www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(3): 356-359
© 2020 TPI
www.thepharmajournal.com
Received: 25-01-2020
Accepted: 27-02-2020

T Rajendrakumar
Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India

Suguna Rao
Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India

ML Satyanarayana
Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India

HD Narayanaswam
Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India

SM Byregowda
Institute of Animal Health and Veterinary Biological KVAFSU, Bangalore, Karnataka, India

KM Purushotham
Institute of Animal Health and Veterinary Biological KVAFSU, Bangalore, Karnataka, India

Corresponding Author:
T Rajendrakumar
Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India

Ameliorative effect of *Andrographis paniculata* against oxidative damage caused by cisplatin in rat kidney

T Rajendrakumar, Suguna Rao, ML Satyanarayana, HD Narayanaswam, SM Byregowda and KM Purushotham

Abstract
The role of ethanolic extract of *Andrographis paniculata* [AP], in preventing cisplatin [CP] induced oxidative damage in rat kidney was studied. Sixty Wistar Albino rats were divided into five groups with twelve rats in each group. Group I served as normal control group. To group II rats, CP was administered at 7.5mg/kg body weight intraperitoneally for single dose. Rats in group III served as AP control and were administered AP at the dose of 500mg/kg body weight for 45 days. Group IV rats were pre-treated with AP fifteen days prior to CP administration and followed by AP treatment for 45 days. Rats in group V were administered with CP and concurrently treated with AP extract at 500mg/kg by oral gavaging for 45 days. The kidney samples collected at 7th, 14th, 28th, and 45th day of the study were subjected for estimation of catalase, superoxide dismutase, glutathione peroxidase and malondialdehyde [MDA]. Significant decrease [$P<0.05$] in the levels of antioxidant enzymes and increase in MDA levels were recorded in CP treatment group. The AP treatment groups revealed a significant recovery [$P<0.05$] all enzymes and decrease in MDA levels which suggested that AP has a good antioxidant effect and could be effectively used for prevention of toxic side effects of CP. The study also indicated that pre-treatment of AP prior to CP administration has good prophylactic effect.

Keywords: *Andrographis paniculata*, Cisplatin, Oxidative damage, Malondialdehyde

1. Introduction
Cisplatin is the most effective and widely used antineoplastic drug employed for treatment of solid tumors. Cisplatin based chemotherapy regimens are presently used in the treatment of testicular cancer, ovarian germ cell tumors, epithelial ovarian cancer, head and neck cancer, advanced cervical cancer, bladder cancer, mesothelioma, endometrial cancer, non-small cell lung cancer, malignant melanoma, carcinoids, penile cancer, adrenocortical carcinoma etc. [1]. Cisplatin interferes with the growth of cells by the formation of intra- and inter-strand covalent cross-linked adduct and interferes with replication and transcription process of malignant cells. Thus, serves as a potent inducer of cell cycle arrest and apoptosis in most cancer cell types [2]. However, several adverse effects of cisplatin have been reported, mainly nephrotoxicity, hepatotoxicity, ototoxicity, neurotoxicity, cardiotoxicity and myelosuppression that limits its clinical use [3]. Along with interaction with DNA, cisplatin also stimulates oxidative stress is one of the important mechanisms involved in cisplatin-induced toxicity resulting in the enhanced production of reactive oxygen species, reduction in the mitochondrial membrane potential and decrease in antioxidant enzymes [4]. The amelioration of cisplatin induced toxicity has been prime concern during therapeutic intervention with cisplatin. Many preclinical trials have been done to evaluate some antioxidants protect against the side effects related with cisplatin [5]. Numerous medicinal plants and their formulations have been claimed to possess protective effects in ethano-medical and traditional system medicine in India.
Andrographis paniculata is one such important medicinal plant widely used around the world. It belongs to the family *Acanthaceae* and used as a traditional herbal medicine in Bangladesh, China, Hong Kong, India, Pakistan, Philippines, Malaysia, Indonesia, and Thailand [6]. It is also known on the Indian subcontinent as Chirayetah and Kalmegh in Urdu and Hindi languages respectively and Nelabeu in Kannada. It is an annual plant, most commonly used in the traditional systems of Unani and Ayurvedic medicines and is considered as “King of Bitters.” It grows in hedge rows throughout the plains of India and is also cultivated in gardens [7]. Phytochemical analyses of *Andrographis paniculata* have revealed that it is a rich source of diterpenoids and 2’-oxygenated flavonoids, including andrographolide.

