

Effect of *Erythrina variegata* and *Spondia spinnata* bark powder on cadmium induced pathological changes in rats

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ABSTRACT

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Forty two, six month old, albino wistar rats were randomly divided into seven groups. All groups except group I which served as control were given cadmium chloride @100mg/L of drinking water. Silymarin @100mg/kg bwt in group III and EVBP and SPBP @ 1 and 2% in groups IV, V, VI and VII, respectively were given in feed for 90 days. After 90 days, the histopathological changes were characterized by vascular and inflammatory changes in kidneys, liver, lungs, heart and brain. Histopathological changes were severe in only Cd treated rats while mild to moderate changes were found in EVBP and SPBP treatment groups. On electron microscopy, ultra structure of the renal and hepatic cells showing disappearance of nucleolus, pyknosis and marginalisation of chromatin material at the periphery of the nucleus were evident in the group II. It is concluded from the present study that the bark powder of *E. variegata* and *Spondia spinnata* have ameliorative effect on tissues of these organs in cadmium chloride induced toxicity in rats.

Keywords: Cadmium, *Erythrina variegata*, pathology, rats, *Spondia spinnata*

INTRODUCTION

Cadmium (Cd) is an element that occurs naturally in the earth's crust. It is known to be one of the most toxic environmental and industrial pollutants present in soil, air, water and even in food. Cadmium enters into food chain through occupational and non-occupational sources which include reünery, smelting of metals such as copper, zinc and combustion of fossil fuel and consumer products like batteries, pigments, metal coatings, plastics and some metal alloys. Subsequently, cadmium gets accumulated in body tissues of man and animals. Major toxic effects of cadmium are due to the oxidative stress leading to damage in the organs like kidney, liver, lung, pancreas, testes, placenta and bone.

In the past decade, several naturally occurring dietary or non-dietary constituents, and parts of several species of medicinal plants like *Ecliptaalba*, *Embllica officinalis*, *Withania somnifera*, *Aloevera*, etc. have been reported to possess various pharmacological activities. *Erythrina variegata orientalis* leaf powder has been widely used for different ailments as folk remedy¹ and it also acts as adsorbent for cadmium and thus reduces its absorption and subsequent toxic effects.

Despite many folklore practices and therapeutic uses of *Erythrina variegata* and *Spondia spinnata* for the treatment of various ailments, there is paucity of data on

their ameliorating potential following cadmium toxicity in animals. Keeping in view the above facts, the present study was undertaken to study the ameliorative efficacy of the bark powder of *E. variegata* (EVBP) and *Spondia spinnata* (SPBP) on pathological and ultrastructural changes in various organs in cadmium chloride induced toxicity in rats.

MATERIALS AND METHODS

Erythrina variegata was collected from the GBPUAT Pantnagar campus and the *Spondia spinnata* from Dumka (Santhal Pargana) region of Jharkhand. Both these plants were confirmed by Botanical Survey of India, Botanical garden, Howrah. The raw bark was gathered from respective plants, chopped fine, shade dried for 6-7 days to remove excess moisture. Later the bark was placed in fan equipped incubator at 45-50°C for 7-10 days, grounded in electric grinder to fine homogenous powder.

Forty two albino wistar rats of six months of age, weighing 170-200 gm were procured from Lab Animal Unit, Indian Veterinary Research Institute, Izatnagar and maintained at Lab Animal Shed, Pantnagar under standard managemental conditions. These rats were divided randomly into seven uniform groups viz. group I, II, III, IV, V, VI and VII after acclimatizing for 15 days. All the groups were fed standard computed ration and deionised water *ad libitum*. The experimental design has been mentioned in the table as given below.

