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Nephroprotective activity of different extracts of *Biophytum sensitivum* (Linn.) DC

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Abstract
The present study was undertaken to evaluate nephroprotective activity of different extracts of whole plant of *Biophytum sensitivum* in Wistar albino rats. Randomly selected animals were divided into five groups of six animals each. The test extracts were administered orally at a dose of 200 mg/kg. Gentamicin was administered at a dose of 40 mg/kg i.p. to the rats for 7 days. On eighth day all the animals were sacrificed and blood was collected. Elevation of urea and creatinine level in the serum was taken as the index of nephrotoxicity. Histopathological examinations of kidneys of all the groups were carried out. The findings revealed that methanol and aqueous extracts of *Biophytum sensitivum* possesses nephroprotective activity. The elevations of serum urea and creatinine produced by Gentamicin were considerably reduced and showed histopathological changes in the kidneys to normal. The study concluded that *Biophytum sensitivum* possess nephroprotective activity.

Keywords: Gentamicin, nephroprotective activity, *Biophytum sensitivum*.

1. Introduction
Renal or kidney failure occurs when the excretory function of the kidney fails. The kidneys are unable to filter out metabolic waste products (creatinine and blood urea nitrogen). Some of the important nephrotoxic agents include heavy metals, antineoplastic agents, antimicrobial agents, aminoglycosides and miscellaneous products [1]. Gentamicin, an important aminoglycoside, is known to cause a number of morphologic, metabolic and functional alterations in the kidney and the specificity of Gentamicin nephrotoxicity is apparently related to its accumulation in the renal proximal convoluted tubules leading to tubular necrosis [2].
The present study was undertaken to evaluate the nephroprotectic potential of different extracts of whole plant of *Biophytum sensitivum* (Linn.) DC. (Family: Oxalidaceae) found in many parts of Africa and Asia including India. The plant grows up to maximum of 20 cm and possess unbranched woody erect stem. The leaves are abruptly pinnate, leaflets opposite, 6 to 12 pairs, and each leaflet is up to 1.5 cm long. The flowers are many and crowded normally yellow, white, or orange with red streak in the center of each of the five petals. Fruits are ellipsoid capsules which are shorter than the persistent calyx [3]. *B. sensitivum* is used traditionally in treating ailments which includes for chest complaints, cramps, inflammatory, tumors, burns, urinary calculi, wounds, gonorrhea, asthma, phthisis, stomachalgia, snake bite, diabetes, diuretic, tuberculosis, antiseptic in boils, gonorrhea, fevers and lithiasis [4-6]. *Biophytum sensitivum* is reported for its chemoprotective effect [7], hypoglycaemic [8], Radioprotective action [9], immunomodulatory, antitumor [10], antibacterial [11], antifertility [12], anti-inflammatory, antipyretic and analgesic activities [13]. Scientifically, there is no report on the nephroprotectic studies of *Biophytum sensitivum* so far, the objective of the present investigation is a systemic approach to explore the nephroprotectic effects of different extracts of *Biophytum sensitivum*.

2. Materials and Methods
2.1 Plant Collection and Authentication
The whole plant of *Biophytum sensitivum* were authenticated at Regional Medical Research Centre (ICMR), Belgaum, Karnataka (Accession number RMRC-588) which were collected from local areas of Goa.

2.2 Plant Extraction
The plants were then dried for two weeks under shade, then at room temperature and were subjected to size reduction with a crusher and then passed through sieve no. 40 to get uniform powder. Around 200 g of powdered plant material were subjected to extraction with various solvents such as petroleum ether (for the purpose of defatting),

chloroform and methanol by successive soxhlet method. The aqueous extract was subjected for maceration process. Each extract was then distilled to dryness under reduced pressure using Buchi rota evaporator to yield the respective dried extracts.

2.3 Animals

Male Wistar albino rats weighing between 150-200 g were obtained from P.E.S's Rajaram and Tarabai Bandekar College of Pharmacy, Ponda, Goa. Animals were housed into groups of six at a temperature of 25 ± 1^0C and relative humidity of 45-55 %. The Institutional Animal Ethical Committee approved the protocol of this study (Resolution number: PESRTBCOP/IAEC; clear/2013-14/R-10).

2.4 Drugs and chemicals used

All standard chemicals of analytical grade were used. Drugs used were Gentamicin injection I.P (Genticyn 80 mg/2 ml, Abbott) and the suspension of extracts (chloroform and methanol) were prepared by triturating accurately weighed quantity of the extract with 1% Tween 80 in a glass mortar, with gradual addition of distilled water, to make up the required volume.

