



Received on 13 February, 2014; received in revised form, 26 March, 2014; accepted, 13 June, 2014; published 01 August, 2014

PHARMACOGNOSTIC, PHYTOCHEMICAL INVESTIGATION & PHARMACOLOGICAL EVALUATION OF *SCOPARIA DULCIS* LINN. PLANT EXTRACTS FOR NEPHRO-PROTECTIVE ACTIVITY

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Keywords:

Scoparia dulcis Linn, Flavonoid, Gentamicin, Nephroprotective

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ABSTRACT: The aerial parts of *Scoparia dulcis* Linn. belonging to family Scrophulariaceae. Traditionally are used as antiulcer, antibacterial, antifungal, cytotoxic, antimicrobial, antianaemic, analgesic, antidiabetic, hyperlipidaemia, hepatoprotective etc. From literature survey the aerial parts of *Scoparia dulcis* Linn. are rich source of flavonoids, tannins, phenolic compounds but not yet scientifically investigated for nephroprotective activity. So in order to give scientific background the aerial parts of *Scoparia dulcis* Linn. was evaluated for nephroprotective activity by gentamicin induced nephrotoxicity. The present study carried out with Pharmacognostic examination like macroscopic, microscopic, proximate values and preliminary phytochemical test evaluation of alcohol and water extract; And their Pharmacological evaluation that gentamicin at dose of 100 mg./kg. BW/day I.P. rout produces significant nephrotoxicity as evidenced by decreased in body weight, urine volume & elevate in kidney weight, serum urea, uric acid, serum creatinine, with renal tubular necrosis. After treatment the alcohol and water extracts each (200 mg./kg. B.W./ day I.P.) of aerial parts of *Scoparia dulcis* Linn. Show significant decreases ($p < 0.001$) serum urea, uric acid, creatinine level, kidney weight & significant increases in urine volume, body weight as compared to negative control group. The finding suggests that the alcohol and water extracts aerial parts of *Scoparia dulcis* Linn. possesses marked nephroprotective activity with minimal toxicity.

INTRODUCTION: *Scoparia dulcis* Linn. (Scrophulariaceae) is a tough, glabrous, leafy, branched green color and odor taste indistinct with smooth fracture, herbaceous plant up to 90 cm. height, indigenous to tropical America and introduced in to India within the last one hundred years, very commonly found as a weed in many parts of India, particularly in Bengal and Tamil Nadu^{1,2}. This plant is also found in Karnataka³.

A survey of the literature revealed that the aerial parts of the plant of *Scoparia dulcis* Linn. are reported to contain flavonoids, terpenoids, phenols, tannins, saponins, amino acids, coumarins and carbohydrates among other components.

Previously isolated phytoconstituents: *Scoparia dulcis* Linn. dulcis is rich in flavones, terpenes and steroids. The main chemicals include scopadulcic acids A and B, scopadiol, scopadulciol, scopadulin, scoparic acids A – C and betulinic acid. Other chemicals include: acacetin, amyryl, apigenin, benzoxazin, benzoxazolin, benzoxazolinone, cirsimaridin, cirsitakooside, coixol, coumaric acid, cynaroside, daucosterol, dulcinol, dulcioic acid, gentisic acid, glutinol, hymenoxin, linarin, luteolin, mannitol, scoparinol, scutellarein, scutellarin,

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.5(8).3342-46</p>
<p>Article can be accessed online on: www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(8).3342-46</p>	

sitosterol, stigmasterol, taraxerol, vicenin, and vitexin^{4,5}.

Gentamicin is an aminoglycoside antibiotic used in a variety of infections caused by Gram negative bacteria. But limiting side effect of these drugs is relatively more nephrotoxic. There is a continuous search for agents which provide nephroprotection against the renal impairment induced by drugs like gentamicin for which allopathy offers no remedial measures. It is thus imperative that we turn towards alternative systems of medicine for solutions⁶.

The wide survey of literature revealed that the aerial parts of *Scoparia dulcis* Linn. are used for the treatment of kidney complaints and renal troubles¹. But no scientific studies source have yet been undertaken to verify these claims. The present study is an attempt to pharmacognostic, phytochemical and pharmacological screening the alcohol and water extracts of the aerial parts for its nephroprotective activity.

