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Research Article
Ameliorative Effect of *Arctium lappa* Against Cadmium Genotoxicity and Histopathology in Kidney of Wistar Rat

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Abstract

Background and Objective: Cadmium (Cd) is a non-essential metal whose dispersion in the environment has increased recently, Cd may enhance cell oxidative stress that leads to DNA damage and apoptotic cell death. The study aimed to evaluate the antioxidative capability of Burdock root '*Arctium lappa*' on cadmium-induced oxidative stress and histopathology of the kidney of Wistar rats. **Methodology:** Cadmium was applied in a form of cadmium chloride to three groups (15 mg Cd kg^{-1}) for five weeks with two groups pre-treated with '*Arctium lappa*' administration, 100 and 200 mg kg^{-1} b.wt. Data were analyzed using one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test to determine the difference among means using the JMP version 12. **Results:** Results revealed that cadmium induced a significant disorganization ($p<0.05$) of renal structure with collapsed tubular lamina and 76 μm tail length of the cells was observed, while histological sections of kidney pre-treated with 100 mg *Arctium lappa* kg^{-1} b.wt., showed a slightly less hypercellularity of glomerulus and reduction in the cell tail (59 μm). Furthermore, histological sections of kidney of rats pre-treated with 200 mg *Arctium lappa* kg^{-1} b.wt., showed high improvement of renal tubules and glomerulus with a prominent urinary space beside tail length of cells was recorded as 39 μm which was lower in comparison to other groups. **Conclusion:** Moreover, cadmium induced cellular destruction of the kidney was resumed with the pre-treatment of the secondary metabolites as an antioxidant compounds that produced from plant extracts. *Arctium lappa* leaf extract was efficient at both applied doses while 200 mg *Arctium lappa* kg^{-1} b.wt., had the most ameliorative effect.

Key words: *Arctium lappa*, cadmium genotoxicity, wistar rat, histopathology

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Competing Interest: The authors has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cadmium (Cd) is a non-essential metal whose dispersion in the environment has increased leading to various degrees of contamination. It is one of the heavy metals that commonly present in contaminated soils and it can be produced as ascertain byproduct of Zn and sometimes lead refining[1]. Cadmium is toxic to animal and human and therefore, it has great concern in the environment[2,3]. In spite of its toxicity, little is known about the effects of Cd on genetic and biochemical adaptive responses of aquatic species under chronic and long-term exposure. Toxicological studies at cellular level have shown that Cd inhibits the mitochondrial electron transfer chain and induces Reactive Oxygen Species (ROS) production[4]. The Cd-promoted oxidative stress leads to DNA damage and apoptotic cell death[4,5]. Different mechanisms could be involved in Cd detoxification. The Cd interferes with DNA repair, which can lead to mutations[6] and eventually carcinogenesis[7]. Therefore, assessment of DNA damage was an important aspect of toxicity testing. The comet assay has been used as a sensitive tool to measure DNA damage in a variety of organisms and therefore, frequently used to assess genotoxicity. *Arctium lappa*, commonly known as burdock, is being promoted/recommended as a healthy and nutritive food in Chinese societies. Burdock has been used therapeutically in Europe, North America and Asia for hundreds of years. The roots, seeds and leaves of burdock have been investigated in view of its popular uses in Traditional Chinese Medicine (TCM)[8]. In this review, the reported therapeutic effects of the active compounds present in the different botanical parts of burdock are summarized in the roots, the active ingredients have been found to "Detoxify" blood in TCM term and promote blood circulation to the skin surface, improving the skin quality/texture and curing skin diseases like eczema. Antioxidants and anti-diabetic compounds have also been found in the root. In the seeds, some active compounds possess anti-inflammatory effects and potent inhibitory effects on the growth of tumors such as the pancreatic carcinoma. In the leaf extract, the active compounds isolated can inhibit the growth of micro-organisms in the oral cavity beside treating chronic diseases like cancers, diabetes and AIDS[8]. *Arctium lappa* root is traditionally used in herbal remedies to treat tonsillitis, throat pain, arthritis, rashes, boils and various skin problems. According to TCM, these pathological events are mainly due to the accumulation of toxin in the body it is suggested that the root of this herb is particularly effective and invaluable in eliminating heavy metals from the body. Therefore, it appears to have the function of draining toxins in terms of TCM theory[7]. *Arctium lappa*, well-known as a traditional plant of Asiatic origin, has a proven hepatoprotective potential against several hepatotoxicants, such as carbon tetrachloride, acetaminophen, carrageenan and chronic ethanol consumption, such property is attributed to its antioxidant and free radical scavenging activity[9-11]. Earlier study was conducted and showed the antioxidative and *in vitro* antiproliferative activity of *Arctium lappa* root extracts as well as the presence of some antioxidative compounds[10]. Encouraging data on the efficacy of *Arctium lappa* against hepatotoxicants prompted us to evaluate its therapeutic efficacy in acute liver Cd intoxication. Pharmacology shows that the extracts from different parts of burdock have long been considered good for health. They help enhance the body's immune system and improve metabolic functions[12]. Biological activities and pharmacological functions reported for the *Arctium* species include anti-inflammatory, anti-cancer, anti-diabetic, anti-microbial and antiviral activities. Now about the useful properties of burdock are due to its components of bioactive secondary metabolites such as lignans and flavonoids, besides its Antioxidants and antidiabetic ability[8,13]. The main objective of the current study was to assess the ability of burdock root (*Arctium lappa*) as an antioxidant rich plant in reducing the oxidative stress of cadmium on the histopathology of the kidney of Wistar rats.