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Groups	Treatment	Duration
I	Control	90 days
II	Cadmium chloride@ 100mg/L in drinking water.	90 days
III	Cadmium chloride@ 100mg/L in drinking water + Silymarin @100mg/kg.b.wt. in feed	90 days
IV	Cadmium chloride@ 100mg/L in drinking water + <i>E. variegata</i> bark powder @ 1% in feed.	90 days
V	Cadmium chloride@ 100mg/L in drinking water + <i>E. variegata</i> bark powder @ 2% in feed.	90 days
VI	Cadmium chloride@ 100mg/L in drinking water + <i>S. pinnata</i> bark powder @ 1% in feed.	90 days
VII	Cadmium chloride@ 100mg/L in drinking water + <i>E. variegata</i> bark powder @ 2% in feed.	90 days

The experiment continued for ninety days. All the rats from all the groups were euthanized using chloroform, as prescribed Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), at the end of experimentation i.e. at 90th day of the study. All the rats were subjected to detailed post mortem examination and the gross lesions were duly recorded. Representative samples from liver, kidney, brain, heart and lungs were collected in 10% neutral buffered formalin and processed for histopathological examination as per standard protocol². Briefly, the tissue samples were washed overnight in running tap water, dehydrated in ascending grades of alcohol, cleared in xylene, impregnated with paraffin wax and finally blocks were made using paraffin wax. Tissue sections were cut at 4 micron thickness. Finally sections were stained with haematoxylin and eosin stain using standard procedures². Ultrastructural studies were considered depending on relevant histopathological findings.

The sample for ultra-structural studies of liver and kidney from group II animals were collected, processed and analysed on Transmission Electron Microscope JEOL 1011 in the Electron Microscopy Lab, Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar. The samples were processed using standard procedures. Glutaraldehyde was used as fixative and acetone was used as dehydrating agent.

RESULTS

Gross pathology

Gross lesions could be observed only in the kidneys, liver and lungs of group II rats which were dark red in colour. Heart showed severe engorgement of blood

vessels. Brain also showed congestion of blood vessels. No other gross lesion could be observed in the organ of any other group.

Histopathology

No microscopic changes were observed in kidney, liver, lungs, heart, brain and spleen of group I rats.

Kidney: In group II, the histopathological changes in cortex of kidney were interstitial hemorrhages, congestion of large blood vessels, and of glomerular capillaries at places, moderate to severe swelling of glomeruli along with coagulative necrosis of renal tubular epithelial cells (Fig. 1). Some of the tubules were showing initial stages of hydronephrosis. In medulla, blood vessels in the interstitium were congested. The treatment with silymarin, EVBP and SPBP also revealed these changes but were of moderate intensity after 90 days of cadmium exposure.

Liver: The histopathological examination of liver from cadmium intoxicated group II revealed, severe congestion of large blood vessels, sinusoidal congestion (Fig. 2), with vacuolar degeneration of hepatocytes. However, these changes were mild to moderate in groups III, IV, V, VI and VII.

Lung: In group II rats, the changes were severe congestion of alveolar capillaries, presence of mononuclear cells at many places throughout the lung, while these changes were mild to moderate in groups III, IV, V, VI and VII.

Heart: The heart revealed congestion and hemorrhages in between the myocardial muscle, degeneration of cardiac muscles and edema in between the cardiac muscle fibres in cadmium intoxicated group II (Fig.3), whereas these changes were mild to moderate in all other treatment groups.

Brain: The histopathological examination of the brain tissues revealed severe congestion of the blood vessels, moderate degeneration of neurons, elongation of neuronal cell bodies with mild gliosis and moderate to severe satellitosis and neuronophagia in cadmium exposed group II. The brain from groups III, IV, V, VI and VII revealed similar lesions but of mild to moderate intensity as compared to group II.

Spleen: Spleen exhibited reduction in red pulp and congestion in group II and IV. No changes were observed in the spleen of rats from other groups.

Ultrastructural pathology

Kidney: Electron microscopy of the renal cells showed disappearance of nucleolus, pyknosis and marginalisation of chromatin material at the periphery of the nucleus (Fig. 4) in the cadmium treated group II. The mitochondria were altered in size and shape and there was extensive loss of the basal plasma membrane

