli, proximal and distal convoluted tubules. Cd + AV200 and Cd + AG200 treated rats showed that the extracts were able to protect the cellular architecture to a significant extent by preventing toxic effects of Cd largely due to their antioxidant properties. In Cd + AV400 and Cd + AG400 treated groups, the dose of 400 mg / Kg b.w was not as effective as 200 mg / kg b.w in maintaining the normal histology by protecting against Cd. Among the rats which were treated with 200 mg / Kg and 400 mg / Kg b.w of hydroalcoholic extracts of AV and AG in combination with Zn, the dose of 200 mg / Kg b.w of both the extracts showed significant protective properties against Cd unlike the dose 400 mg / Kg b.w which were less effective (Figure 2).

Figure 2 Photomicrograph of H&E stained section of kidney in cadmium (Cd, 10 mg / kg, p.o.) toxicity and its protection by zinc (Zn, 40 mg / kg, p.o.), *Aloe vera* extract (AV, 200 and 400 mg / kg, p.o.), *Apium graveolens* extract (AG, 200 and 400 mg / kg, p.o.) and their combinations.

DISCUSSION

Cd causes injury to several tissues, including liver and kidney.[7] Cd is an exceptionally poisonous metal that is extremely harmful to people because its accumulation in tissues causes metabolic, histological, and pathological changes.[8] The Kupffer cells (KC) in liver can directly damage its cells by releasing wide variety of cytotoxic mediators that include reactive oxygen species (ROS), nitric oxide (NO), and cytotoxic proteins. Apart from these, KC promote infiltration of other inflammatory cells by releasing chemokines into the liver.[9] A large number of ROS are released by infiltrating neutrophils. This results in oxidative stress and lipid peroxidation (LPO).[10]

There are evidences suggesting direct hepatocyte damage due to Cd. Additionally, new evidences suggest that ischemia may also play a role in Cd - induced liver injury.[11] Ischemia that occurs due to liver damage may result from direct effects of Cd on hepatic EC. The damaged cells that enter the sinusoidal space alter hepatic microcirculation and cause hypoxia that further makes hepatocytes more prone for harmful effects due to Cd.[12] The hepatocyte damage was evident in H & E stained sections of the liver tissue of rats in Cd group in the present study. In general, Cd induced oxidative stress plays a major role in inducing toxicities in kidney, liver and testes.[13]

In the present study, alterations observed in normal histology of kidney was due to cadmium induced toxicities. The major pathological changes observed were damage of tubule epithelium and surrounding blood vessels, increase in interstitial space due to loss of connective tissue integrity, glomerular lesion involving the epithelial cells and the capillary loop. So, it is evident that microvascular damages have also taken place. Aughey[14] and Mitsumori[15] reported similar or more advanced changes in liver and kidney histology and functions under the influence of Cd.

Zn exhibits its protective effect in Cd exposed rats in various organs and tissues.[16,17] Zn protects renal tissues against the toxicity of Cd.[18] Similar result was obtained in the present study also. It could be due to the protective nature of Zn by acting as an antioxidant.[19,20,21] Furthermore, Zn supplementation prevents increased rate of lipid peroxidation.[22] It was found that when Cd was given in combination with Zn simultaneously, the protective effect of Zn was exhibited clearly, and the toxicity caused was less when Cd was administered alone.[23] Similar results were found in the present study.

The Cd treated rats in their liver showed several degenerative changes and these findings of the present study were in agreement with the previous studies.[24,25,26] Also subchronic exposure with $CdCl_2$ caused liver damage, demonstrated by histopathological alterations; moderate degeneration (ballooning) and discrete necrosis.[27]

The histopathological changes in liver could be because of the highly reactive radicals and the lipid peroxidation that results following it due to Cd. The accumulated hydroperoxidase can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxidase, the basis for hepatocellular damage.[26]

The histopathological changes observed in the kidney of Cd treated rats in the present study is in accordance with a study[28] which found that Cd affected the glomeruli especially glomerular capillaries in Bowman's space and atrophy of some glomerulus.

Several histopathological studies revealed the toxic effect of Cd in the kidney and one such change was proximal tubular necrosis, apoptosis, and tubular degeneration.[29]

Tubular necrosis and degeneration was found in this study also. These changes could be due to the accumulation of free radicals as a consequence of increased lipid peroxidation by free Cd ions in the renal tissues of Cd - treated rats.[26]

The kidney and liver treated with hydroalcoholic extracts of AV and AG showed minimal histopathological changes especially at the dose of 200 mg / Kg than 400 mg / Kg bw. Both these plants are rich in antioxidants (such as vitamin C) and antioxidants counter the deleterious effects of ROS that

were generated in this case due to Cd. Earlier studies on the beneficial role of vitamin C on Cd - induced organ toxicity reported that antioxidant supplements with vitamin C have a prophylactic effect.[30]

Zn is an essential component of the oxidant defense system with participation at multiple cellular levels.[31]

In the present work, treatment with Cd and Zn effectively protected liver and kidney against Cd - induced damage. Zn has a protective effect on histological damage by maintaining membrane integrity due to its direct action on free radicals.[32] When the extracts were supplemented with Zn, due to the combined actions of Zn and antioxidants in the extracts, they were able to very effectively protect the tissues from damages due to Cd. On comparison, AG at the dose of 200 mg / kg bw was better protective than 200 mg / Kg bw of AV. With Zn supplementation the protective nature was enhanced.

CONCLUSIONS

The Cd is, very much a potential threat to biological system. Zn acts as a chelator and antioxidant and is capable of maintaining structural and functional integrity of tissues. Among antioxidant rich AG and AV, AG offers better protection against pathological effects of Cd in combination with Zn without any side effects.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jemds.com.

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