Andrographolide is the primary bioactive phytochemical of *Andrographis paniculata* and it exhibits significant anti-oxidant, anti-inflammatory activity and has also chemo-protective potential towards normal cells [8]. The aim of the present study was to evaluate the level of oxidative stress in cisplatin mediated nephrotoxicity and to investigate possible protective effect of *Andrographis paniculata* on cisplatin-induced renal damage in rats.

2. Materials and Methods

2.1 Drugs and chemicals

Cisplatin [Kemoplat] was procured from Fresenius Kabi India Pvt. Ltd. Pune, India. and the ethanolic extract of *Andrographis paniculata* (AP) was obtained from Himalaya Herbal Pvt Ltd. Bangalore, India

2.2 Animals

Normal adult Wistar albino rats weighing approximately 180-200 grams were procured from commercial animal facility, Bangalore for the study. They were maintained under standard laboratory conditions and fed with *ad libitum* standard commercial rat feed and clean drinking water. The duration of experiment was for a period of 45 days and a prior permission was obtained from the Institutional Animal Ethics Committee [IAEC] for the conduct of the experiment.

2.3 Experimental design

The rats were maintained under standard laboratory conditions for a period of 15 days for acclimatization in the experimental animal house. The rats were divided, based on the body weight, into five groups with twelve rats in each group.

Group I: Negative control - injected with 0.5ml sterile PBS intraperitoneally on Day 1 and gavaged with PBS daily.

Group II: Positive control- nephrotoxicity induced with administration of cisplatin at 7.5mg/kg body weight intraperitoneally for single dose.

Group III: *Andrographis paniculata* control-animals supplemented with ethanolic extract of *Andrographis paniculata* alone at the dose rate of 500 mg/kg body weight.

Group IV: Supplemented with *Andrographis paniculata* extract at the dose rate of 200mg/kg bodyweight 15 days prior to induction of nephrotoxicity by CP.

Group V*:* Supplemented with *Andrographis paniculata* extract at the dose rate of 500mg/kg bodyweight concurrently with administration of CP.

2.4 Estimation of superoxide dismutase [SOD]

Superoxide dismutase activity was determined by the method described by Marklund and Marklund [9]. The enzyme activity was expressed in terms of units per minute per mg of protein. One unit of SOD was defined as the amount of enzyme required to inhibit pyrogallol auto-oxidation reaction by 50 per cent.

2.5 Estimation of catalase [CAT]

Catalase was estimated by the method described by Calliborne [10]. Enzyme activity was expressed as µmol of H_2O_2 decomposed per minute per mg of protein.

2.6 Estimation of glutathione peroxidase [GPx]

Glutathione peroxidase was determined by the method described by Rotruck *et al* [11]. Enzyme activity was expressed as units per mg protein.

2.7 Estimation of TBARS [Malondialdehyde]

Lipid peroxidation in the kidney tissue was determined by estimation of thiobarbituric acid reactive substance (TBARS) by the method of Yagi [12]. The unit of activity was expressed as n moles of MDA /mg of tissue.

2.8 Statistical analysis

Statistical analysis was performed using the statistical software Graph Pad Prism, version 6.0 for Windows. Mean values and standard error were calculated and all values were expressed as Mean [± SE]. The data were analyzed by two-way analysis of variance [ANOVA].

3. Results

The effect of CP administration on the antioxidant enzyme status and lipid peroxidation in kidney of rats was analyzed. The results indicated that CP caused a significant decrease [$P<0.05$] in the levels of SOD, CAT and GPx [Tables 1,2,3] and significant increase [$P<0.05$] in the level of MDA [Table 4] in Group- II rats when compared to other groups, throughout the duration of the experiment. Administration of ethanolic extract of *Andrographis paniculata* ameliorated the deleterious effects of CP which was reflected by significant improvement [$P<0.05$] in the levels of SOD, GPx and CAT in the animals of Group-IV and Group-V [Tables 1, 2, 3] and also by significant reduction [$P<0.05$] in the levels of MDA [Table 4]. Improvement in the levels of antioxidants [$P<0.05$] and MDA [$P<0.05$] was significant in the AP pretreated group [Group-IV] when compared to concurrent AP treatment group [Group-III]. No significant difference in antioxidant levels and MDA was recorded in the AP control group [Group-III] when compared to the normal control group [Group-I] throughout the duration of the experiment.

