

Antioxidant and Nephroprotective Activities of the Extract and Fractions of *Homonoia riparia* Lour

Seena kanniparambil Xavier, Shoja Muhammed Haneefa¹, Devkar Raviraj Anand, Picheswara Rao polo, Rajalekshmi Maheshwari, Chandrashekara Shastry Shreedhara, Manganahalli Manjunath Setty

Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, ¹Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India

Submitted: 15-06-2016

Revised: 11-07-2016

Published: 06-01-2017

ABSTRACT

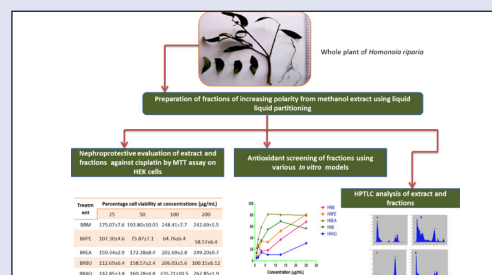
Background: *Homonoia riparia* is a plant, which is widely used in the indigenous system of medicine for the treatment of urolithiasis, renal disorders and inflammatory conditions. This is the first report on the antioxidant and nephroprotective activities of whole plant of *H. riparia*.

Objective: The present study aims at investigating the *in vitro* antioxidant and nephroprotective activity of the methanol extract and its different fractions of *H. riparia*. **Methods:** Petroleum ether (HRPE), Ethyl acetate (HREA), Butanol (HRBU), aqueous fractions (HRAQ) were prepared from the crude methanol extract of *H. riparia* (HRM) using liquid partitioning. Total phenolic content, flavonoid content and antioxidant activity assay were performed according to suitable methods. Nephroprotective activities were evaluated by MTT assay using Human Embryonic Kidney cells against cisplatin induced toxicity. Quantification of gallic acid was performed using validated HPTLC method. **Results:** The studies showed that extract and fractions possess significant nephroprotective activity against cisplatin induced renal toxicity. All the extracts/fractions of whole plant of *Homonoia riparia* was found to be significantly reducing cisplatin induced toxicity (< 0.05). The highest activity was observed with HRBU and HRAQ with a percentage viability of 293.09 ± 4.3 and 345.07 ± 3.2 at a concentration of 200 µg/ml. Gallic acid was detected in the HRM/fractions using HPTLC.

Key words: Antioxidant activity, gallic acid, *Homonoia riparia*, HEK 293, nephroprotective

SUMMARY

- Cisplatin (8 µg/ml) exhibited 50 % inhibition in cell viability in HEK 293 cells
- Butanol and aqueous fractions of *Homonoia riparia* showed significant nephroprotective activity against cisplatin induced cell damage in HEK cells.
- Gallic acid was detected and quantified in the extract and fractions of whole plant of *Homonoia riparia*



Abbreviations used: HPTLC: High Performance Thin Layer Chromatography, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, MTT: 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyl tetrazolium bromide, GAE: Gallic acid equivalents, QE: Quercetin equivalents, HEK: Human Embryonic Kidney, HRM: Methanol extract of *H. riparia*, HRPE: Petroleum ether fraction of *H. riparia*, HREA: ethyl acetate fraction of *H. riparia*, HRBU: Butanol fraction of *H. riparia*, HRAQ: Aqueous fraction of *H. riparia*, DMEM: Dulbecco's minimum essential medium, FBS: Foetal bovine serum, DMSO: Dimethyl sulfoxide, ANOVA: One way analysis of variance.

Correspondence:

Dr. Manganahalli Manjunath Setty,
Professor and Head, Department of
Pharmacognosy,
Manipal College of Pharmaceutical Sciences,
Manipal University, Manipal, Karnataka, India.
E-mail: mm.setty@manipal.edu
DOI: 10.4103/0973-1296.197647

Access this article online

Website: www.phcog.com

Quick Response Code:



INTRODUCTION

Cisplatin is one of the highly effective inorganic platinum based oncologic medications used in the chemotherapy for treating ovarian, bladder, testicular, cervical, and various solid or hematologic tumors. However, its use is limited by tumor cell resistance and various side effects such as suppression of bone marrow, renal toxicity, ototoxicity, emesis and neural toxicity.^[1,2] Among them, nephrotoxicity has been reported as the major limiter in 25-30% of the patients receiving single dose of cisplatin.^[3] Even though various platinum drugs are currently available in the market; still cisplatin is the most commonly used drug in platinum based therapy.^[4] The development of cisplatin nephrotoxicity is complex and a number of interrelated factors such as transporter mediated cisplatin accumulation, conversion into nephrotoxins, formation of DNA adducts, mitochondrial dysfunction, nitrosative and oxidative stress, inflammation, signal transducers and apoptotic pathway activation are involved. The various *in vitro* and *in vivo* studies suggest that oxidative stress and inflammation play an important role in the pathogenesis of cisplatin induced renal toxicity.^[5,6]

Cisplatin administration leads to the formation reactive oxygen species such as hydroxyl radical by mitochondrial injury and that may lead to the activation of inflammatory mediators and number of apoptotic pathways mediated by caspase and mitogen activated protein kinase.^[7] Plants are a rich source of a number of phytochemicals such as phenolic compounds, mainly flavonoids and phenolic acids, known to exert antioxidant property by their redox nature, which allows them to act as a hydrogen donors or

