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In vitro antioxidant and nephroprotective properties of *Polyalthia longifolia*: A traditional medicinal plant

Research Article

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Abstract

Polyalthia longifolia (Sonn.) Thwaites is a traditional medicinal plant with diverse medicinal properties including kidney protection. The present investigation was aimed to assess the phytochemical content, antioxidant activity and evaluate nephroprotective activity of *Polyalthia longifolia* (PL) 70% ethanolic leaf extract against cisplatin and gentamicin-induced toxicity on HEK293 cell line. Phytochemical analysis confirmed the presence of flavonoids, reducing sugars, tannins, coumarin, saponins, alkaloids, and glycosides, as well as the abundant total phenolic and total flavonoid content in the PL leaf extract. The extract exhibited significant free radical scavenging activity ($p<0.001$) in ABTS (IC_{50} 224 µg/mL), DPPH (IC_{50} 150.1 µg/mL) and H_2O_2 (IC_{50} 271.1 µg/mL) assays. *In vitro* MTT assay validated remarkable cytoprotective activity ($p<0.001$) of PL leaf extract at three concentrations (125 µg/mL, 250 µg/mL, 500 µg/mL) against cisplatin and gentamicin treated HEK293 cell line in dose dependent manner with 70% cell viability (IC_{50} 312.6 µg/mL) and 72% cell viability (IC_{50} 307.3 µg/mL), respectively, at PL dose 500 µg/mL. The cytotoxicity assay also confirmed no adverse effect of PL leaf extract on cell viability in the HEK293 and considered as nontoxic when compared with normal control group. The present study revealed that *Polyalthia longifolia* leaf extract has remarkable antioxidant activity and nephroprotective properties against cisplatin and gentamicin induced nephrotoxicity in *in vitro* model which might be due the presence of free radical scavenging phytochemicals.

Key Words: *Polyalthia longifolia,* Antioxidant, Nephroprotective, Cisplatin, Gentamicin, Cytotoxicity, HEK293.

Introduction

Today, kidney disease is one of the most common health concerns in society. Chronic kidney disease affects approximately 843 million people worldwide (1). Nowadays, drug-induced nephrotoxicity (DIN) is a common problem in people due to prolonged exposure to synthetic drugs, alcohol, unhealthy foods, and smoking. Around 20% of nephrotoxicity occurs worldwide as a result of such causative factors. Acute kidney injury (AKI), nephrolithiasis, and glomerular and tubular damage are all common consequences of DIN (3). Cisplatin and gentamicin, among other synthetic drugs, have been reported to cause side effects on the kidneys. For a long time, traditional or alternative systems of Indian medicine have relied heavily on herbal medicine and plant products. *Polyalthia longifolia* (Sonn.) Thwaites is well known medicinal plant with many medicinal properties. However, there is a dire need to validate the traditional claim of medicinal plants by experimental study. In this study, cisplatin and gentamicin were used as nephrotoxic models to evaluate the protective effect of *Polyalthia longifolia* leaves. Cisplatin is a known antineoplastic chemotherapy agent and as a result of the side effects, its induces nephrotoxicity. It cause mitochondrial and nuclear DNA damage, oxidative stress, inflammatory responses and activation of tumour necrosis factor (TNF), resulting in cell death (4-7). Gentamicin is an aminoglycoside antibiotic which causes renal damage by free radical generation, inflammation, tubular necrosis, leukocyte infiltration and glomerular filtration rate (GFR) reduction, on prolonged administration (8-11). It triggers a demand for alternative therapies that are beneficial, cost-effective and non-toxic to human health. Medicinal

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plants are potential candidates, known to cure various human ailments traditionally in Ayurveda and can be scientifically validated into standardized herbal drugs (12, 13).

Polyalthia longifolia (PL), also known as false Ashoka, is a member of the family Annonaceae. The word "Polyalthia" derives from Greek roots, with "poly" meaning "many" and "althia" meaning "cure," implying that this plant has been used to treat a wide range of diseases (14). Herbal preparations of *P. longifolia* have traditionally been used to treat ulcers, fever, diabetes, cardiovascular diseases, urinary tract disorders and skin diseases (14, 15). This plant is also known and validated for its antimicrobial, anti-inflammatory, anti-ulcer, anti-cancer, hypoglycemic and antipyretic activities (16-19). Even with all of the attention this plant has garnered, there have been insufficient reports on scientific research to determine its nephroprotective potential and medicinal properties. As a result, the purpose of this research is to assess the phytochemical constituents, antioxidant activity and *in vitro* nephroprotective effect of *Polyalthia longifolia* (Sonn.) Thwaites against cisplatin and gentamicin-induced toxicity in the HEK293 cell line.