4. Discussion

In the present study, there was a significant ($P<0.05$) increase in the MDA level in cisplatin group (Group II) compared to normal control associated with significant ($P<0.05$) decrease in the level of antioxidants CAT, SOD and GPx in the renal tissue throughout the study period. Such observations were also made by several workers [13-16].

Nephrotoxicity is a major side effect caused by cisplatin treatment. Kidney is primarily affected during cisplatin treatment as it serves as the main route of cisplatin excretion. Reports of past studies have suggested that kidney has a tendency for accumulating cisplatin at higher levels compared to any other organ [17, 18].

Disproportionate retention of cisplatin within the tissues of the kidney has been recognised as an important factor that contributes cisplatin nephrotoxicity [19]. The accumulation and concentration of cisplatin within the proximal tubular epithelial cells is approximately five times that of the serum concentration and promotes the cellular damage involving multiple mechanisms including oxidative stress, DNA damage, apoptosis and inflammation [20]. In proximal tubular cells, the cisplatin hydrolyzes into highly reactive form, which can rapidly react with thiol-containing molecules including glutathione. Depletion or inactivation of endogenous glutathione and other related antioxidants by cisplatin is expected to shift the cellular redox status, leading to the accumulation of ROS and oxidative stress within the cells. The cisplatin also implicated in the mitochondrial dysfunction and increase in ROS production *via* the disrupted respiratory chain [21]. Intracellular generation and accumulation of

reactive oxygen species such as superoxide anion, hydrogen peroxide, singlet oxygen, hydroxyl radical and peroxyl radical in the stressed cells overcomes the natural antioxidant defense causing damage to biological macromolecules including nucleic acids, proteins and lipids [22]. Our findings revealed that the administration of the ethanolic extract of *Andrographis paniculata* significantly ($P<0.05$) decreased MDA level and increased antioxidant enzymes CAT, SOD and GPx levels in both the AP treatment groups (Group IV and V) compared to those of cisplatin control (Group II) and indicated that *Andrographis paniculata* could alleviate cisplatin induced oxidant injury in the kidney. Among the *Andrographis paniculata* treatment groups (IV and V), the pre-treatment of *Andrographis paniculata* provided better nephroprotective effect with a progressive improvement in the antioxidant enzyme levels and significant decrease in the MDA level from Day 7 to Day 45. Nephroprotective effect *Andrographis paniculata* has also been reported by many earlier workers against chemical induced kidney injury [23-26]. Nephroprotective effect of AP could be attributed to the antioxidant and free radical-scavenging properties. As indicated earlier, antioxidant effect is attributable to its diterpene phytochemcials such as andrographalides, neoandrographalide and deoxy andrographalide etc. [27]

5. Conclusion
In conclusion, the present study supports the role of oxidative stress in cisplatin induced nephrotoxicity and ethanolic extract *Andrographis paniculata* was able to produce considerable alleviation from nephrotoxic action of cisplatin through suppression of oxidative stress.

Table 1: The mean (±SE) values of superoxide dismutase (SOD) levels (U/min/mg protein) in kidney of rats in different groups at different time intervals

Groups	Days post treatment			
	07th	14th	28th	45th
Group-I: Negative control	36.27±0.06^{ax}	34.5±0.137^{ay}	34.99±0.03^{ay}	36.02±0.20^{ax}
Group-II: CP control	13.64±0.06^{bx}	13.57±0.01^{bx}	14.06±0.004^{by}	15.05±0.14^{bz}
Group-III: AP Control	36.06±0.21^{ax}	36.98±0.03^{ax}	37.54±0.03^{ay}	37.50±0.17^{ay}
Group-IV: AP pre-treatment group	25.10±0.01^{cx}	26.98±0.026^{cy}	27.72±0.04^{cz}	29.63±0.42^{cw}
Group-V: AP concurrent group	15.24±0.018^{dx}	16.89±0.06^{dy}	18.57±0.09^{dz}	21.45±0.61^{dw}

Values with different superscripts in a row and column vary significantly at $P<0.05$

Table 2: The mean (±SE) values of catalase (µmol/min/mg protein) levels in kidney of rats in different groups at different time intervals