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Xavier Sk, Haneefa SM, Anand DR, polo PR, Maheshwari R, Shreedhara CS, Setty MM. Antioxidant and nephroprotective activities of the extract and fractions of *Homonoia riparia* Lour. Phcog Mag 2017;13:25-30.

metal chelating agents.^[7] Further, they are reported to possess a number of bioactivities such as anticarcinogenic, neuroprotective, cardioprotective, nephroprotective, and antiinflammatory properties. Moreover, researchers suggested that phenolic compounds may be useful in reducing the cytotoxic effect on normal cells caused by chemotherapeutic agents.^[8]

Homonoia riparia is a widely seen small shrub or tree in the tropical region and has been extensively used in indigenous system of medicine for treating a wide range of ailments.^[9] The roots of the plant have diuretic, laxative, emetic properties and root decoction is used for treating bladder stones, urinary discharges, inflammation, ulcers, uterine disorders, cardiac disorders, blood disorders, urinary infections and malaria.^[10-12] The leaves and fruits are used in the treatment of inflammation, wounds and skin diseases. An ethanol extract of root of *H. riparia* was reported to possess antiurolithiatic activity in male Wistar rats.^[13] Phytochemical investigation on *H. riparia* reported the isolation of sterols, phenolic compounds, gallic acid, taxerone, triterpenoids, quercetin glycosides etc.^[14-16]

Therefore, to investigate the nephroprotective effects of *H. riparia*, screening of the extract and fractions of whole plant of *H. riparia* against cisplatin induced nephrotoxicity was undertaken.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, curcumin, ascorbic acid, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and 2, 2 -Azino-bis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS), 3-(4,5-dimethylthiazolyl-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), were obtained from Sigma Aldrich Co, Cisplatin. All other chemicals and solvents used were of analytical grade.

Plant material

H. riparia was procured from the shores of Seetha River, Karnataka in the month of February and authenticated by botanist Dr. Gopala Krishna Bhat, Poorna Prajna College, Udupi and deposited the specimen for future reference in Manipal college of Pharmaceutical Sciences, Manipal.

Preparation of the extract/fractions

Powdered *H. riparia* was extracted with methanol for three days at 65°C using Soxhlet extraction method and was concentrated by rotary evaporator using controlled temperature and pressure. The resultant crude methanol extract (HME) was dispersed in distilled water to get an aqueous methanol solution and successively fractionated into petroleum ether (HRPE), ethyl acetate (HREA), n-butanol (HRBU) and the remaining aqueous (HRAQ) fractions. Extract and all the fractions were stored in a refrigerator.

Phytochemical screening

Methanol extract and fractions of *H. riparia* was subjected to chemical screening using different chemical tests to check the presence of secondary metabolites such as sterols, steroids, fatty acid, alkaloids, glycosides, flavonoids, and tannins in the plant by standard methods.^[17]

Total phenolic content

The total phenolic content of the methanol extract and its fractions were performed using Folin-Ciocalteu colorimetric method.^[18] Briefly, 200 µL of the extracts/fractions in methanol was mixed with Folin-Ciocalteu reagent and sodium carbonate and the mixture was kept in room temperature for 120 min and absorbance was measured at 750 nm using UV spectrophotometer. The assay was performed in triplicate. Quantification was carried out using standard Gallic acid. The total phenolic content was expressed as Gallic acid equivalents (GAE) in mg/g of dry extract/fractions.

Total flavonoid content

The Aluminum chloride colorimetric assay was adopted for the determination of total flavonoid content present in the extract/fractions of *H. riparia*.^[19] Briefly, 0.5 mL of the crude methanol extract/fractions (1 mg/mL) was added with 1.5 mL methanol, 0.1 mL 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water and was incubated for 30 min at room temperature and read the absorbance at 415 nm against a blank sample consisting of 0.5 mL of extract/fractions and 4.5 mL of distilled water. A standard calibration curve of quercetin was used to estimate total flavonoid content and was expressed as mg Quercetin equivalents (QE)/g of extracts.

Evaluation of antioxidant activity

DPPH radical scavenging assay

Hundred microliters of the different concentration of extract/ fractions (3.12 -250 µg/ml) were allowed to react with 0.1mL of DPPH (100 mM) in methanol in a multi-well plate. After 30 min of incubation at 37°C in the dark, the absorbance of extract/fractions was measured at 517 nm using an Elisa reader. Ascorbic acid, a widely used antioxidant was used as positive control and 100 µL of DPPH and 100 µL of methanol was kept as blank.^[20] The radical scavenging percentage (%) was calculated as: Percent scavenging = $((A_o - A_t)/A_o) \times 100$; where A_o = Absorbance of control (without extract) and A_t = Absorbance of sample. Assay was performed in triplicate.