MATERIALS AND METHODS

Experimental design

Animal grouping: Adult male Wistar rats (120-135 g) were divided into four groups randomly (n = 10). All animals were kept in cages in the laboratory under environmentally controlled conditions. Burdock root (*Arctium lappa*) were commercially obtained from General Nutrition Center (GNC), *Arctium lappa* were administrated orally through a syringe 30 min before the cadmium chloride administration ($CdCl_2$) (Lobachemi Co, India) for five weeks. The experimental groups were as follows:

Group 1: Control

Group 2: Exposed to cadmium (15 mg Cd kg^{-1} b.wt., day^{-1})

Group 3: Exposed to cadmium (15 mg Cd kg^{-1} b.wt., day^{-1})+ treated with a single dose daily of (100 mg kg^{-1} b.wt.) *Arctium lappa*

Group 4: Exposed to cadmium (15 mg Cd kg^{-1} b.wt., day^{-1})+ treated with a single dose daily of (200 mg kg^{-1} b.wt.) *Arctium lappa*

Specimen collection: During the experimental period, all rats were observed for the appearance of morphological changes. At the end of the experimental period, venous retro orbital blood samples for comet assay. After the blood collection, the rats were immediately sacrificed from each group and the kidney was excised out and weighed. Samples of kidney were fixed in 10% buffered formaldehyde solution for histological investigation.

Single Cell Gel Electrophoresis (SCGE) assay

Lymphocytes isolation: A volume of 20 µL of whole blood was mixed with 1 mL RPMI 1640 in a micro-centrifuge tube, to which 100 µL ficoll histopaque was added below the blood/media mixture. The contents were centrifuged for 3 min at 2000×g. Thereafter, 100 µL of bottom of the media/top of Ficoll layer was removed. To this was added to 1 mL media and contents were mixed and centrifuged for 3 min at 2000×g to get a pellet of lymphocytes. The supernatant was poured off and the pellet was re-suspended in 75 µL of Low Melting Point Agarose (LMPA).