MATERIALS AND METHODS:

Plant material: The fresh plant of *Scoparia dulcis* Linn. was collected during month of October & November 2011 from the Western ghat near Dandeli, Karwar district in Karnataka state. The plant material was taxonomically identified & authenticated by Dr. B. D. Huddar, Professor and Head, Department of Botany, H. S. K. Kotambari Science College, Vidyanagar, Hubli. The voucher specimen (KLESCOP/HBL/AUTH/2011-12) has been deposited in the herbarium section of the Pharmacognosy Division, K. L. E. University College of Pharmacy, Vidyanagar, Hubli; For future & further reference.

Pharmacognostic study: The aerial parts of *Scoparia dulcis* Linn. The habitat is shrubs, height-90 cm. stem-non-woody, quadrangular, leaves-opposite or whorled, lanceolate, coarsely serrate, Flowers-Small, white, 2-4 to 5-flowered inflorescence. And fresh fruit is Color Green after dry it forms faint yellow. The aerial parts of the powder characters are present phloem fiber, covering trichomes, epidermis cell, cortex, calcium oxalate crystal, & cuticle⁵.

Various proximate values parameters such as total ash (8.5% w/w), water soluble ash (6.5% w/w), acid insoluble ash (0.5% w/w), sulphated ash (0.59% w/w), Petroleum ether soluble extractive value (1.6% w/w), water soluble extractive value (7.8% w/w), alcohol soluble extractive value (4% w/w) and moisture contents (8% w/w) were calculated^{7,8,9,10}.

Phytochemical investigation: The shade dried aerial parts of *Scoparia dulcis* Linn. 5.0 gm. were ground to coarse powder and soxhleted with 90% alcohol and 5.0 gm. macerated with water. The alcohol and water extracts was concentrated by rotary flash evaporator and then evaporated to dryness on water bath. The collected extracts was dried and put in desiccators obtained dark green color sticky mass of alcohol and dark brown color solid mass of water extract.

Then this alcohol and water extracts is used for Phytochemical and Pharmacological screening was conducted with various qualitative test to identify the various chemical constituents (**Table 1**) and Nephroprotective action. To perform test the following chemicals and reagents were used^{7,8,9,10}.

TABLE 1: QUALITATIVE PHYTOCHEMICAL TESTS

Phytoconstituents	Chemical tests	Alcohol extract	Water extract
Carbohydrate	Molisch's test	+ ve	+ ve
	Fehling test	+ ve	+ ve
Sterol & triterpenoids	Salkowski test	+ ve	- ve
	Liebermann-Burchard tests	+ ve	- ve
Glycoside	Water solution	+ ve	- ve
	Sodium hydroxide solution	+ ve	- ve
Flavonoids	Shinoda test	+ ve	+ ve
	Ferric chloride test	+ ve	+ ve
Tannins	Phenazone test	+ ve	+ ve
	Gelatin test	+ ve	+ ve
Saponin	Haemolysis test	- ve	+ ve
	Foam tests	- ve	+ ve

(+ ve) = Present; (- ve) = Absent

Pharmacological evaluation: The study of acute toxicity and Nephroprotective activity used alcohol and water extract of aerial parts of *Scoparia dulcis* Linn plant. The study was conducted after obtaining Institutional animal ethical committee clearance (Ref. KLESCOPH/IAEC. Clear/201-2012/Pharmacognosy/01).

Acute toxicity study: Acute toxicity study was performed on healthy female albino mice weighing between 25-30 gm. As per the OECD guidelines no.423 fixed dose method procedure; the maximum nonlethal dose was found to be 2000 mg./kg. body weight; hence 1/10th of alcohol and water extract to evaluate nephroprotective activity. 24 Healthy adult male albino wistar rats weighing between 100-150 gm. were selected for the nephroprotective activity each group contains 6 rats, They were housed in polypropylene cages and maintained at ambient temperature of 27°C ± 2°C under 12:12 hours, dark: light cycle^{11, 12, 13}.