ABTS radical scavenging assay

In this method, the ABTS radical cation was prepared by the reaction of ABTS solution (7 mM) and potassium persulfate (2.45 mM) and kept for incubation in the dark for 16 h. The absorbance of 1.1 ± 0.2 units was obtained after diluting the solution with methanol at 734 nm. Hundred microliters of various concentrations of extract/fractions (3.12 -250 µg/ml) were allowed to react with 100 µL of ABTS free radical cations for 5 min and the absorbance was measured using ELISA reader at 735 nm.^[21] Ascorbic acid was used as positive control. The percentage of radical scavenging activity was calculated using the formula: Percent scavenging = $(A_o - A_t)/A_o \times 100$; where A_o = Absorbance of control (without extract) and A_t = Absorbance of sample.

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was evaluated by griess reagent method. 0.5 mL of extract/fractions (3.12 -250 µg/ml) were treated with 2 mL of 10 mM sodium nitroprusside and 0.5 mL phosphate buffer saline (PBS) and incubated at 25°C for 150 min in light followed by the addition of 0.5 mL of the reaction mixture with 1mL of sulphanilic acid reagent and allowed to stand for 5 min and then 1 mL of N- (1- naphthyl) - ethylenediamine dichloride (1%) was added and kept for 30 min. Curcumin was used as positive control. Read the absorbance at 540 nm against corresponding blank by ELISA reader.^[22] Nitric oxide scavenging activity was calculated as Percent scavenging = $[(A_o - A_t)/A_o] \times 100$; where A_o = Absorbance of control (without extract) and A_t = Absorbance of sample.

Total antioxidant Capacity assay

Phosphomolybdenam method was adapted to quantify the total antioxidant capacity of extracts/fractions of *H. riparia*.^[23,24] 0.1 mL of the extract/fractions (1mg/mL in DMSO) was added 0.3 mL of reagent solution consisting of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate and were incubated at 95°C for 90 min. Then, it was cooled and the absorbance was recorded at 695 nm using spectrophotometer against blank solution consisted of 1 mL of the reagent solution without sample. A calibration curve was prepared using different concentrations of ascorbic acid. The results were expressed as ascorbic acid equivalents.

Quantification of gallic acid in the extract/fractions using HPTLC

CAMAG High Performance Thin Layer Chromatography instrument was used for the quantification of gallic acid in the methanol extract of *H. riparia*. 100 µg/mL of gallic acid (standard) and 2 mg/mL solution of test solutions were prepared. 10 µL of the sample was spotted on pre-coated silica gel plate using Camag Linomat 5 applicator in duplicate. The mobile phase consisted of toluene: ethyl acetate: formic acid: methanol (6:6:1.8:0.25 v/v) was used for the development of HPTLC plates.^[25] The plate was dried using drier and scanned at 280 nm using CAMAG TLC scanner 3. The peak areas were recorded and percentage content of gallic acid was determined.

Cell culture

HEK 293 cells (Human Embryonic Kidney) were collected from School of Life Science, Manipal University, Manipal. The cells were cultured in 25 cm² tissue culture flask and maintained at 37°C under atmosphere of 5% CO₂ and humidified air. Dulbecc's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 50 µg/mL gentamycin and trypsinized was used for culturing the cells and are used for assay whenever the cellular confluency was 70 %.

Nephroprotective evaluation

Here, cell viability of crude extract/fractions was determined by MTT assay. Briefly, HEK cells were allowed to grow in 96 well plates for one day and were pretreated with 100 µl of different concentrations of crude extract/fractions at 37 for 2 h and then 50 µl of 8 µg/mL cisplatin were added to wells and incubated for an additional 48 h. The media was removed and washed with 100 µl of PBS, after which 20 µl of the MTT reagent (3 mg/ml in PBS) was added to each wells and kept for 3 h of incubation. The supernatant was removed and formazan crystals formed were dissolved in 20 µl of DMSO and absorbance was noted at 540 nm using micro plate ELISA reader.^[26,27] Quercetin was used as a positive control and 0.4 % DMSO as negative control. The percentage of cell viability was calculated using Graph pad prism 5.

Statistical analysis

Statistical analysis was performed using Graph pad prism software. All the values are presented as means ± SEM. One way analysis of variance followed by post-hoc turkeys test was used to find out the difference in the means.

RESULTS

Phytochemical screening, extract/fractions yield, total phenolic and flavonoid contents

Extract and fractions yield, total phenolic and flavonoid contents of whole plant of *H. Riparia* are documented in Table 1.

Phytochemical screening of the total HRM exhibited the presence of sterols, tannins, flavonoids, phenolic acids. Salkowski's and Lieberman's

Table 1: Percent yield, total phenolic and flavonoid content of extract and fractions of *H. riparia*

Extract/ Fractions	Percent field (%)	Total phenolic content (mg GAE/g of plant extract)	Total flavonoid content (mg GAE/g of plant extract)
HRM	6.90	64.75 ± 0.14 ^a	4.50 ± 0.02 ^a
HRPE	35.54	17.25 ± 0.79 ^b	2.66 ± 0.09 ^b
HREA	3.36	223.75 ± 0.14 ^c	2.26 ± 0.58 ^b
HRBU	4.58	106.08 ± 0.16 ^d	2.74 ± 0.15 ^b
HRAQ	56.71	18.50 ± 0.83 ^b	3.12 ± 0.09 ^{a,b}

All values are expressed as mean ± SEM (n=3). ^{a-d} Column wise values with different

Burchard tests confirm the presence of sterols and triterpenoids in HRPE, HREA, and HRBU. The foam test was positive for HREA, HRBU, HRAQ documented the presence of saponins. HREA, HRBU AND HRAQ exhibited the presence of phenols in Ferric chloride test. Lead acetate test was positive for tannins in HRBU and HRAQ. The presences of flavonols, flavones, flavonones and flavononols have been confirmed in HREA and HRBU using shinoda test.