Preparation of slide: The cells (1×10^7 cells) were collected and equal volume of cell suspension (4×10^5) was mixed with 0.7% (w/v) Low Melting Agarose (LMA). The mixture was pipetted onto the frosted slides pre-coated with 1.0% (w/v) normal melting agarose. After solidification of agarose, the slides were covered with another 100 mL of 0.7% (w/v) LMA and immersed in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl buffer, 0.1% SDS and 1% Triton X-100 and 10% DMSO, pH 10.0) for 90 min to lyse the cellular and nuclear membranes. The slides were then washed twice with neutralizing buffer (0.4 M Tris-HCl; pH 7.5) for 10 min and treated with ethanol for another 5 min. The slides were stained with 40 mL of SYBR green (20 mg mL^{-1}) and DNA damage was visualized by using fluorescence microscope ("Olympus, Japan" equipped with Cool SNAP1 Pro color digital camera). The damage appeared as a 'comet' with fragmented DNA (tail) being separated from undamaged nuclear DNA (head) and measurements were made by a Comet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK) linked to a CCD camera to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the tail length of DNA migration (µm) and the percentage of migrated DNA. Finally, the program calculates tail moment. Generally, 50-100 randomly selected cells were analyzed per sample[14,15].

Histological analysis: Samples of fragments of fresh kidney was fixed in buffered 10% formalin solution. Thereafter the tissues were dehydrated through a graded series of ethanol (from 70-100% alcohol in subsequent steps). Xylene was used as a clearing agent. Tissues were embedded in paraffin (58.6°C). Sections of paraffin blocks were cut by a rotary microtome (5 µm). Sections were stained with hematoxylin and eosin and were examined and photographed using a photomicroscope (Nikon, Japan).

Statistical analysis: Results were expressed as Mean±Standard Error of Mean (SEM). Statistical significance at the $p\leq0.05$ were considered significant and determined by one-way analysis of variance (ANOVA) and *post hoc* Least-Significant Difference (LSD) using JMP statistical software (Start Statistics, 8th edition (SAS Institute, Inc., Cary, North Carolina, USA)[16]. The data obtained from only cadmium toxicity studies was analyzed using Student's t-test.

RESULTS

Histopathological effects on the kidney: The sections of the control kidney showed normal structural information and the transverse section of kidney control (group 1) showed well defined renal tubules both proximal convoluted tubules and distal convoluted tubule and normal glomerulus (Fig. 1a). Sections of kidney of rats from group 2 exposed to Cd alone showed disorganized renal structure with collapsed tubular lamina with diffused hemorrhage (Fig. 1b), while histological sections of kidney of rats from group of (15 mg Cd kg^{-1} b.wt.+ 100 mg *Arctium lappa* kg^{-1} b.wt.) showed a minor restoration in the organization of the structure in relation of less hyper-cellularity of glomerulus and normal renal tubules with lumina compared to control group (Fig. 1c). Furthermore, the histological sections of kidney of rats from the group of (15 mg Cd kg^{-1} b.wt.+200 mg *Arctium lappa* kg^{-1} b.wt.) showed more improvement and retained the normal appearance of renal tubules and glomerulus with a prominent urinary space and decreased number of destructive tubules in comparison to group 2 and 3 (Fig. 1d).

DNA damage: In the current study, the tail length of cells from the rats of control group was about 7.0 µm. While in cells from the positive control group (Cd only) a highly significant ($p\leq0.01$) increase was observed with a tail length of 76 µm. In the Cd exposed group treated with *Arctium lappa* (100 mg kg^{-1} b.wt.) the tail length of cells was recorded as 59 µm which was significantly ($p\leq0.001$) higher compared to control group. In the Cd exposed group treated with *Arctium lappa* (200 mg kg^{-1} b.wt.) the tail length of cells was recorded as 39µm which was significantly ($p\leq0.001$) lower in comparison to group 2 and 3 (Fig. 2).

