Panacea Journal of Pharmacy and Pharmaceutical Sciences 2023:12(1), 01-11
International Journal

Panacea Journal of Pharmacy and Pharmaceutical Sciences
ISSN: 2349 7025

PJPPS
Panacea Research Library
http://internationaljournal.org.in/journal/index.php/pjpps

Original Research Article **Volume 12 Issue 1** **Jan-March 2023**

CISPLATIN INDUCES NEPHROTOXICITY IN RATS: MODULATORY ROLE OF HERBAL EXTRACTS AGAINST INFLAMMATION

Divyanshu Dubey, Neha Maheshwari, *Dr. Sujata Kushwaha, Dr C. K. Tyagi

College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M.P.)

*Corresponding Author's Email id: sujatakushwahasehore@gmail.com

Abstract: In the present study, we attempted to investigate the effect of hydroalcoholic and *Curcuma caesia Roxb* rhizomes on CP- induced nephrotoxicity in rats. Our results clearly showed that the hydroalcoholic extract of *Curcuma caesia Roxb* (rhizomes) inhibited the CP-induced increases of kidney damage biomarkers (BUN and creatinine). Pathological findings also confirmed the protective effect of the hydroalcoholic extract of *Curcuma caesia Roxb*. Hydroalcoholic extract of *Curcuma caesia Roxb* 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 *Curcuma caesia Roxb* 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 *Curcuma caesia Roxb* 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 *Curcuma caesia Roxb* 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: *Curcuma caesia Roxb,* Cisplatin, Nephrotoxicity,

Introduction

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 asvery 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 rhizomes of *Curcuma caesia Roxb* 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-diarrhoetic. 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 *Curcuma caesia Roxb* rhizomes on CP- induced nephrotoxicity in rats.

Collection of plant materials

The rhizomes of *Curcuma caesia Roxb* were collected from local area 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) [5]

Maceration

Collected plant drugs namely *Curcuma caesia Roxb* (rhizomes) 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 *Curcuma caesia Roxb*, 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 [6].

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 [7].

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 [8]. 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 [9]

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 -20ºC until analysis. Both kidneys were removed and weighed. The left kidneys were prepared for pathological examinations.

Measurement of biochemical parameters [10]

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 [11]

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 ± SEM. All results except for pathologic findings were analyzed using one way analysis of variations (ANOVA) followed by Tukey post hoc test.

Results

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 *Curcuma caesia Roxb* rhizomes. Obtained results were recorded in table

The percentage yield of Hydroalcoholic extract of *Curcuma caesia Roxb* 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 *Curcuma caesia Roxb* rhizomes. The results of phytochemical revels that the all polar and Methanolic and aqueous soluble compound was found to be present in *Curcuma caesia Roxb* rhizomes 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.

Table: 1 Absorbance of standard and sample at 760nm

Standard	Concentration (µg/ml)	Mean Absorbance
Gallic acid	5	0.125
	10	0.226
	15	0.325
	20	0.415
	25	0.526

n= 3, values are given in SEM

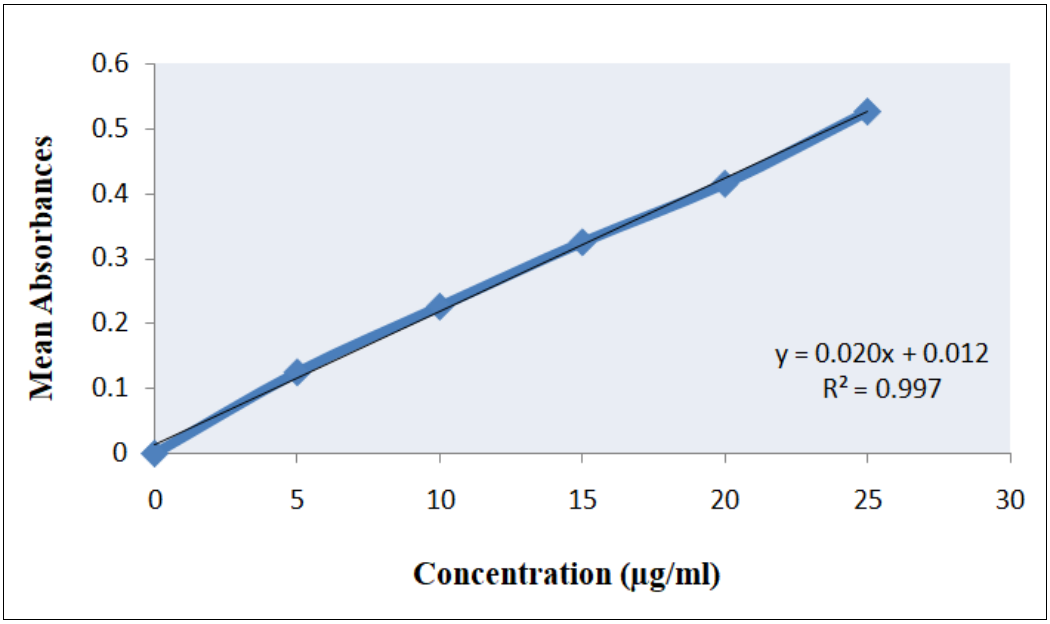

Figure: 1 Standard (Gallic acid) Calibration curves

Table: 2 Total Phenolic Content of Hydroalcoholic extract of *Curcuma caesia Roxb*

Sample	Total phenolic content GAE mg/100mg
Hydroalcoholic extract 100µg/ml	0.214

n=3, values are given in SEM

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.