Phenolic compounds are the secondary metabolic phytoconstituents found in all plants and participate in the protection against oxidation process. Plants may contain simple phenolic compounds (phenolic acid, anthocyaninetc.) to highly polymerised compounds (tannins) in various quantities.^[28] Total phenolic content of extract/fractions of *H. riparia* was expressed as milligrams of Gallic acid equivalents (GAE). Table 1 shows that total phenolic content in fraction varied widely from 28.50 ± 0.28 and 147 ± 0.14 mg/g expressed as gallic acid equivalents (GAE). HREA exhibited the highest total phenolic content (223.75 ± 0.14) followed by HRBU (106.08 ± 0.16) and HRM (64.75 ± 0.5), HRAQ (18.50 ± 0.83) and HRPE (17.67 ± 0.26) had lowest amount of total phenolic content compared to other fractions.

Plant flavonoids are polyphenolic compounds, which shows antioxidant activity mainly due to their ability to inhibit the formation of ROS by the suppression of enzymes or chelating trace elements or by scavenging the free radicals or by the upregulation of antioxidant enzymes.^[29] The total flavonoid content of the fractions was expressed in quercetin equivalents/g of extracts and was found to be high in HRM (4.50±0.94). The total flavonoid content was in the following order HRM>HRAQ>HREA>HRBU>HRPE [Table 1].

In vitro antioxidant activity

Various studies reported the role of ROS/RNS in cisplatin induced nephrotoxicity. In the present study, we have performed the antioxidant activity of crude methanol extract and fractions of *H. riparia* using four assays such as DPPH assay, ABTS assay, Nitric oxide radical scavenging assay and total antioxidant assay. The extract and fractions showed different level of antioxidant activity in different systems performed.

DPPH is a stable organic free radical having an absorption band at 517 nm. It loses this absorption when it accepts an electron or a free radical species, resulting in a visually noticeable discoloration from purple to yellow.^[30] Analysis of the results shows that HREA shows significant DPPH radical scavenging activity with an IC₅₀ value of 2.24 µg/ml followed by HRBU with IC₅₀ value 7.11 µg/ml and HRAQ shows lowest activity with 43.58 µg/ml and is presented in Table 2.

Table 2: Free radical scavenging and antioxidant activities of extract/fractions of *H. riparia*.

Extract/ fractions	DPPH Scavenging IC ₅₀ (µg/ml)	ABTS scavenging IC ₅₀ (µg/ml)	Nitric oxide Scavenging IC ₅₀ (µg/ml)	Total antioxidant capacity (AAE/mg extract) ^f
HRM	17.12 ± 0.34 ^a	15.18 ± 1.70 ^a	67.27 ± 1.34 ^a	372.13±1.22 ^a
HRPE	9.39 ± 5.45 ^b	128.3 ± 0.62 ^b	186.6 ± 9.67 ^b	108.46±1.41 ^b
HREA	2.29 ± 2.14 ^b	71.23 ± 3.94 ^c	23.83 ± 0.87 ^c	209.53±1.30 ^c
HRBU	7.11 ± 1.20 ^b	88.52 ± 0.87 ^c	16.97 ± 1.34 ^c	412.80±5.62 ^d
HRAQ	43.58 ± 1.48 ^c	74.13 ± 1.34 ^c	109.3 ± 10.23 ^d	438.66±1.27 ^c
Ascorbic acid	7.003 ± 0.1 ^b	2.35 ± 5.63 ^d	-	-
Curcumin	-	-	24.48± 0.12 ^c	-

All values are expressed as mean ± SEM (n=3). ^{a-d} Column wise values with different superscripts of this type indicate significant difference (p>0.05). ^f Total antioxidant capacity expressed as µg ascorbic acid equivalents/mg extract

Table 3: *In vitro* evaluation of fractions of *H. riparia* in cisplatin induced nephrotoxicity in HEK 293 by MTT assay.