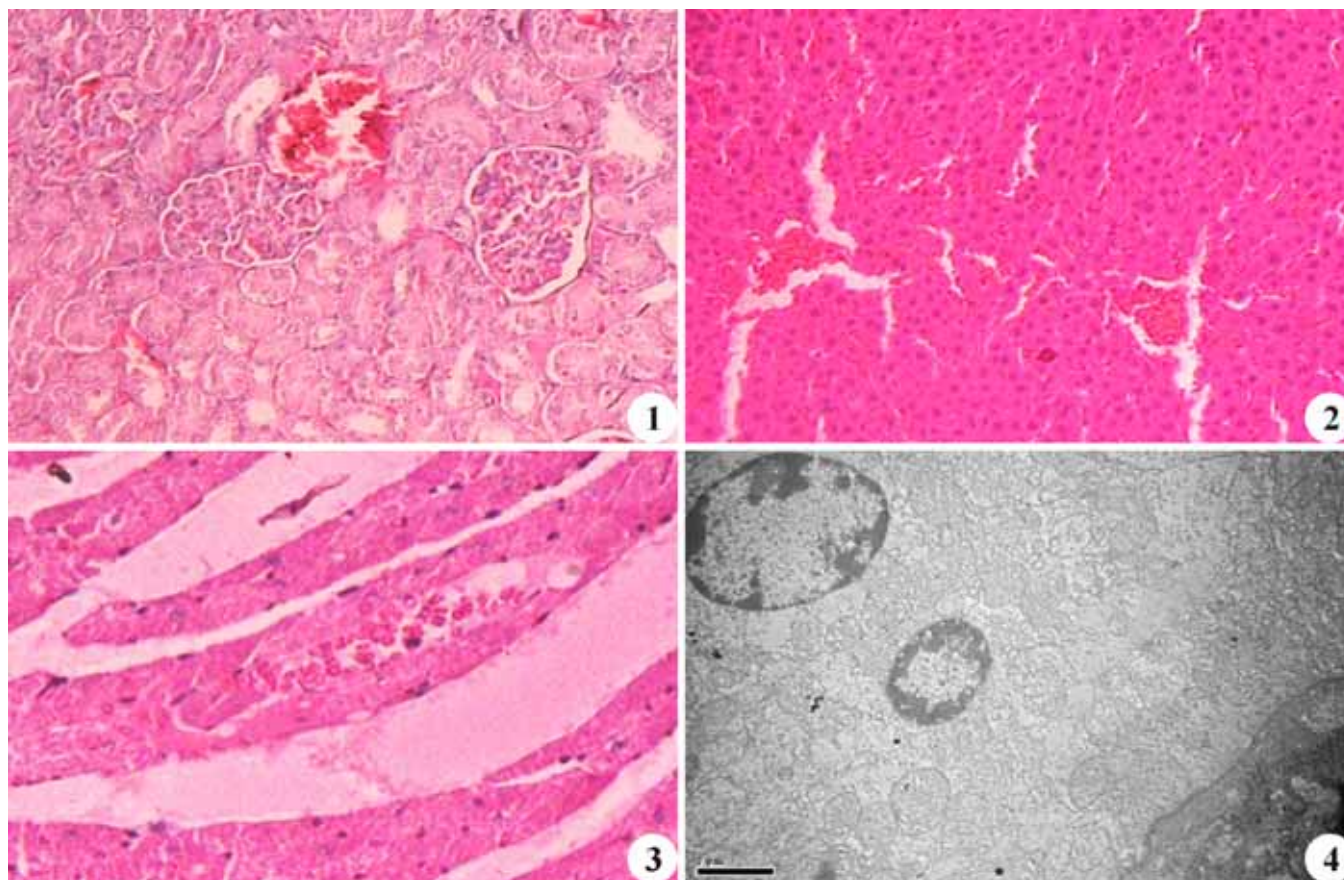


Fig.1. Kidney (Group II) showing congestion of blood vessel, interstitial haemorrhage and coagulative necrosis in most of the tubular epithelial cells. H&E x100; **Fig. 2.** Liver (Group II) showing cellular swelling of hepatocytes and severe congestion of blood vessel. H&E x100; **Fig.3.** Heart (Group II) exhibiting separation of cardiac muscle fibres with severe oedema. H&E x100; **Fig.4.** Ultrastructure of the renal cell (Group II) showing marginalisation of chromatin material at the periphery of the nucleus. x10600.

infoldings together with complex cisternal proliferation in proximal tubular cells.