Table: 3 Absorbance of standard and Hydroalcoholic extract of *Curcuma caesia Roxb*

S. No	Concentration of Quercetin (µg/ml)	Mean absorbance
1	10	0.225
2	20	0.452
3	30	0.615
4	40	0.812
5	50	1.012

n=3, values are given in SEM

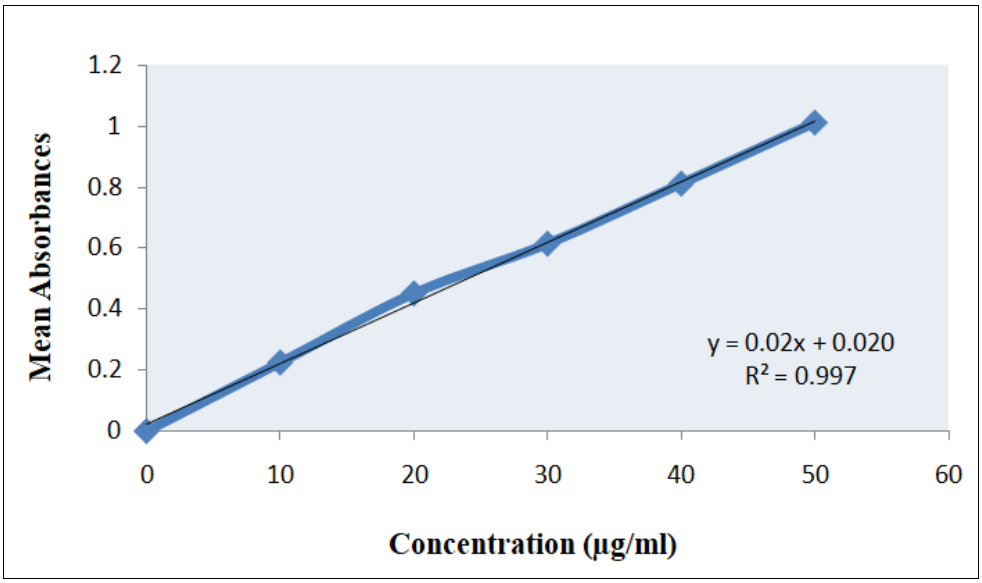

Figure: 2Standard (Quercetin) Calibration curves

Table: 3 Total Flavonoid content of Hydroalcoholic extract *Curcuma caesia Roxb*

S. N.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mg/100mg
1	Hydroalcoholic extract (100µg/ml)	0.167

n=3, values are given in SEM

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.

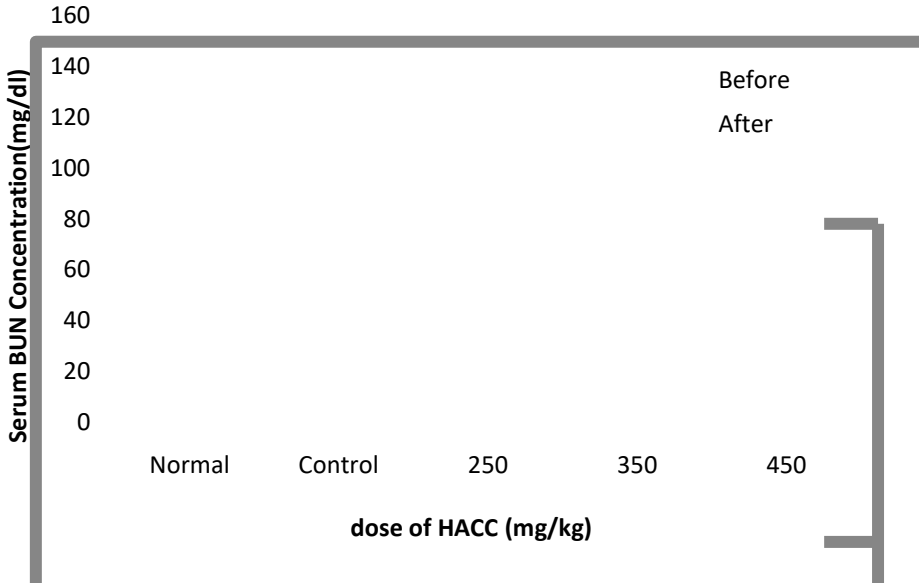

Fig. 3 Effect of different doses of hydroalcoholic extract of *Curcuma caesia Roxb* on serum BUN concentration in cisplatin-treated rats.

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.