Evaluation of Nephroprotective activity: Animals will be divided in to four groups, each group comprising of six animals. The groups were divided as follows (**Table 2**): **Group I:** Normal control animals received vehicle 10% DMSO sterile water for injection solution IP for 8 days (5 ml./Kg.). **Group II:** Negative control animals received Gentamicin sulphate injection (100 mg./kg. B.W./day I.P.) for 8 days. (Gentamicin injection Piramal healthcare, Pritampur, Madhya Pradesh, Batch no. PT14O7). **Group III:** Curative control animals received *Scoparia dulcis* Linn alcohol extract (200 mg./kg. B.W./day I.P.) + Gentamicin sulphate injection (100 m.g./kg. B.W./day I.P.) for 8 days. **Group IV:** Curative control animals received *Scoparia dulcis* Linn water extract (200 mg./kg. B.W./day I.P.) + Gentamicin sulphate injection (100 m.g./k.g. B.W./day I.P.) for 8 days^{12, 13}.

Parameters assessed for renal function:

1. **Body weight:** The weight of animals was measured before and after treatment.
2. **Kidney weight:** The weight of animal's kidney was measured before and after treatment.

3. **Urine volume:** The urine volume of animals was measured before and after treatment.
4. **Serum creatinine:** The creatinine level in serum was estimated by the alkaline picrate method, using creatinine kit (Modified Jaffe's reaction). Absorbance was read from a UV-520 on Autoanalyser (ARTOS- Autoanalyser).
5. **Serum urea:** The urea level in serum was estimated by urease method (Talke & Schubert, Tiffany et al.) and absorbance at 340 nm. in a fixed time which is proportional to the urea Concentration in the sample.
6. **Uric acid:** The urea level in serum was estimated by modified trinder method (Trivedi & Kabasakalian with modified trinder peroxidase method) & Intensity of the color formed which is measured at 505 nm. is directly proportional to the amount of uric acid present in the sample^{14, 15, 16}.

Histopathological studies: Two animals from each group were sacrificed 9th with diethyl ether anaesthesia on the day of blood withdrawal puncturing the retro-orbit plexus for evaluating the serum biochemical parameters. The blood so collected was centrifuged at 2500 rpm. For 15 min. to get clear serum & analyzed for various biochemical parameters. And kidneys were isolated, processed and embedded in paraffin wax. The sections were stained with Eosin is an acidic stain & hemotoxylin is basic stains, which are used staining and observed under light microscope¹³.

Statistical analysis: The results obtained from the present study were analyzed using one-way ANOVA followed by Dunnett's - t test, n=6 animals. The data are expressed as mean ± SEM. The gentamicin group ### p<0.001 as compared to normal group *p<0.05, **p<0.01, ***p<0.001.

RESULTS AND DISCUSSION: The pharmacognostic & phytochemical evaluation *Scoparia dulcis* Linn. was study macroscopic characters, microscopic powder characteristics contain present phloem fibre, covering trichome, cortex, calcium oxalate crystal, cutine layer, cuticle (pink color after staining) epidermis cell etc. & physicochemical evaluation.

Qualitative identification tests presence of many constituents like carbohydrate, sterol & triterpenoids, glycoside, flavonoids, tannins, etc. in alcohol and water extract.

In nephroprotective activity alcohol and water extract of *Scoparia dulcis* Linn. were performed by using gentamicin induced nephrotoxicity. The aerial parts shows prevent nephrotoxicity & the activity may due to the presence of flavonoids, steroids & triterpenes in *Scoparia dulcis* Linn plant.

Group-I is normal not changes in biochemical parameters with histological photograph of rat kidney showing normal glomeruli & tubules,

group-II is negative group Gentamicin 100 mg./kg. B.W./day, producing nephrotoxicity confirmed by decreased body weight & urine volume, increase kidney weight, serum creatinine, serum urea & serum uric acid with histological changes showing glomerular & peritubular congestion and inflammatory cells infiltration. Group III & IV is curative group give Gentamicin 100 mg./kg. B.W./day with alcohol and water extract 200 mg./kg. B.W./day shows the nephroprotective activity confirmed by significant increased body weight & urine volume, significant decreased kidney weight, serum creatinine, serum urea & serum uric acid (p<0.001).