Extracts/ fractions	Percentage cell viability at concentrations ($\mu\text{g/mL}$)			
	25	50	100	200
HRM	240.31 \pm 3.2 ^a	273.17 \pm 2.6 ^a	348.57 \pm 1.1 ^a	352.38 \pm 2.3 ^a
HRPE	141.74 \pm 1.8 ^b	147.46 \pm 7.4 ^b	193.49 \pm 1.7 ^b	202.53 \pm 8.4 ^b
HREA	172.38 \pm 9.4 ^b	175.07 \pm 1.2 ^b	195.39 \pm 2.4 ^b	269.68 \pm 4.7 ^c
HRBU	258.57 \pm 5.8 ^a	262.61 \pm 1.2 ^{a,c}	285.56 \pm 2.5 ^c	293.09 \pm 4.3 ^{a,c}
HRAQ	207.30 \pm 8.1 ^a	238.88 \pm 0.1 ^{a,c}	293.96 \pm 1.2 ^{a,c}	345.07 \pm 3.2 ^{a,c}
Quercetin	6.25	12.5	25	50
Quercetin	173.49 \pm 4.5	187.46 \pm 3.5	290.63 \pm 4.3	309.20 \pm 1.4

All values are expressed as mean \pm SEM (n=3). ^{a-d} Column wise values with different superscripts of this type indicate significant difference (p>0.05).

ABTS assay is a well-established method for estimating the electron donating /accepting abilities of extract and fractions.^[31] Scavenging effect of extract and fractions of *H. riparia* was concentration dependant and the results showed that the ABTS radical scavenging activity of the extract and fractions can be ranked as HRM>HRAQ>HREA>HRBU>HRPE and reported in Table 2.

Nitric Oxide is a diffusible radical and displays its toxicity when it comes in reaction with certain types of proteins and other free radicals and forms highly reactive peroxy nitrite anions. The assay is based on the principle that sodium nitroprusside spontaneously generates nitric acid at physiological solution and the resultant nitric oxide will interact with oxygen to form nitrite ions. The extract /fraction, the scavengers of nitric oxide compete with oxygen leading to the decreased generation of nitrite ions.^[32] Various studies suggest that renal cells produce peroxy nitrite during cisplatin induced nephrotoxicity.^[33] Highest radical scavenging activity was observed with aqueous fraction with an IC_{50} value of 5.96 $\mu\text{g/mL}$ followed by HRBU with IC_{50} value of 16.97 $\mu\text{g/mL}$ and is presented in Table 2.

The total antioxidant capacities of extract/fractions were determined in ascorbic acid equivalents using phosphomolybdenum assay^[23,24] and Table 2 shows total antioxidant activities of the extract and fractions. HRM exhibited highest antioxidant capacity of 372.13 \pm 1.22 AAE/mg followed by HRBU, HRAQ, HREA and HRPE at a concentration of 1000 $\mu\text{g/mL}$.

The various radical scavenging assays revealed that extract/fractions can effectively prevent the biomolecules such as DNA, Poly unsaturated fatty acids, amino acids, proteins from the damage caused by the attack of reactive radical species. However, some of the factors like solubility of the extract/fraction in different antioxidant system and stereospecificity of radical may affect the capacity of the radical cation to react and quench different radical. Also, some of the reports show that some extract, which exhibit good radical scavenging ability in one antioxidant assay may not show that much activity in another antioxidant model.^[34]

Nephroprotective effect of *H. riparia* against cisplatin toxicity in human kidney cells

Here, nephroprotective activity of extracts and fractions were evaluated in Human embryonic kidney cells. HEK 293 cells are considered to be a good model to study the *in vitro* cytotoxicity to kidney because it has many of the specialized characteristic functions of human kidney cells and are originated from the embryonic neuronal cells in kidney.^[35]