Materials and methods

Plant material collection

Healthy *Polyalthia longifolia* (Sonn.) Thwaites specimen was collected in the month of September, from a population growing at the Herbal Garden of the Indira Gandhi National Open University, Maidan Garhi, New Delhi, India, at latitudes and longitudes (28.5026" and 77.1986", respectively). It was identified and authenticated by Prof. Prem Lal Uniyal, Department of Botany, University of Delhi and a voucher specimen (accession number DUH14499) was submitted to the herbarium unit. The leaves were washed using distilled water and dried at room temperature (25-30°C) for 15 days. The dried PL leaves were ground into fine powder and subjected to defatting and 70% ethanolic extract preparation. All chemicals used in this experimental study were of analytical grade.

Defatting and Preparation of 70% ethanolic extract

Polyalthia longifolia (PL) leaf powder was defatted using petroleum ether at 1:3 (w/v) and 70% ethanolic extract was prepared from the defatted residue using absolute ethanol at 1:5 (w/v) (20). The extract obtained was preserved in a vial and kept at 4 °C. The formula used for the calculation of the percentage yield of extract was: (Formula 1)

Percent Yield (%) = (W_1/W_2) x 100 where W_1 is the weight of the extract obtained and W_2 is the weight of the leaves taken.

Phytochemical screening

Qualitative phytochemical screening was performed to detect the presence of phytoconstituents (i.e. flavonoids, reducing sugar, tannins, coumarin, saponins, alkaloids, glycosides, anthocyanin and steroids) using standard procedures as shown in Table 1 (21).

Determination of total phenol and total flavonoid content

The Folin-Ciocalteu method was used to determine the total phenolic content of PL 70% ethanolic leaf extract (22). The total flavonoid content of PL extract was estimated using the aluminium chloride colorimetric method on standardization of the Woisky and Salatino procedure (23). The test was performed in triplicate for statistical analysis.

In vitro antioxidant activity

To evaluate the antioxidant activity of PL 70% ethanolic leaf extract, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazine (DPPH) and Hydrogen peroxide (H_2O_2) radical assays were performed at five different concentrations (100, 200, 300, 400 and 500 µg/mL respectively) (24-26). Ascorbic acid was used as the standard (10-50 µg/mL). IC_{50} values (Inhibitory concentrations of a sample to scavenge 50% of free radicals) were calculated using linear regression analysis. All chemicals were purchased from Himedia India.

Percent antioxidant activity was calculated using this formula: (Formula 2)

Percent inhibition (%) = (Absorbance of control – Absorbance of Sample)/ (Absorbance of control x 100)

Nephroprotective activity of PL extract

The nephroprotective activity of PL 70% ethanolic leaf extract was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay. Human Embryonic Kidney cell line (HEK293) was procured from the National Centre for Cell Sciences (NCCS), Pune, India and cultured *in vitro* (27). Silymarin, Cisplatin and Gentamicin were sourced from Sigma Aldrich (USA).

MTT assay was performed to assess the cytotoxic effect of PL 70% ethanolic leaf extract at three concentrations (125 µg/mL, 250 µg/mL and 500 µg/mL) on HEK293 cell lines and compared with the positive control Silymarin. The percent (%) cell viability was calculated using the following formula: (Formula 3)

Cell viability (%) = (Mean O.D. of Treated group/ Mean O.D. of Control group) x 100

Effect of PL extract on Cisplatin-induced toxicity

HEK293 cells (1×10^5 cells/well) were seeded in a 96 well culture plate with eight groups (1–8) in triplicate, and incubated for 24 hours in CO_2 incubator. Group 1 (Normal control) was given DMEM medium; Groups 2-8 received Cisplatin at a concentration of 25 µl/well; Groups 3-5 were treated with PL extract (125 µg/mL, 250 µg/mL, and 500 µg/mL) and Groups 6-8 were given Silymarin (125 µg/mL, 250 µg/mL, and 500 µg/mL) respectively, as a conjoint treatment (28). After 24 hours of incubation, 10 µl MTT dye was added into each well and incubated in dark for 4 hours, After incubation, media was removed from each well and

then 100 µl of DMSO was added to all wells including control to dissolve the colored formazan crystals and kept on a orbital shaker for 10 minutes and the absorbance of each well plate was measured with Elisa reader at 570 nm.