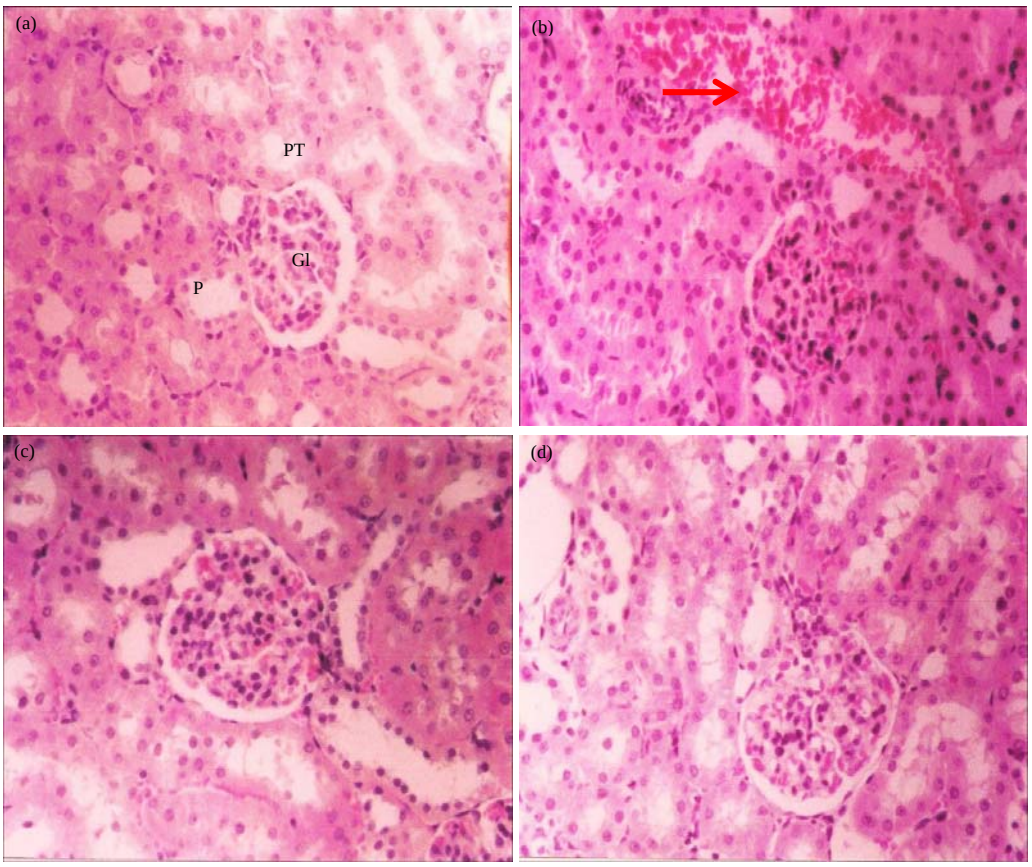

Fig. 1(a-d): Kidney sections of male Wistar rats (a) Control group, (b) Cadmium treated rat from group two, (c) Cadmium treated rat from group three and (d) Cadmium treated rat from group four

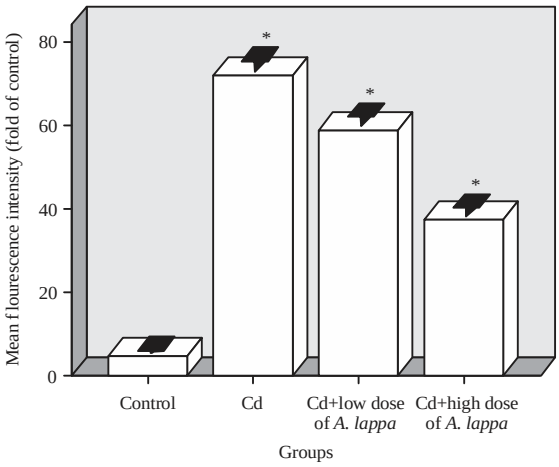

Fig. 2: Effect of aqueous extract of *Arctium lappa* against cadmium-induced DNA damage in the kidney of male Wistar rats

Control shows DNA damage in control group; Cd shows DNA damage in group two, Cd+low dose of *Arctium lappa* shows DNA damage in group 3, Cd+high dose of *Arctium lappa* shows DNA damage in group 4. Data are presented as Mean±SEM (n = 5). *Significant differences from control group at p<0.05, Error bars: 95% CI