Liver: Ultrastructural details of hepatocyte were represented through vacuolation and marginalisation of chromatin material. There were presences of electron dense particles in nucleus and vacuoles in hepatocytes as well as karyorrhexis were observed at places in hepatocytes in rats of group II.

DISCUSSION

The lesions in the kidney are of moderate to severe intensity in all the treatment. These moderate degenerative changes in the renal tubular epithelial cells might be due to the damage by free oxidative radicals produced in excess by the presence of cadmium in the renal tissues overwhelming the capacity of the treatment with silymarin, EVBP and SPBP. These moderate nephrotoxicity can also be correlated as evidenced by elevated value of BUN and creatinine in cadmium intoxicated rats as well as in the treatment groups³. Earlier workers have also reported toxic effect in kidney

following cadmium exposure in albino rats^{4,5}. However there are very limited studies regarding the protective effect of any plant in cadmium toxicity on kidney. It is inferred from histopathological examination of liver of various groups that EVBP and SPBP and even silymarin failed to completely reverse the cadmium induced histopathological changes in liver. This might be due to the persistence of cadmium in liver as also evident from residual analysis in this study³. The presence of cadmium in liver tissue might be responsible for the persistence of microscopic changes in liver. Hepatotoxic effects following cadmium exposure in albino rats have already been reported⁵ and in bank vole⁶. However there is paucity of literature regarding any protective effect of any plant in cadmium toxicity over liver. Previous studies have also reported that antioxidant defence system components of lungs and heart i.e. GPX might be potential modulator of the Cd-induced LPO reaction in lungs and heart tissues⁷. Our study revealed mild to moderate histopathological changes in lungs as compared to the severe changes in cadmium exposed control group which indicated protective action of the

treatments of silymarin, EVBP and SPBP. In heart, cadmium intoxication induces cardiovascular abnormality⁸. The susceptibility to lipid peroxidation of liver, kidney, brain, testis, lungs and heart was investigated in tissue homogenates incubated with various concentrations of cadmium by earlier workers⁷. They found that liver, heart and testis exhibited greatest release of breakdown products of cell components from Cd exposure along with significant increase in lipid peroxidation. As the injury caused by Cd is mediated by lipid peroxidation and EVBP and SPBP have free radical scavenging action. Thus, EVBP and SPBP treatment would have exerted protective effects by reducing Cd induced lipid peroxidation. Cd has been shown to produce free radicals in the brain, which may potentially damage both neurons and glial cells which myelinate axons in the CNS. Cd toxicity affected CNS white matter⁹ and later workers demonstrated that glial cells are direct targets of Cd¹⁰. Experimental studies have shown that Cd can be a potent neurotoxicant for the peripheral nervous system and directly destroys the plexus ultrastructure¹¹. Cd-induced injury in the cerebral microvessels is thought to be associated with oxidative stress. Thus, a depletion of microvessel antioxidant defense systems and a resultant increase in lipid peroxidation (LPO) may provoke microvessel damage¹². As such the treatment with EVBP and SPBP promoted radical scavenging action and thus reduced the Cd induced oxidative damage in brain, thereby the Cd induced damage was lesser in comparison to the Cd treated group II. In spleen, hyperplasia of lymph follicles in the spleen has been reported earlier at doses of more than 1.0 mg Cd/kg in 4- and 8-month-old rats thrice weekly⁴. Spleen of guinea pig seven weeks post-cadmium chloride exposure exhibited focal hemorrhagic areas with diffuse hemosiderosis¹³. It can be inferred that the treatment excluding group II and IV manifested no reduction of red pulp may be due to the beneficial effects of the silymarin, EVBP (2%) and SPBP as a result of radical scavenging properties. Furthermore it can be added that EVBP fed @ 2% were more efficient as compared to 1% EVBP.

Similar findings have also been reported by earlier workers with regard to ultrastructural changes in the kidney¹⁴. They reported focal loss of brush border, swelling of mitochondria, loss of cristae, and condensation of chromatin materials, along with terminal nucleoli appeared with heterochromatin. The ultrastructural changes observed in liver in this study are in accordance with the earlier reports⁵.

It is concluded from the study that the bark powder of *E. variegata* and *Spondia spinnata* have ameliorative effect on tissues of these organs in cadmium chloride induced toxicity in rats.

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