Effect of PL extract on Gentamicin-induced toxicity

Similar to the cisplatin treatment, HEK293 cell lines were seeded and treated in the 96 Elisa well plate in eight groups (1–8) for *in vitro* study of Gentamicin. After 24 hours of incubation, all groups were treated in the same pattern except Groups 2-8, which received Gentamicin at a concentration of 30 µl/well. The concentrations of PL extract and Silymarin used as a conjoint treatment were same as in the previous experiment (29). After 24 hours of incubation, the same procedure was followed as of the cisplatin *in vitro* study. The colour change in cell suspension (from yellow to purple) was recorded by Elisa reader at 570 nm wavelength.

Statistical analysis

All experiments were performed in triplicate (n = 3) and calculated results were presented as mean ± SD. InStat-Graph Pad software, (an open source statistical software) was used for statistical analysis and data presentation. Differences and correlations among the groups were inferred using ANOVA. Probability (p) value of < 0.001 was considered statistically significant.

Results

Table 1: Phytochemicals present in *Polyalthia longifolia* leaf extract

Tests performed	Expected result	Results
Test for Flavonoid PL extract + 1N NAOH + dil. HCl	Decoloration of yellow color on adding acid	Present
Test for Reducing sugar Fehling solutions A and B + PL extract + Heat (60°C)	Formation of Brick red precipitation	Present
Test for Tannins PL extract + 5% FeCl3	Green black coloration	Present
Test for Coumarins PL extract + 10% NAOH	Yellow color/ orange coloration	Present
Test for Anthocyanins PL extract + dil. H_2SO_4 + Ammonia	Pink red coloration	Absent
Test for Saponins PL extract was agitated in a graduated cylinder for 15 minutes.	Formation of 1cm layer of foam.	Present
Test for Steroids PL extract + Chloroform + Conc. H_2SO_4	Red upper layer with green fluorescent acid layer	Absent
Test for Alkaloids PL extract + dil. HCl + Wagner's reagent.	Reddish brown colored precipitation	Present
Test for Glycosides PL extract + alcoholic α–naphthol + Conc. H_2SO_4	Violet ring formation	Present

The yield of PL 70% ethanolic leaf extract corresponding to the initial dry plant material was 14.62% as calculated using Formula 1. On qualitative phytochemical analysis of the PL leaf extract, the phytochemicals found present were flavonoids, reducing sugar, tannins, coumarin, saponins, alkaloids, and glycosides (Table 1). Anthocyanin and steroids were found to be absent in the PL extract.

The total phenolic content in the leaf extract was found to be 3263.5 ± 0.55 mg of gallic acid equivalent per gram of PL extract and the total flavonoid content was estimated to be 1201.4 ± 0.71 mg quercetin equivalent per gram of PL extract (Table 2).

Table 2: Total phenolic and Total flavonoid content of the *Polyalthia longifolia* leaf extract

Plant Extract	Phenolic content (Mg Gallic acid equivalents/ g of plant material)	Flavonoid content (mg Quercetin equivalents/ g of plant material)
PL extract	3263.5 ± 0.55	1201.4 ± 0.71

The radical scavenging activity of PL leaf extract was shown in a concentration-dependent manner ($p<0.001$) in Table 3. ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) is a standard antioxidant activity assay used to determine the free radical scavenging activity of plant extract. At the highest concentration of 500 µg/mL, ABTS percent inhibition was found to be 78%. The IC_{50} value of ABTS inhibition was found to be 224 ± 1.49 µg/mL. DPPH (2,2-diphenyl-1-picrylhydrazyl), which is a free radical blue-colored dye that is used to determine the free radical scavenging activity of plant extracts, is another common antioxidant activity evaluation assay. PL extract showed significant DPPH free radical inhibition in a dose-dependent manner in comparison with ascorbic acid (10-50 µg/mL) used as a standard, with a maximum DPPH inhibition of 89% at 500 µg/mL concentration. The IC_{50} value was found to be 150.2 ± 0.86 µg/mL**.** The H_2O_2 scavenging activity of PL extract was also found in a concentration dependent manner, with the maximum inhibition by the PL extract found to be 86% at 500 µg/mL and IC_{50} of 271.1 ± 0.95 µg/mL**.**