DISCUSSION

The results showed that the oral administration of cadmium chloride caused geno/cytotoxicity depend on the amount of cadmium absorbed by the tissues of the body. The Cd tended to accumulate in liver and kidney more in gills, muscles, blood and brain when applied in *Cyprinus carpi*[17,18]. Oral administration of $CdCl_2$ (15 mg Cd kg^{-1} b.wt.) in drinking water of rats enhances oxidative stress and subsequently the excess of Reactive Oxygen Species (ROS) my induce DNA damage[19-22]. The DNA were affected adversely after rats were exposed to cadmium chloride alone. Cadmium caused DNA damage through the breakage of single or double strand, thus resulting and showing comet formation. The traditional treatment containing *Arctium lappa* is consumed 30 min before cadmium exposure as pre-treatment in order to prevent toxic effect of cadmium chloride due to protective effect of *Arctium lappa* against $CdCl_2$, *Arctium lappa* is a natural antioxidants products which possess free radical scavenging activity. Therefore, *Arctium lappa* was designed and tested against cadmium toxicity[21]. In the present study,

the low dose of *Arctium lappa* with cadmium chloride (15 mg Cd kg^{-1} b.wt.+100 mg *Arctium lappa* kg^{-1} b.wt.) recorded slight reduction. However, after administration of $CdCl_2$ with high dose of *Arctium lappa* (15 mg Cd kg^{-1} b.wt.+ 200 mg *Arctium lappa* kg^{-1} b.wt.) was restored and showed apparent signs of renal protective effect against cadmium chloride compared to cadmium alone[21,23]. In consensus to with previous studies on cadmium exposure effects on histopathological sections[23-25]. Sections of kidney of rats from group 2 exposed to Cd alone showed disorganized renal structure with collapsed tubular lamina compared to control group. Supplementation of *A. lappa* reduces cadmium damage levels in the kidney sections, the oral administration of $CdCl_2$ with high dose of *Arctium lappa* (15 mg Cd kg^{-1} b.wt.+200 mg *Arctium lappa* kg^{-1} b.wt.) clearly showed alleviation of $CdCl_2$ in comparison to low dose of *A. lappa* (15 mg Cd kg^{-1} b.wt.+100 mg *Arctium lappa* kg^{-1} b.wt.) and Cd-exposed group. Several investigations have confirmed that after *Arctium lappa* extract administration, even after Cd exposure, showed marked therapeutic effects[7,19]. Results from the current investigation suggested that plant rich in phytochemical such as *Arctium lappa* was efficient in relieving the Cd-induced oxidative stress in rat. High amount of applied plant extract provided more improvement in cellular organization in kidney as well as the DNA which might be related to the a free radical quenching effect. Therefore, *Arctium lappa* extract showed remarkable ameliorative effect on the oxidative damage that subsequently led to tissue destruction.

CONCLUSION

Results from the current study revealed that, a tissue and DNA damage were induced by cadmium application but, significant outcome on such damage was obtained when *Arctium lappa* was orally pre-applied to the Wister rabbits. Efficiency of *Arctium lappa* extract was dose dependent since application of 200 mg *Arctium lappa* kg^{-1} b.wt., proved to be more effective than the lower dose (100 mg *Arctium lappa* kg^{-1} b.wt.). The supplementation of *Arctium lappa* used in the present study have a wide therapeutic use against toxicity due to its antioxidant role. The use of natural products such as plant phytochemical to fight against metal toxicity and combat their oxidative damage does provide a good therapeutic field to restore the normal function of the body organs.

SIGNIFICANCE STATEMENTS

This study discovers the positive effect of plant extract *Arctium lappa* against cadmium genotoxicity and histopathology in the kidney of Wistar rat. Oral application of plant extract in different concentration provided histology and genetic improvements of kidney suggesting high progress after application of high dose of *Arctium lappa*. This study will help researcher to decide about such plant extract since better health was resulted.

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