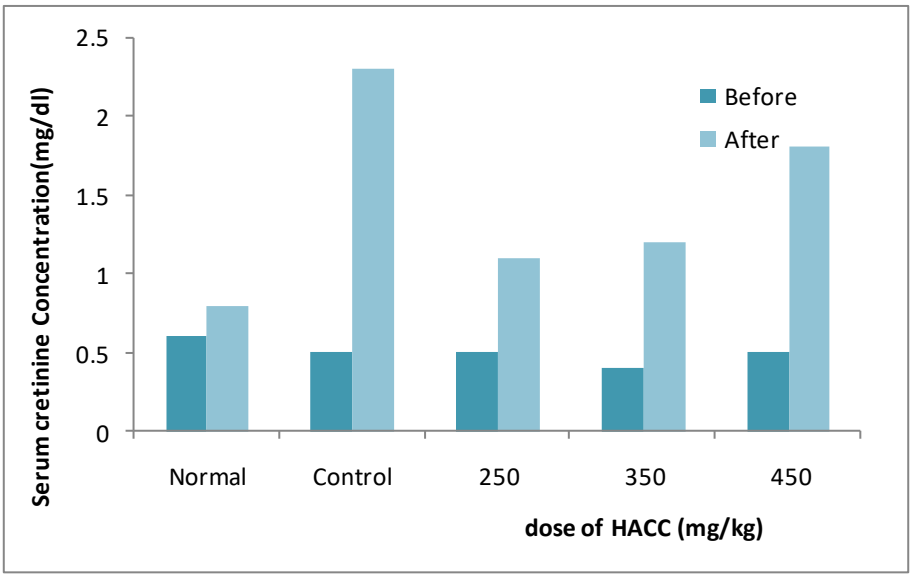

Fig. 4 Effect of different doses of hydroalcoholic extract of *Curcuma caesia Roxb* on serum creatinine concentration in cisplatin-treated rats.

Normal group received 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.

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 *Curcuma caesia Roxb* could not prevent the increase of CP-induced kidney weight. The pathological findings clearly showed that CP significantly damaged the kidney tissue .

Table : 5 Effect of different doses of *Curcuma caesia Roxb* hydroalcoholic extract on kidney weight , serum osmolality and nitric oxide (NO) concentration in male rats.

Group	Dose (mg/kg)	Kidney weight (g/100 g BW)	Serum osmolality (mM/L)	Serum NO concentration (µM/L)
Normal	-	0.311±0.02	272.0±5.2	8.93±1.94
Control	-	0.496±0.03	323.6±25.2	16.57±2.13
HACC	250	0.489±0.05	298.4±9.3	21.64±8.44
HACC	350	0.561±0.04	343.7±25.8	12.40±2.71
HACC	450	0.425±0.02	312.1±25.2	16.73±2.73

Animals of all groups except normal group received cisplatin (7 mg/kg , i.p.). HACC; *Curcuma caesia Roxb* hydroalcoholic extract.

Table:6 Effect of different doses of *Curcuma caesia Roxb* hydroalcoholic extract on pathological scores

Group	Pathologic grading					
	0	1	2	3	4	n
Normal	7					7
Control (only CP) #		1	4	2	1	8
CP + HACC (250 mg/kg)	2		6			8
CP + HACC (350 mg/kg)	4	1	3			8
CP + HACC (450 mg/kg)	2	1	3	1		7

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, ≤ 25% cortex affected

2 = mild, > 25% and ≤ 50% cortex affected

3 = moderate, > 50% and ≤ 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.

References

1. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. N Engl J Med 1996;334:1448–60.
2. Arany I, Safirstein RL. Cisplatin nephrotoxicity. SeminNephrol2003;23:460–4.
3. Verma D, Srivastava S, Singh V, Rawat AK. Pharmacognostic evaluation of Curcuma caesia Roxb. rhizome. Natural Product Sciences. 2010;16(2):107-10.
4. Behar N, Tiwari KL, Jadhav SK. A review on non-conventional turmeric: Curcuma caesia Roxb. Current Trends in Biotechnology and Pharmacy. 2014;8(1):91-101.
5. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015 Jul 6;4(196):2167-0412.
6. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020 Mar;8(2):603-8.
7. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols. 2007 Apr;2(4):875-7.
8. Marjanovic A, Djedjibegovic J, Lugusic A, Sober M, Saso L. Multivariate analysis of polyphenolic content and in vitro antioxidant capacity of wild and cultivated berries from Bosnia and Herzegovina. Scientific reports. 2021 Sep 28;11(1):19259.
9. dos Santos NA, Carvalho Rodrigues MA, Martins NM, Dos Santos AC. Cisplatin-induced nephrotoxicity and targets of nephroprotection: an update. Archives of toxicology. 2012 Aug;86:1233-50.
10. Eraslan G, Kanbur M, Silici S. Evaluation of propolis effects on some biochemical parameters in rats treated with sodium fluoride. Pesticide Biochemistry and Physiology. 2007 Jul 1;88(3):273-83.
11. Padmini MP, Kumar JV. A histopathological study on gentamycin induced nephrotoxicity in experimental albino rats. IOSR J Dent Med Sci. 2012 Jul;1(1):14-7.