Table 3: Antioxidant activity of *Polyalthia longifolia* 70% ethanolic leaf extract

Treatment	% ABTS inhibition	% DPPH inhibition	% H_2O_2 inhibition
PL extract (µg/mL)			
100	34.67 ± 0.35	44.44 ± 1.06	19.31 ± 1.63
200	53.03 ± 1.78	55.32 ± 1.01	39.08 ± 0.46
300	56.11 ± 1.13	66.94 ± 1.90	56.20 ± 1.68
400	67.67 ± 1.38	78.29 ± 1.35	74.24 ± 0.53
500	77.75 ± 1.62	88.99 ± 0.02	85.88 ± 0.47
IC_{50} value (µg/mL)	224 ± 1.49	150.18 ± 0.86	271.1 ± 0.95
Ascorbic acid (µg/mL) (Used as standard)			
10	52.43 ± 0.22	66.23 ± 0.74	35.72 ± 0.42
20	78.84 ± 0.58	69.68 ± 0.08	51.83 ± 0.21
30	82.96 ± 0.60	72.13 ± 0.30	57.07 ± 0.35
40	91.60 ± 0.29	74.46 ± 0.94	68.40 ± 0.16
50	98.01 ± 1.98	79.80 ± 0.61	83.36 ± 0.55

Values are expressed as Mean ± Standard Deviation. IC_{50} values are the results of linear regression of % scavenging activities with PL extract at 50% inhibition.

In vitro studies are extensively used as the primary level of screening before moving further to the *in vivo* methods of analysis to evaluate the potential therapeutic activities, like nephroprotection, of various medicinal plants. PL extract was found to have no toxic effect on the cell population for all three concentrations, and the results were similar to those of the normal cell group and the silymarin treated cell group with the MTT assay (Figure 1). According to the results, cell viability was found to be 99% at the highest concentration, i.e., 500 µg/mL of PL extract.

The protective effect of PL extract was analyzed against cisplatin-induced *in vitro* toxicity at three different concentrations (125, 250 and 500 µg/mL) on the HEK293 cell line (Figure 2). In comparison with the normal control group (Group 1), HEK293 cell lines exposed to cisplatin toxicity show a drop of 80% in cell viability (Group 2). Cell line groups treated with PL extract showed a significant increase in cell viability in a dose-dependent manner against cisplatin-induced toxicity (Groups 3-5). Cell viability was recorded to be 70% at the highest concentration of PL extract (Group 5). These results were compared to the cell viability effect of Silymarin, used as positive control, which showed 88% cell viability at 500 µg/mL. The IC_{50} value of PL extract against cisplatin was found to be 312.6 ± 0.45 µg/mL with ($p<0.001$) significance.

