



## Cisplatin induces Nephrotoxicity in Swiss Albino Mice: Modulatory role of herbal extract of *Articum Lappa* (Roots) against inflammation

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### Abstract

**Abstract:** In the present study, we attempted to investigate the effect of hydroalcoholic and *Articum Lappa* on CP- induced nephrotoxicity in rats. Our results clearly showed that the hydroalcoholic extract of *Articum Lappa* (Roots) inhibited the CP-induced increases of kidney damage biomarkers (BUN and creatinine). Pathological findings also confirmed the protective effect of the hydroalcoholic extract of *Articum Lappa*. Hydroalcoholic extract of *Articum Lappa* showed significant protective effect at doses of 250 and 350 mg/kg and unexpectedly the dose of 450 mg/kg was ineffective.

It seems that the dose of hydroalcoholic extract of *Articum Lappa* is detrimental and higher doses lose activity. At present we do not have explanation for it and further studies are required to find out the reason. The hydroalcoholic extract of *Articum Lappa* are good source of flavonoid compounds such as quercetin. The findings of many researches confirmed that these compounds are responsible for useful effects in oxidative stress.

On the other hand, several studies reported that the mechanism of CP nephrotoxicity can be associated with oxidative stress. As a result, the protective effect of hydroalcoholic extract of *Articum Lappa* on CP-induced nephrotoxicity might be partly due to antioxidant potential of these compounds. Some investigators have reported that CP-induced nephrotoxicity has an inflammatory component and pro-inflammatory cytokines are involved in its pathogenesis.

**Key words:** *Articum Lappa* , Cisplatin, Nephrotoxicity.

### Introduction :

Microcapsules have focal points like proficient retention and upgraded bioavailability of the medications because of a high surface to volume proportion, a substantially more cozy contact with the bodily fluid layer and particular focusing of medications to the ingestion site.

Cisplatin is a major anti neoplastic drug used for the treatment of solid tumors. Its chief dose limiting side effect is nephrotoxicity; 20% of patients receiving high-dose Cisplatin have severe renal dysfunction. Cisplatin-DNA crosslink's cause cytotoxicity lesions in tumors and other dividing cells. DNA damaging agents usually have less toxicity in non-proliferating cells, yet the quiescent proximal tubule cells are selectively damaged by Cisplatin [1]. The mechanism for this renal cell injury has been the focus of intense investigation for many years, and recent studies suggest that inflammation, oxidative stress injury, and apoptosis probably explain part of this injury. Understanding the mechanism(s) for this side effect should allow clinicians to prevent and/or treat this problem better and provides a model for investigating drug-induced nephrotoxicity in general. [2]

In Madhya Pradesh, the plant is regarded as very auspicious and is stated that a person who possess it will never experience shortage of cereals and food. The rhizomes of the plant are aromatic in nature. The inner part of the rhizome is bluish-black in colour and emits a characteristic sweet smell, due to presence of essential oil. Traditionally, the Roots of *Articum Lappa* are used in treating leucoderma, asthma, tumours, piles, bronchitis etc. The paste is applied on bruises, contusions and rheumatic pains in Manipur [3]. In Arunachal Pradesh, Adi tribes use decoction of fresh rhizome as anti-diarrhoeic. The Khamti tribe of Lohit district applied the paste of fresh rhizome in case of snake and scorpion bite [4]. In the present study, we attempted to investigate the effect of hydroalcoholic and *Articum Lappa* on CP- induced nephrotoxicity in rats.

### Collection of plant materials

The rhizomes of *Articum Lappa* were Purchased from Akshat Nursary of Bhopal in the period of March 2022, considering the seasonal conditions for obtaining maximum phytoconstituents. Authentication of collected plant materials were confirmed by HOD Department of Botany, Barkatullaha University, Bhopal, Madhya Pradesh, India.

### Extraction (By Maceration Method) <sup>[5]</sup>

#### Maceration

Collected plant drugs namely *Articum Lappa*) were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (50 gm) and packed in (1 liter) air tight glass Bottle. The plant drugs were subjected to extraction by Metahnol: water (20:80) as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

### Phytochemical Analysis

#### Preliminary Phytochemical Screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the Hydroalcoholic extract of rhizomes of *Articum Lappa*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids <sup>[6]</sup>.

#### Estimation of total phenolic content

Estimation of total phenolic content Total phenolic content of all the extracts was evaluated with Folin-Ciocalteu method. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, aliquots of 5, 10, 15, 20 and 25µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin– Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer 1650 Shimadzu, Japan. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extracts as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract <sup>[7]</sup>.

#### 5.5.2 Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified from the procedure reported by Marjanovic A et al.,2021 <sup>[8]</sup>. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 10 to 50 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of Hydroalcoholic extracts andv The aluminum chloride colorimetric method was modified from the

procedure reported by Marjanovic A et al.,2021<sup>[8]</sup>. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 10 to 50 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of Hydroalcoholic extracts and Flavonoid standard solutions (100 ppm) were reacted with aluminum chloride for determination of Flavonoid content as described.

## Pharmacology

### Experimental animals

Healthy adult Wistar albino rats of either sex were selected randomly for the study. The rats were obtained from College of Veterinary Sciences and Animal Husbandry, Mhow, Indore. Rats of 12–16 weeks, weighing 160–200 g, were used for the experiment. Each rat was housed in a plastic box cage under standard conditions at 19–25°C and was kept under 12/12 h light/dark cycle. The rats were allowed free access to standard pellet feed and water ad libitum. The study was carried out according to the CPCSEA and Organization of Economic Co-operation and Development (OECD) guidelines. Approval from Institutional animal ethical committee (IAEC) was also obtained.