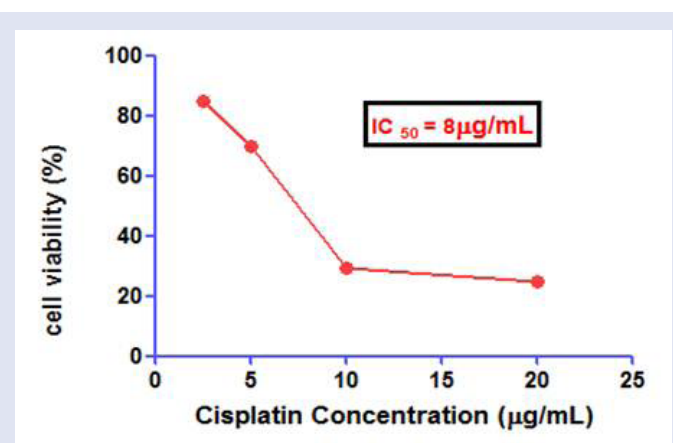


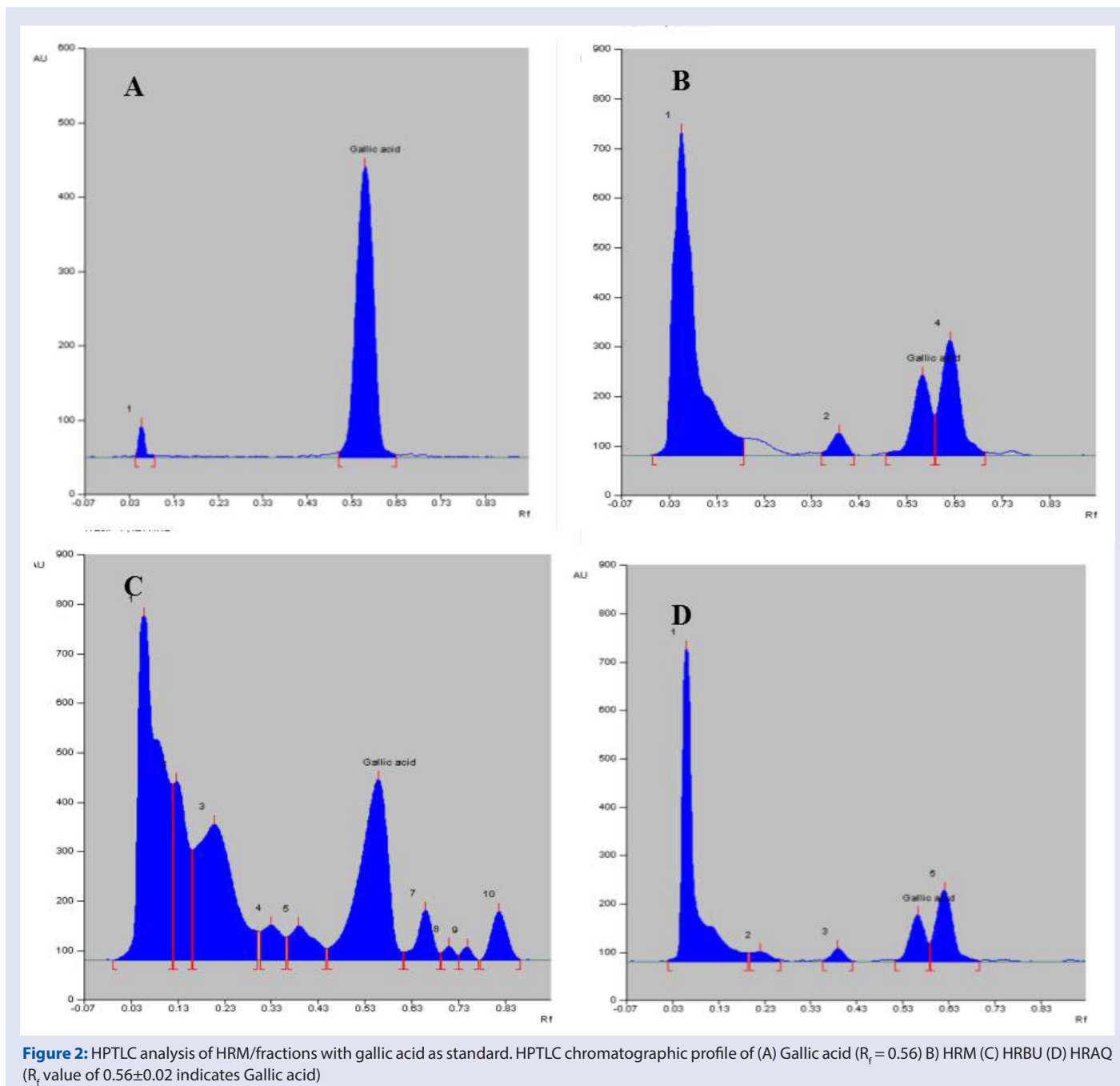
Figure 1: Effect of cisplatin on cell viability. HEK 293 cells were treated with various concentrations of cisplatin for 24 h.

Viability of HEK cells

HEK 293 cells were treated with different concentration of cisplatin and then incubated for 24 h to evaluate its activity on viability. Figure 1 shows Cisplatin inhibited the cell viability in a dose dependent manner. The concentration of cisplatin that resulted in suppression of 50% cell viability was determined using nonlinear regression analysis. The IC_{50} of cisplatin drug was found to be 8 $\mu\text{g/mL}$ and this concentration was used for further assay to find out the recovery effect of methanol extract and fractions of whole plant of *H. riparia*.

Potential nephroprotective activity against cisplatin toxicity in human kidney cells

To select suitable concentration of extract/fractions of *H. riparia* for the nephroprotective study, nontoxic concentration were first determined. Exposure of different concentrations (0.2 -2 mg/mL) of extract and fractions on HEK 293 cell line did not alter the cell viability (data not provided). Therefore, extract concentrations of 25-200 $\mu\text{g/mL}$ were selected to further evaluate protective nature of extract/fractions of *H. riparia* against cisplatin induced toxicity. HEK 293 cell lines were treated with different concentrations of extracts/fractions for 2 hours after which 8 $\mu\text{g/mL}$ cisplatin were added to the cells. This was followed by incubation for additional 48 hours and the recovery effects of the extract/fractions were determined by MTT assay. The data shows that viability of the cells treated with extract/fractions of *H. riparia* was higher than that of the non-treated control cells (HEK 293 cells treated with cisplatin). All the extracts/fractions of whole plant of *H. riparia* was found to be significantly reducing cisplatin induced toxicity (< 0.05) and the highest activity was observed with HRBU and HRAQ with percentage viability of 293.09 % and 345.07 % at a concentration of 200 $\mu\text{g/mL}$, when compared to non-treated control cells and was not significantly different [Table 3]. The standard quercetin showed a dose dependent increase in cell viability against cisplatin toxicity and it showed an increased viability of 319.08% at higher concentration of 50 $\mu\text{g/mL}$. It has been reported that Quercetin is a renoprotective agent, which effectively reduces cisplatin induced renal oxidative stress, inflammation and apoptosis without compromising the antitumor activity of cisplatin.^[36,37] Many substances including antioxidants have been reported for their positive effects on nephrotoxicity induced by cisplatin. However, most of these studies have been performed *in vitro* using cells and in *in vivo* models using non malignant animals. Because potential nephroprotective compounds might adversely affect the anticancer activity of cisplatin and also tumours may change the metabolic and endocrine equilibrium.^[36]