Figure 1: Cytotoxic effect of PL 70% ethanolic leaf extract on HEK293 cell line

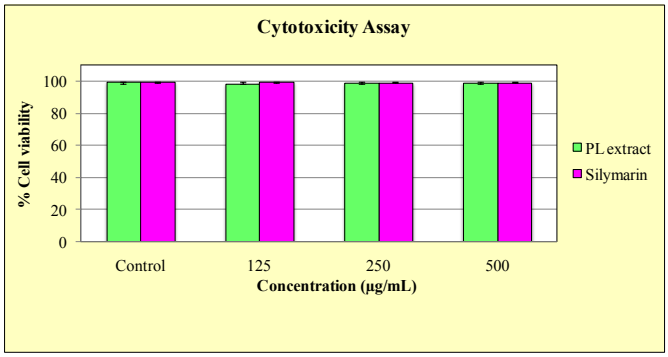
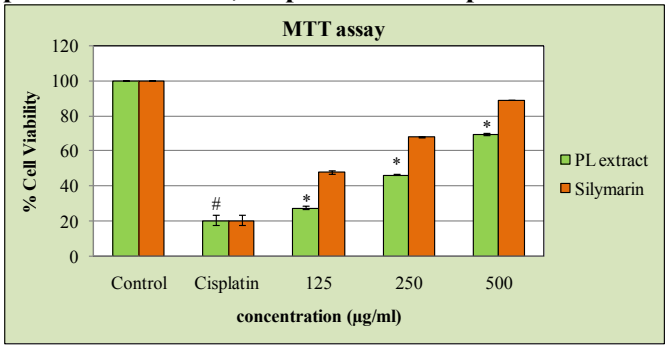
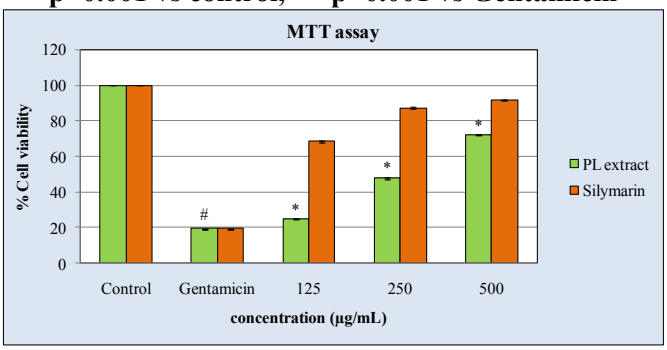


Figure 2: Protective effect of PL 70% ethanolic leaf extract on Cisplatin treated HEK293 cell line; # = p<0.001 vs control, *= p<0.001 vs Cisplatin



The cytoprotective effect of PL extract against gentamicin-treated HEK293 cells is shown in (Figure 3). Gentamicin toxicity in the HEK293 cell lines decreased the cell viability rate to a low of 19%, which was subsequently improved by the PL extract administration at three doses (125-500 µg/mL). The maximum percent cytoprotective effect was found to be 72% at 500 µg/mL of PL extract ($p<0.001$) against gentamicin induced cell damage. The results were compared with the positive control silymarin, which showed 90% cell viability at 500 µg/mL. The IC_{50} value of PL extract against gentamicin was found to be 307.3 ± 0.92 µg/mL.

Figure 3: Protective effect of PL 70% ethanolic leaf extract on Gentamicin treated HEK293 cell; # = p<0.001 vs control, *= p<0.001 vs Gentamicin



Discussion

Polyalthia longifolia is a popular medicinal plant with elaborate mention and therapeutic usage in Indian Ayurveda (13). It has a variety of medicinal properties, including antimicrobial, anticancer, hepatoprotective, and hypoglycemic properties, according to previous research (14). The nephroprotective activity of this plant is also mentioned in traditional medicinal literature; however, there is very little knowledge and scientific evidence to validate the same. In this study of PL 70% ethanolic leaf extract, qualitative phytochemical screening, total phenolic and flavonoid content, along with antioxidant activity, were analyzed. *In vitro* nephroprotective activity was evaluated using HEK293 cell line culture by MTT assay against cisplatin and gentamicin toxicity so to predict the suitability of *Polyalthia longifolia* as nephroprotective agent to investigate further in *in vivo* models (30). *In vitro* studies are extensively used as the primary level of screening before moving toward the *in vivo* methods of analysis to evaluate the potential therapeutic activities, like nephroprotection, of various medicinal plants. Cisplatin is a popular antineoplastic drug used as chemotherapy for treating different types of cancer but is also criticized for its nephrotoxic side effects; similarly, Gentamicin is an aminoglycoside prescribed to treat broad spectrum bacterial infections with a limitation of serious kidney damage as a side effect of its administration. Previous experimental studies have demonstrated a prominent relationship between oxidative stress and nephrotoxicity in different models (31). Cisplatin and gentamicin are known to cause nephrotoxicity as a consequence of oxidative stress.

Cisplatin damages the kidney tissues by producing free radicals, causing altered arginine metabolism and increased calcium independent nitric oxide synthase activity (32, 33). Gentamicin triggers oxidative stress coupled with phospholipases activation and disrupts the lysosomal membrane (32-34). *Polyalthia longifolia* extract demonstrated remarkable antioxidant activity in three different assays (DPPH, ABTS, H_2O_2) with total phenolic and flavonoid content revealed the presence of phytochemicals such as alkaloids, phenols, and flavonoids, with other important phytoconstituents. The phytochemicals as antioxidants might have showed positive modulations in enzyme system activities and reduce cellular damage caused by toxicant-induced oxidative stress (35, 36).