Groups	Days post treatment			
	07th	14th	28th	45th
Group-I: Negative control	40.82±0.19^{ax}	43.025±0.03^{ay}	40.44±0.12^{ax}	40.74±0.29^{ax}
Group-II: CP control	17.81±0.07^{bx}	15.92±0.03^{by}	17.015±0.05^{bx}	19.34±0.30^{bz}
Group-III: AP Control	41.72±0.15^{ax}	43.795±0.06^{ay}	43.15±0.01^{ay}	43.63±0.28^{ay}
Group-IV: AP pre-treatment group	22.47±0.08^{cx}	23.49±0.07^{cy}	25.03±0.2^{cz}	26.74±0.32^{cw}
Group-V: AP concurrent group	19.08±0.08^{dx}	19.37±0.01^{dx}	19.855±0.06^{dx}	20.32±0.10^{dy}

Values with different superscripts in a row and column vary significantly at $P<0.05$

Table 3: The mean (±SE) values of glutathione peroxidase (GPx) levels (U/mg protein) in kidney of rats in different groups at different time intervals

Groups	Days post treatment			
	07th	14th	28th	45th
Group-I: Negative control	46.77±0.08^{ax}	48.4±0.13^{ay}	48.52±0.08^{ay}	49.15±0.20^{az}
Group-II: CP control	10.64±0.14^{bx}	11.305±0.20^{by}	13.105±0.01^{bz}	15.20±0.08^{bw}
Group-III: AP Control	47.56±0.16^{ax}	48.72±0.01^{ay}	49.15±0.01^{ay}	47.92±0.18^{az}
Group-IV: AP pre-treatment group	25.09±0.05^{cx}	26.895±0.09^{cy}	27.14±0.01^{cy}	30.005±0.51^{cz}
Group-V: AP concurrent group	15.24±0.03^{dx}	16.66±0.03^{dy}	21.22±0.02^{dz}	23.54±0.19^{dw}

Values with different superscripts in a row and column vary significantly at $P<0.05$

Table 4: The mean (±SE) values of malondialdehyde (MDA) levels (n moles/mg tissue) in kidney of rats in different groups at different time intervals

Groups	Days post treatment			
	07th	14th	28th	45th
Group-I: Negative control	0.781±0.03^{ax}	1.0605±0.01^{ax}	0.9625±0.01^{ax}	1.05±0.05^{ax}
Group-II: CP control	5.83±0.05^{bx}	8.78±0.021^{by}	8.95±0.04^{by}	8.58±0.32^{by}
Group-III: AP Control	0.706±0.03^{ax}	1.03±0.04^{ax}	0.82±0.021^{ax}	0.97±0.04^{ax}
Group-IV: AP pre-treatment group	4.26±0.036^{cx}	3.435±0.019^{cy}	3.165±0.09^{cyz}	2.78±0.11^{cz}
Group-V: AP concurrent group	5.38±0.065^{bex}	7.375±0.092^{dy}	7.02±0.023^{dyz}	6.76±0.42^{dz}

Values with different superscripts in a row and column vary significantly at $P<0.05$

6. Acknowledgment
Authors are thankful to the Head of the Department, Department of Veterinary pathology and Dean, Veterinary College, Bangalore, for providing facilities to carry out the present study.

7. References
1. Katzung BG, Masters SB, Trevor AJ. Basic and Clinical Pharmacology. Edn 12, New York: McGraw-Hill. Medical, London, 2012, 881-890.
2. Cohen SM, Lippard SJ. Cisplatin: From DNA damage to