### Experimental procedure<sup>[9]</sup>

Rats were randomly divided into five groups of 6 in each. One group received normal saline (0.2 ml, i.p) from the beginning to tenth day (end of the study). The second group received saline (0.2 ml, i.p.) for 2 days and in the third day CP (7 mg/kg, i.p) was injected. Other groups

received either hydroalcoholic extract (250, 350 and 450 mg/kg, i.p.) for two days before CP administration and thereafter until the tenth day.

In the last day, rats were anesthetized with ketamine (100 mg/kg) and then sacrificed. Blood samples were collected, centrifuged at 1000 rpm for 20 min, serum was removed and stored at -20°C until analysis. Both kidneys were removed and weighed. The left kidneys were prepared for pathological examinations.

### Measurement of biochemical parameters<sup>[10]</sup>

Serum creatinine concentration was measured by the Jaffe method and BUN (blood urea nitrogen) by Berthelot method using commercial kits. Serum nitric oxide was measured by a quantitative diagnostic kit (a colorimetric method). Osmolality of serum samples was determined by an Osmometer.

### Histopathological examination<sup>[11]</sup>

The left kidneys of animals halved through a coronal section after removal from the body. Then the two halves were fixed by a 10% solution of formalin for several days. After processing, they were embedded in paraffin and cut into 3-4 micrometer slices. The slices were mounted on glass slides and stained with hematoxylin and eosin (H & E) for light microscopic analysis. The assessment was conducted by a pathologist in a blinded way. The pathologic changes of the kidneys were recorded using a grading scale of 0-4 which was based on a subjective impression of the extent of cortical changes as follows:

- 0 = indistinguishable from controls
- 1 = minimal, ≤ 25% cortex affected
- 2 = mild, > 25% and ≤ 50% cortex affected
- 3 = moderate, > 50% and ≤ 75% cortex affected
- 4 = severe, >75% cortex affected

This grading scale is adapted from Goering and coworkers with minor modification.

### Statistical analysis

The analysis of data was performed with SPSS statistical analysis software (version 15.0). Data was reported as the mean  $\pm$  SEM. All results except for pathologic findings were analyzed using one way analysis of variations (ANOVA) followed by Tukey post hoc test.

### Result and Discussion

The plant drug (100g) was subjected to extraction by (maceration) using Hydroalcohol as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated. The weighed extract of plant drug was stored in desiccators for further use.

The yields were found to be (11.2% w/w of crude drug) of Hydroalcoholic extract *Articum Lappa*. Obtained results were recorded in table

The percentage yield of Hydroalcoholic extract of *Articum Lappa* was found to 11.2% by using maceration method.

Results of Phytochemical test showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Phenols, Diterpenes and Saponins, Alkaloid was found to absent in extract *Articum Lappa*. The results of phytochemical reveals that the all polar and Methanolic and aqueous soluble compound was found to be present in *Articum Lappa* extract.

#### Estimation of total phenolic content

Total phenolic content was estimated by gallic acid and expressed as milligrams of gallic acid equivalent (GAE). The extract contained a considerable amount of phenolic contents of GAE/g of extract. The results were presented in Fig 1.

#### 6.3 Estimation of total flavonoids content

Flavonoid content was calculated from the regression equation of the standard plot ( $y=0.02x+0.020$ ,  $R^2 =0.995$ ) and is expressed as quercetin equivalents (QE) (fig.). Total Flavonoid content was 0.165mg/100mg quercetin equivalent in HECC. Flavonoids are the most common and widely distributed group of plant's phenolic compounds.

#### Pharmacology

Treatment of animals with CP significantly increased serum concentrations of BUN and Cr. Hydroalcoholic extract (250, 350 and 450 mg/kg, i.p.) inhibited CP-induced increases of BUN and Cr. As it is seen in Table 1, the hydroalcoholic extract could not change serum osmolality and nitric oxide concentration.

Normal group received normal saline (0.2 ml, i.p.) once daily for 10 days. Control group received saline (0.2 ml, i.p.) for 2 days and in the third day cisplatin (7 mg/kg, i.p.) was injected. Other groups received hydroalcoholic extract (250, 350 and 450 mg/kg, i.p.) for two days before cisplatin administration and thereafter until tenth day.

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The weight of rat kidneys received CP was significantly greater than those of normal rats. Treatment of animals with different doses of hydroalcoholic extract of *Articum Lappa* could not prevent the increase of CP-induced kidney weight. The pathological findings clearly showed that CP significantly damaged the kidney tissue.

The numbers in the table are representative of the numbers of kidneys in each grade of a total of n in each group. Grading scale is as follows:

**0 = indistinguishable from controls**

**1 = minimal,  $\leq 25\%$  cortex affected**

**2 = mild,  $> 25\%$  and  $\leq 50\%$  cortex affected**

**3 = moderate,  $> 50\%$  and  $\leq 75\%$  cortex affected**

**4 = severe,  $> 75\%$  cortex affected.**

P<0.05 compared to control group and # P<0.01 compared to normal group

### Conclusion

Cisplatin nephrotoxicity is very complex and several mechanisms including accumulation of the drug in renal epithelial cells, attack of the drug to nuclear and mitochondrial DNA, initiation of a severe inflammatory response, induction of oxidative stress have been proposed. Based on these pathways, several in vitro and in vivo researches have been performed and a relatively large number of chemicals and natural compounds have been tested for their potential protection against CP-induced nephrotoxicity.

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