In vitro cell viability assessment is done using the colorimetric MTT assay. The working principle of this assay is based on the conversion of yellow tetrazolium salt into blue formazan crystals by the mitochondrial succinate dehydrogenase enzyme (37). The MTT results showed that the rate of cell viability was reduced (19-20%) in the Cisplatin and Gentamicin treated groups, which increased considerably on treating with PL extract at three concentrations (125 µg/mL, 250 µg/mL, 500 µg/mL). The antioxidant activity of PL extract might possibly be co-related to the improvement of cell viability rate in cell line groups against drug induced toxicity (38, 39). PL extract showed no toxicity or negligible cell population decline in HEK293 cell lines indicating no adverse effect on cultured cell lines.

At 500 µg/mL, the PL extract demonstrated maximum cellular protection with 70% and 72% cell viability against cisplatin and gentamicin-induced toxicity in the HEK293 cell line, respectively. This protective efficacy of PL can be linked with the remarkable free radical scavenging property of PL extract that prevents cellular damage associated with the cisplatin and gentamicin in the *in vitro* model. The antioxidant activity of PL extracts was determined to be (78%–89%) percent inhibition of DPPH, ABTS, and H_2O_2 assays in a dose-dependent manner.

The antioxidant and nephroprotective activities of PL extracts might be due to the presence of flavonoids and phenolic compounds that are widely studied and known for having free radical scavenging properties. *Polyalthia longifolia* has also been reported in the past for its antioxidant activity and quantitative phytochemical evaluation in various studies to back our findings (14, 16, 17, 40). The presence of phenols and flavonoids may also have contributed to the scavenging of DPPH, ABTS and H_2O_2 radicals.

Conclusion

The results of the present study reveal that PL extract has promising antioxidant activity and protective effect against Cisplatin and Gentamicin induced toxicity in the HEK293 cell line *in vitro* model. The presence of various phytochemicals in significant concentrations, along with free radical scavenging properties, possibly accounts for its antioxidant and nephroprotective activity. Further *in vivo* studies are required to validate the in vitro nephroprotective effects of *Polyalthia longifolia* (Sonn.) Thwaites.

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Abbreviations

- **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
- **AKI:** Acute kidney injury; **DIN:** Drug-induced nephrotoxicity
- **DMEM:** Dulbecco's modified eagle medium
- **DPPH:** 2,2-diphenyl-1-picrylhydrazyl
- **ELISA:** Enzyme-linked immunosorbent assay
- **FBS:** Fetal bovine serum
- **GFR:** Glomerular filtration rate
- **H_2O_2:** Hydrogen peroxide
- **HEK293:** Human Embryonic Kidney 293 cell line
- **MTT:** 3- (4,5 dimethyl thiazol-2yl) -2,5 diphenyl tetrazolium bromide
- **NCCS:** National Center for Cell Sciences
- **O.D.:** Optical density
- **PL:** *Polyalthia longifolia*
- **TNF:** Tumour necrosis factor

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. Kidney Int Suppl (2011). 2022; 12(1); 7-11. https://doi.org/10.1016/j.kisu.2021.11.003
2. Al-Naimi M.S, Rasheed H.A, Hussien N.R, Al-Kuraishy H.M, Al-Gareeb A.I. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharm Technol Res. 2019; 10(3); 95-99. https://doi.org/10.4103/japtr.JAPTR_336_18
3. Sales G.T.M and Foresto R.D. Drug-induced nephrotoxicity. Rev Assoc Med Bras (1992). 2020; 66Suppl 1(Suppl 1); 82-90. https://doi.org/10.1590/1806-9282.66.S1.82
4. Barnett L.M.A and Cummings B.S. Cellular and Molecular Mechanisms of Kidney Toxicity. Semin Nephrol. 2019; 39(2); 141-151. https://doi.org/10.1016/j.semnephrol.2018.12.004
5. Tang C, Livingston M.J, Safirstein R, Dong Z. Cisplatin nephrotoxicity: new insights and therapeutic implications. Nat Rev Nephrol. 2022; 2022. https://doi.org/10.1038/s41581-022-00631-7
6. Dupre T.V, Sharp C.N, Siskind L.J. Renal Toxicology/Nephrotoxicity of Cisplatin and Other Chemotherapeutic Agents Comprehensive