cancer chemotherapy. Progress in Nucleic Acid Research and Molecular Biology. 2001; 67:93-130.
3. Barabas K, Milner R, Lurie D, Adin C. Cisplatin: a review of toxicities and therapeutic applications. Veterinary Comparative Oncology. 2008; 6(1):1-18.
4. Saad SY, Najjar TA, Alashari M. Role of non-selective adenosine receptor blockade and phosphodiesterase inhibition in cisplatin-induced nephrogonadal toxicity in rats. Clinical and experimental pharmacology and physiology. 2004; 31(12):862-867.
5. Kim HJ, Park DJ, Kim JH, Jeong EY, Jung MH, Kim TH *et al*. Glutamine protects against cisplatin-induced nephrotoxicity by decreasing cisplatin accumulation. Journal of pharmacological sciences. 2015; 127(1):117-126.
6. Akbar S. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Alternative Medicine Review. 2011; 16(1):66-77.
7. Hossain MD, Urbi Z, Sule A, Rahman KM. *Andrographis paniculata* (*Burm*. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. The Scientific World Journal. 2014, 1-28.
8. Bardi DA, Halabi MF, Hassandarvish P, Rouhollahi E, Paydar M, Moghadamtousi SZ *et al*. *Andrographis paniculata* leaf extract prevents thioacetamide-induced liver cirrhosis in rats. PLoS One. 2014; 9(10):1-13.
9. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry. 1974; 47(3):469-474.
10. Calliborne AL, Assay of catalase: Handbook of oxygen radical research. CRC Press. Boco-Raton, 1985, 283-284.
11. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973; 179(4073):588-590.
12. Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochemical Medicine. 1976; 15:212-216.
13. Sherif IO. Amelioration of cisplatin-induced nephrotoxicity in rats by triterpenoid saponin of Terminalia arjuna. Clinical and experimental nephrology. 2015; 19(4):591-597.
14. Bishr A, Sallam N, Nour El-Din M, Awad AS, Kenawy SA. Ambroxol attenuates cisplatin-induced hepatotoxicity and nephrotoxicity via inhibition of p-JNK/p-ERK. Canadian journal of physiology and pharmacology. 2019; 97(1):55-64.
15. Janakiraman M. Protective efficacy of silver nanoparticles synthesized from silymarin on cisplatin induced renal oxidative stress in albino rat. International Journal of Applied Pharmacy. 2018; 10:110-116.
16. Xiao G, Peng L, Liu Y, Xiao X. Bacoside a Attenuates Nephrotoxicity and Acute Kidney Injury in Male Albino Rats Induced by Cisplatin. Pharmacology. 2019; 15(2):257-264.
17. Dulz S, Asselborn NH, Dieckmann KP, Matthies C, Wagner W, Weidmann J *et al*. Retinal toxicity after cisplatin-based chemotherapy in patients with germ cell cancer. Journal of cancer research and clinical oncology. 2017; 143(7):1319-1325.
18. Sarin N, Engel F, Rothweiler F, Cinatl J, Michaelis M, Frötschl R *et al*. Key players of cisplatin resistance: towards a systems pharmacology approach. International journal of molecular sciences. 2018; 19(3):767-772.
19. Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. Drug metabolism reviews. 1999; 31(4):971-97.
20. Mohan IK, Khan M, Shobha JC, Naidu MU, Prayag A, Kuppusamy P *et al*. Protection against cisplatin-induced nephrotoxicity by Spirulina in rats. Cancer chemotherapy and pharmacology. 2006; 58(6):802.
21. Kruidering M, Van De Water B, De Heer E, Mulder GJ, Nagelkerke JF. Cisplatin-induced nephrotoxicity in porcine proximal tubular cells: mitochondrial dysfunction by inhibition of complexes I to IV of the respiratory chain. Journal of Pharmacology and Experimental Therapeutics. 1997; 280(2):638-649.
22. Chirino YI, Hernández-Pando R, Pedraza-Chaverrí J. Peroxynitrite decomposition catalyst ameliorates renal damage and protein nitration in cisplatin-induced nephrotoxicity in rats. BMC pharmacology. 2004; 4(1):20-29.
23. Padmalochana K, Dhana Rajan KS. *In-vivo* nephroprotective potential from leaves extracts of *Andrographis paniculata*. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4:1416-1425.
24. Adejo GO, Gnimintakpa JM, Olowoniyi OD, Matthew PO. *Andrographis paniculata*: Capabilities against Free Radicals, Lipid Peroxidation, Hepatotoxicity, and Nephrotoxicity. Open Access Library Journal. 2016; 3(7):1-9.
25. Bijoy B, Meghali C. Reno-protective action of the ethanolic extracts of *Andrographis paniculata* leaves in albino rats. Indian Journal of Basic and Applied Medical Research. 2017; 6(2):436-446.
26. Adeoye BO, Oyagbemi AA, Asenuga ER, Omobowale TO, Adedapo AA. The ethanol leaf extract of *Andrographis paniculata* blunts acute renal failure in cisplatin-induced injury in rats through inhibition of Kim-1 and upregulation of Nrf2 pathway. Journal of basic and clinical physiology and pharmacology. 2018; 30(2):205-217.
27. Verma H, Negi MS, Mahapatra BS, Paul AS. Evaluation of an emerging medicinal crop Kalmegh [*Andrographis paniculata* (Burm. F.) Wall. Ex. Nees] for commercial cultivation and pharmaceutical & industrial uses: A review. Journal of Pharmacognosy and Phytochemistry. 2019; 8(4):835-848.