Therefore, renoprotective activities should be tested in malignant tumor bearing animals. However, anticancer effects of the extract and fractions of *H. riparia* were not envisaged. Further work is required on *H. riparia* to investigate its attenuating effect on the therapeutic effect of cisplatin. Various studies also reported that antioxidants decrease the toxicities and lipid peroxidation associated with cisplatin. This is the first report on the nephroprotective activity of whole plant of *H. riparia* against cisplatin induced nephrotoxicity.

Gallic acid quantification in HRM/fractions by HPTLC

Gallic acid was quantified in the methanol and fractions of *H. riparia* by HPTLC analysis. HRM was found to contain 0.9% w/w gallic acid. The

content of gallic acid in HRBU and HRAQ was found to be 3.5% w/w and 0.5% w/w respectively and are shown in Figure 2. Gallic acid and its derivatives are polyphenolic compounds, which are well known for their antioxidant activity by exhibiting free radical scavenging abilities. It has variety of therapeutic activities including antimicrobial, anti-inflammatory, renoprotective and anticancer properties. Recently, it was reported to protect the kidney from oxidative damage caused by cisplatin induced toxicity through the reinforcement of antioxidant defense of renal tissues.^[38,39] Further, reports on structure activity relationship of gallic acid reveals that free radical scavenging activity is mainly dependent on the presence of phenolic hydroxyl group and steric freedom in the molecule. Moreover, literature reports support that gallic acid and other phenolic compounds can effectively scavenge free radical

that may be responsible for its cytoprotective activity and an inducer in the apoptotic pathway in various tumor cells. HRM, HRBU HRAQ was found to be enriched with gallic acid and other phenolic compounds, which might have been responsible for the nephroprotective potential of the extract and fractions.

CONCLUSIONS

Our study showed that whole plant of *H. riparia* had a protective activity against toxicity by cisplatin, which might be associated with the antioxidant property of the phenolic compounds present in the plant. Further work to separate and identify the bioactive compounds responsible for the nephroprotective activity is undergoing in our lab, which would be followed by pharmacological screening in animal models.