Toxicology. 3ed. Elsevier; Oxford; 2018; 452-486. https://doi.org/10.1016/B978-0-12-801238-3.64183-1

7. Latcha S, Jaimes E.A, Patil S, Glezerman I.G, Mehta S, Flombaum C.D. Long-Term Renal Outcomes after Cisplatin Treatment. Clin J Am Soc Nephrol. 2016; 11(7); 1173-1179. https://doi.org/10.2215/CJN.08070715
8. Boroushaki M.T, Fanoudi S, Mollazadeh H, Boroumand-Noughabi S, Hosseini A. Reno-protective effect of *Rheum turkestanicum* against gentamicin-induced nephrotoxicity. Iran J Basic Med Sci. 2019; 22; 328-333. https://doi.org/10.22038/ijbms.2019.31552.7597
9. Morales-Alvarez M.C. Nephrotoxicity of Antimicrobials and Antibiotics. Adv Chronic Kidney Dis. 2020; 27(1); 31-37. https://doi.org/10.1053/j.ackd.2019.08.001
10. Udupa V, Prakash V. Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. Toxicol Rep. 2018; 6; 91-99. https://doi.org/10.1016/j.toxrep.2018.11.015
11. Mahi-Birjand M, Yaghoubi S, Abdollahpour-Alitappeh M. Protective effects of pharmacological agents against aminoglycoside-induced nephrotoxicity: A systematic review. Expert Opin Drug Saf. 2020; 19(2); 167-186. https://doi.org/10.1080/14740338.2020.1712357
12. Shakya A.K. Medicinal plants: Future source of new drugs. Int J Herb Med. 2016; 4(4); 59-64. http://dx.doi.org/10.13140/RG.2.1.1395.6085
13. Katkar K.V, Suthar A.C, Chauhan V.S. The chemistry, pharmacologic, and therapeutic applications of Polyalthia longifolia. Pharmacogn Rev. 2010; 4(7); 62-68. doi:10.4103/0973-7847.65329
14. Firdous S.M, Ahmed S.N, Hossain S.M. *Polyalthia longifolia*: phytochemistry, ethnomedicinal importance, nutritive value, and pharmacological activities review. Med Chem Res. 2022; 31; 1252–1264. http://dx.doi.org/10.1007/s00044-022-02917-8
15. Chatrou L, Erkens R.J, Richardson J.E, Saunders R.K, Fay M. The natural history of Annonaceae. Bot J Linn Soc. 2012; 169; 1–4. https://doi.org/10.1111/j.1095-8339.2012.01242.x
16. Chen Y.C, Chia Y.C, Huang B.M. Phytochemicals from *Polyalthia* Species: Potential and Implication on Anti-Oxidant, Anti-Inflammatory, Anti-Cancer, and Chemoprevention Activities. Molecules. 2021; 26(17); 5369. https://doi.org/10.3390/molecules26175369
17. Mudhafar M. Review of Photochemistry for *Polyalithia longiflia*. Discov Phytomed. 2019; 6(2); 33-55. http://dx.doi.org/10.15562/phytomedicine.2019.82
18. Yao L.J, Jalil J, Attiq A, Hui C.C, Zakaria N.A. The medicinal uses, toxicities and anti-inflammatory activity of Polyalthia species (Annonaceae). J Ethnopharmacol. 2019; 229; 303–325. https://doi.org/10.1016/j.jep.2018.10.001
19. Rai A.K, Singh S.P, Pandey A.R. Flavonoids from *Polyalthia longifolia* prevents advanced glycation end products formation and protein oxidation aligned with fructose-induced protein glycation. Nat Prod Res. 2021; 35(17); 2921-2925. https://doi.org/10.1080/14786419.2019.1672690
20. Kath R.K and Gupta R.K. Antioxidant activity of hydroalcoholic leaf extract of ocimum sanctum in animal models of peptic ulcer. Indian J Physiol Pharmacol. 2006; 50(4); 391-396.
21. Harborne J.B. 'Phytochemical methods: A guide to modern technique of plant analysis', Champman and Hall, London, 1998.
22. Lowry O.H, Rosebrough N.J, Farr A.I, Randall R.J. Protein determination with the folin's phenol reagent. J Biol Chem. 1951; 193; 265-275.
23. Gonsales G.Z, Orsi R.O, Fernandes Júnior A, Rodrigues P, Funari S.R. Antibacterial activity of propolis collected in different regions of Brazil. J Venom Anim Toxins Incl Trop Dis. 2006; 12; (2). https://doi.org/10.1590/S1678-91992006000200009
24. Re R, Proteggente P.N, Pannala A, Ananth R.E, Catherine. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999; 26; 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3
25. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of *Xanthan* on the autoxidation of soybean oil in cyclodextrin emulsion. J AgricFood Chem. 1992; 40; 945–948. https://doi.org/10.1021/jf00018a005
26. Ruch R, Cheng S.J, Klaunig J.E. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10; 1003-1008. https://doi.org/10.1093/carcin/10.6.1003
27. Khan M.U, Gaurav, Zahiruddin S, Basist P, Krishnan A, Parveen R, et al. Nephroprotective potential of Sharbat-e-Bazoori Motadil (sugar-free) in HEK-293 cells and wistar rats against cisplatin induced nephrotoxicity. J King Saud Univ Sci. 2022; 34(3); 101839. https://doi.org/10.1016/j.jksus.2022.101839
28. Halabian R, Roudkenar M.H, Jahanian-Najafabadi A, Hosseini K.M, Tehrani H.A. Co-culture of bone marrow-derived mesenchymal stem cells overexpressing lipocalin 2 with HK-2 and HEK293 cells protects the kidney cells against cisplatin-induced injury. Cell Biol Int. 2015; 39(2); 152-163. https://doi.org/10.1002/cbin.10344
29. Sun H, Yang H, Ruan H. The protective effect of sika deer antler protein on gentamicin-induced nephrotoxicity in vitro and in vivo. Cell Physiol Biochem. 2018; 50(3); 841-850. https://doi.org/10.1159/000494471
30. Tiong H.Y, Huang P, Xiong S, Li Y, Vathsala A, Zink D. Drug-induced nephrotoxicity: clinical impact and preclinical in vitro models. Mol Pharm. 2014; 11(7); 1933-1948. https://doi.org/10.1021/mp400720w