TABLE 2: PARAMETERS STUDIED FOR THE NEPHROPROTECTIVE ACTIVITY OF THE ALCOHOL AND WATER EXTRACTS OF AERIAL PARTS OF SCOPARIA DULCIS LINN.

Parameters	Group-I	Group-II	Group-III	Group-IV
Body weight (gm)	173.00 ± 6.608	114.7 ± 3.159 ^{###}	168.7 ± 6.951 ^{***}	167.07 ± 8.466 ^{***}
Kidney weight (gm)	1.222 ± 0.033	1.697 ± 0.064 ^{###}	1.297 ± 0.037 ^{***}	1.355 ± 0.028 ^{***}
Urine volume (ml)	10.52 ± 0.852	7.900 ± 0.409 ^{###}	12.48 ± 0.817 ^{***}	12.72 ± 0.867 ^{**}
Serum creatinine (mg/dl)	1.067 ± 0.098	2.400 ± 0.141 ^{###}	1.283 ± 0.047 ^{***}	1.433 ± 0.084 ^{***}
Serum urea (mg/dl)	35.01 ± 2.697	148.0 ± 5.842 ^{###}	82.63 ± 5.727 ^{***}	92.048 ± 3.642 ^{***}
Uric acid (mg/dl)	4.300 ± 0.402	11.01 ± 0.366 ^{###}	6.103 ± 0.542 ^{***}	6.450 ± 0.275 ^{***}

The histological study of rat kidneys showing Group III normal glomerular cells, tubular cells and inflammatory cells, Group IV showing normal

blood vessel congestion, glomerular & peritubular congestion, inflammatory cells. All histological study rat kidney photograph shown in (Figure 1).

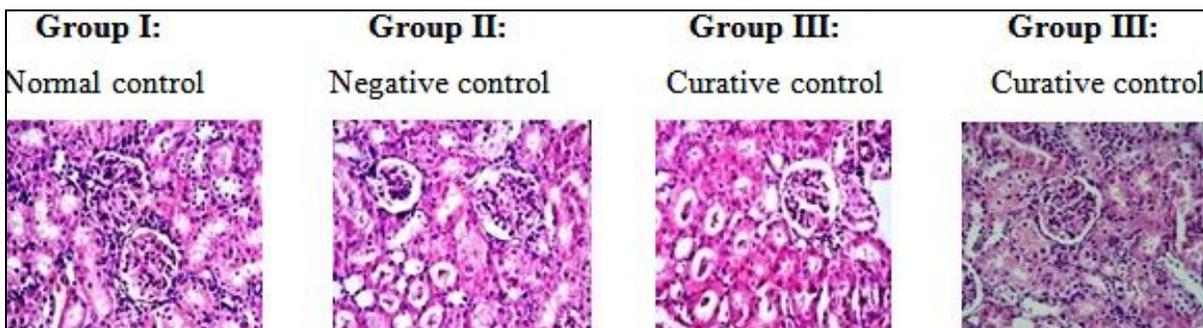


FIGURE 1: HISTOPATHOLOGICAL STUDY PHOTOGRAPH

These results suggest the therapeutic utility of herbal *Scoparia dulcis* Linn. aerial parts extracts in renal injury. The flavonoids, terpenes, steroids & tannins have a antioxidant & organ protective properties of it could be attributed to the presence of these constituents^{13, 17, 18}.

CONCLUSION: From the above findings it can be concluded that the alcohol and water extracts of *Scoparia dulcis* Linn. possess significant nephroprotective activity. These results support the ethno medical uses of *Scoparia dulcis* Linn. in the

treatment of Diabetes mellitus, hypertension and kidney disorders etc.

Further pharmacodynamic studies will help in understanding the precise mechanism of action of the drug.

ACKNOWLEDGEMENT: The author is thankful to Research guide Dr. K. P. Manjunath, Associate Professor & Head Department of Pharmacognosy, KLE University, College of Pharmacy, Hubli, Karnataka state for providing support for this study.

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How to cite this article:

Jedage HD: Pharmacognostic, phytochemical investigation & pharmacological evaluation of *Scoparia dulcis* Linn. plant extracts for nephroprotective activity. Int J Pharm Sci Res 2014; 5(8): 3342-46. doi: 10.13040/IJPSR.0975-8232.5(8).3342-46

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