Acknowledgement

The authors are thankful to Manipal College of Pharmaceutical Sciences, Manipal University, Manipal for providing facilities and QIP, AICTE, New Delhi for the financial support.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Thulasi GP, Mathew J, Gifty ST. Prevention of cisplatin induced nephrotoxicity by terpenes isolated from *Ganoderma lucidum* occurring in Southern Parts of India. *Exp Toxicol Pathol* 2011;63:157-60.
- Tae-Won K, In-Bae S, Hong-Ki L, Jong-Hwan L, Eun-Sang C, Hwa-Young S, *et al.* Platycodin D, a triterpenoid apoinin from *Platycodon grandiflorum* ameliorates cisplatin-induced nephrotoxicity in mice. *Food Chem Toxicol* 2012;50:4254-9.
- Neife Aparecida DS, Maria Augusta CR, Nadia MM, Antonio Cardozo DS. Cisplatin-induced nephrotoxicity and targets of nephroprotection: an update. *Arch Toxicol* 2012;86:1233-50.
- Abir AS, Mokhtar I, Lamiaa Youssef, El-Shennawy K. Cisplatin induced damage in kidney genomic DNA and nephrotoxicity in male rats: The protective effect of grape seed proanthocyanidin extract. *Food Chem Toxicol* 2009;47:1499-506.
- Lin L, Jing Z, Weiping Z, Ning J. Nephroprotective Effect of Gelsemine Against Cisplatin-Induced Toxicity is mediated via Attenuation of Oxidative Stress. *Cell Biochem Biophys* 2015;71:535-41.
- Ashraf Y N, Hamid AM. Aged garlic extract protects against oxidative stress and renal changes in cisplatin-treated adult male rats. *Cancer Cell Int* 2014;14:92.
- Penelope DS, Francisco JL, Fernando PB, Ana IM, Lopez-N Jose M. Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. *Nephrol Dial Transplant* 2011;26:3484-95.
- Padma Vijaya, Sowmya P, Arun F, Baskaran R, Poornima P. Protective effect of gallic acid against lindane induced toxicity in experimental rats. *Food Chem Toxicol* 2011;49:991-8.
- Hanum F, Van der Maesen L.J.G. PROSEA: Plant Resources of South-East Asia 11, Auxiliary Plants. Indonesia: Prosea Foundation; 2007. p. 155.
- Khare CP. Indian medicinal plants-An illustrated dictionary. Newyork: Springer Science Business media; 2007. p. 314.
- Quaatrocchi U. CRC World dictionary of medicinal plants and poisonous plants. Boca Raton: Taylor and Francis group; 2012. p. 2002.
- Singh MP, Himadri P. Medicinal Herbs with Their Formulations. India: Daya Publishing House 2005;478.
- Prasad KVSRG, Abraham R, Bharathi K. Srinivasan Evaluation of *Homonoiariparia* Lour for antiurtrolithiatic activity in albino rats. *Int J Pharmacogn* 1997;35:278-82.
- GS Viswanad, Atchutha R, Laatsch H, Rajendar M. Chemical constituents of heartwood and bark of *Homonoiariparia*. *J Trop Med Plants* 2006;7:267-73.
- Yang SM, Liu XK, Qing C, Wu DG, Zhu DY. Chemical constituents from root of *Homonoiariparia*. *Yao Xue Xue Bao* 2007;42:292-96.
- Fan X, Xueqian Z, Lingli Y, Xiuhua W, Jing Z. A New Cycloartane-Type Triterpenoid Saponin Xanthine Oxidase Inhibitor from *Homonoiariparia* Lour. *Molecules* 2014;19:13422-431.
- Harbone JB. Methods of extraction and isolation, In: *Phytochemical Methods*. London: Chapman and Hall 1998;60-66.
- Slinkard J, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic* 1977;28:49-55.
- Chang C, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002;10:178-82.
- Bansal P, Paul P, Nayak PG, Pannakal ST, J-H Zou, Laatsch H, *et al.* Phenolic compounds isolated from *Pileamicrophylla* prevent radiation-induced cellular DNA damage. *Acta Pharm Sin B* 2011;1:226-35.
- Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. *Food Chem* 2007;103:381-8.
- Gouthamchandra K, Mahmood R, Manjunatha H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from *Clerodendrum infortunatum* L. *Environ Toxicol Pharmacol* 2010;30:11-8.
- Berker KI, Guclu K, Tor I, Apak R. Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP) and ferricyanide reagents. *Talanta* 2007;72:1157-65.
- Prieto P, Pineda M, Aguilar MM. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 1999;269:337-41.
- Tandon N, Sharma M. Quality standards of Indian medicinal plants. New Delhi: Indian Council of Medical Research (ICMR) 2003;1:203.
- Mustafa IW, Mansour SA, Badreldin HA. Effect of Curcumin on Cisplatin- and Oxaliplatin-Induced Oxidative Stress in Human Embryonic Kidney (HEK) 293 Cells. *Ren Fail* 2011;33:518-23.
- Sung-Hwa S, Hyoung L, Ji-young N, Sung KI, Hee-J J, Yangseok K. Screening of herbal medicines for the recovery of cisplatin-induced Nephrotoxicity. *Environ Toxicol Pharmacol* 2009;28:206-12.
- Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. *Molecules* 2013;18:2328-75.
- Zhishen Jia, Cheng Tang Men, Jianming Wu. Determination of flavonoid content in mulberry and their scavenging effect on superoxide radicals. *Food chem* 1999;64:555-59.
- Jonathan PA, Prince A. Antioxidant and free radical scavenging activity of iron chelators. *Toxicol Rep* 2015;2:721-28.
- Roberta R, Nicoletta P, Anna P, Ananth P, Min Y, Catherine R.E. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999;26:1231-37.
- Tsai PJ, Tsai TH, Yu CH, Ho SC. Evaluation of NO-suppressing activity of several Mediterranean culinary spices. *Food Chem Toxicol* 2007;47:440-44.
- Bidya DS, Kiran Kumar RR, Uday KP, Madhusudana K, Ganga Modi NV, Ramakrishna S. Carnosic acid attenuates renal injury in an experimental model of rat cisplatin-induced nephrotoxicity. *Food Chem Toxicol* 2011;49:3090-97.
- Hadi G, Behrouz JG, Chandra N, Ramachandra K, Prakash HS. Antioxidant and Neuroprotective Activities of *Hyptissuaveolens* (L.) Poit. Against Oxidative Stress-Induced Neurotoxicity. *Cell Mol Neurobiol* 2014;34:323-31.
- Shampur NM, Subha S, Padinjarematatthil TU. Utility of human embryonic kidney cell line HEK-293 for rapid isolation of fixed and street rabies viruses: comparison with Neuro-2a and BHK-21 cell lines. *Int J Infect Dis* 2010;14:1067-71.
- Penelope DS, Francisc JL, Fernando PB, Ana IM, Jose ML. Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. *Nephrol Dial Transplant* 2011;26:3484-95.
- Estela BB, Milena CS, Heloisa DC, Lusania MGA, Roberto SC. Maria de Lourdes PB. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. *Pharmacol Rep* 2006;58:526-32.
- Sayed MN, Solomon H, Seyed FN, Antoni S, Maria D, Akbar HM, *et al.* Protective effect of gallic acid isolated from *Peltiphyllum peltatum* against sodium fluoride-induced oxidative stress in rat's kidney. *Mol Cell Biochem* 2013;372:233-9.
- Vijaya Padma V, Sowmya P, Arun Felix T, Baskaran R, Poornima P. Protective effect of gallic acid against lindane induced toxicity in experimental rats. *Food Chemical Toxicol* 2011;49:991-98.