31. Negi K, Mirza A. Nephroprotective and Therapeutic Potential of Traditional Medicinal Plants in Renal Diseases. J Drug Res Ayurvedic Sci. 2020; 5(3); 177–185. http://dx.doi.org/10.5005/jdras-10059-0079
32. Prasanthi D, Adikay S. Amelioration of cisplatin and gentamicin –induced nephrotoxicity by seeds of Cucumis sativus. Int J Pharma Bio. 2016; 7(4); 245-53. https://doi.org/10.4103/0973-1482.43139
33. Annie S, Rajagopal P.L, Malini S. Effect of Cassia auriculata Linn. root extract on cisplatin and gentamicin-induced renal injury. Phytomedicine. 2005; 12(8); 555-560. https://doi.org/10.1016/j.phymed.2003.11.010
34. Zaman R, Alam A, Jafri M.A, Sofi G, Ahmad G. Nephroprotective effect of Beekh Kasni (Roots of Cichorium intybus) in the form of methanolic and aqueous extract in Gentamycin induced rat models. J pharmacogn phytochem. 2017; 6; 337–341.
35. Bjørklund G and Chirumbolo S. Role of oxidative stress and antioxidants in daily nutrition and human health. Nutrition. 2017; 33; 311-321. https://doi.org/10.1016/j.nut.2016.07.018
36. Athira K, Madhana R.M, Lahkar M, Flavonoids, the emerging dietary supplement against cisplatin-induced nephrotoxicity. Chem Biol Interact. 2016; 248; 18-20. https://doi.org/10.1016/j.cbi.2016.02.005
37. Berridge M.V, Tan A.S, McCoy K.D, Wang R. The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. Roche Appl Sci. 1996; 14–19.
38. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol. 2004; 142; 231–255. https://doi.org/10.1038/sj.bjp.0705776
39. Vargas F, et al. Flavonoids in Kidney Health and Disease. Front Physiol. 2018; 9; 394. https://doi.org/10.3389/fphys.2018.00394
40. Yu M, Gouvinhas I, Rocha J, Barros A.I.R.N.A. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Sci Rep. 2021; 11(1); 10041. https://doi.org/10.1038/s41598-021-89437-4.

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