2.5 Acute toxicity studies

Literature survey found that the plant extracts were administered in the dose of 200 mg/kg body weight in animals [13].

2.6 Gentamicin induced nephrotoxicity in rats

Animals were divided in total of five groups containing six animals in each group. On the day of experiment, the dosing was scheduled as follows: Group I served as control was given normal saline in the dose of 30ml/Kg, twice a day for seven days. Group II, III and IV received chloroform, methanol and aqueous plant extracts respectively along with Gentamicin in the dose of 40 mg/kg, twice a day for seven days. Group V animals were treated with only Gentamicin in the dose of 40 mg/kg, twice a day for seven days. Gentamicin was administered by i.p. route. On eighth day 12 h after the drug/vehicle administration all the animals were sacrificed by over dosing of anesthetic ether and blood was collected by cervical decapitation. Serum was separated from the blood and the level of urea and creatinine were estimated. Elevation of urea and creatinine level in the serum was taken as the index of nephrotoxicity [14].

2.7 Histopathological examination of rat kidneys

After all the animals were sacrificed, rat kidneys were dissected. The kidneys were rinsed in normal saline. Small piece of kidney from each group were preserved in 10% formalin and stained with Ehrlich's hematoxylin and sections were taken from them. The sections were evaluated for the pathological symptoms of nephrotoxicity.

3. Statistical analysis

All data are analyzed by one way analysis of variance test (ANOVA), followed by dunnet's multiple comparison test.

4. Results and discussion

The present study indicates pharmacological evaluation of nephroprotective action of different extracts of *Biophytum sensitivum* and in each case the dose of the extract administrated was 200 mg/kg body weight. Gentamicin (GM) which is widely used aminoglycoside antibiotic, is recognized as possessing significant nephrotoxic potential in man and experimental animals [15]. GM induced nephrotoxicity is characterized by elevated levels of urea and creatinine in plasma as well as urine, uric acid severe proximal tubular necrosis, renal failure [16, 17]. Methanol and aqueous extract of *Biophytum sensitivum* supplementation to GM treated rats recorded significantly decrement in levels of urea and creatinine. Methanol extract showed significant decrease in uric acid level, and chloroform and aqueous extract showed marked decrease in levels of uric acid compared to GM induced rats. GM administration to control rats produced a typical pattern of nephrotoxicity which was manifested by marked increase in serum creatinine and BUN accompanied by significant decrease in serum glucose, inorganic phosphate (Pi) and increase in serum cholesterol, phospholipids [18]. Methanol, aqueous and chloroform extract of *Biophytum sensitivum* showed very less marked decrease in the levels of blood urea compared to control. These observations indicate at an improved renal function by methanol and aqueous extract than chloroform extract as shown in table 1. Decrement in activity levels of renal SOD and CAT after GM treatment suppresses endogenous enzymatic antioxidant machinery [19]. Treatment with methanol and aqueous extract of *Biophytum sensitivum* increased in activity levels of SOD and CAT significantly compared to gentamycin induced rats. GSH depletion is linked to a number of diseases including cancer, neurodegenerative diseases, kidney and cardiovascular diseases [20]. It has been observed that, there is decreased in GSH level in gentamicin induced rats but treatment with methanol and aqueous extract of *Biophytum sensitivum* increased the GSH level for the non-enzymatic antioxidant activity in rats. Also observed was decrement in renal enzymatic and non-enzymatic antioxidants coupled with increment in LPO in GM treated rats are indicative of compromised antioxidant machinery and a higher susceptibility towards oxidative damage [21]. However, *Biophytum sensitivum* lowered LPO levels recorded in methanol and aqueous extract of *Biophytum sensitivum* treated groups than chloroform extract that prevent membrane lipid peroxidation and subsequent depletion of enzymatic and non-enzymatic antioxidants during GM induced nephrotoxicity as shown in table 2. Normal rats showed prominent critical tubules and bowman's capsules and showing normal glomerular structure (Fig. A). Gentamicin induced rats produced renal tubular damage, tubular dilatation, and glomerular atrophy interruption in the basement membrane around the necrotic tubules and narrowing of the Bowman's space (Fig. B). Figures C, D, and E indicate treatment with methanol, aqueous and chloroform extracts of *Biophytum sensitivum* showing marked reduced in renal tubular and intracellular edema membrane, tubular dilatation, and glomerular atrophy when compared to GM induced group respectively. Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, carbohydrates, flavonoids, tannins and phenolic compounds. As per the findings, the secondary metabolite, flavonoids is present in the plant which is antioxidant in nature [22]. This may be responsible for kidney protective activity. The results show that *Biophytum sensitivum* protects against Gentamicin induced kidney injury.

Table 1: Parameters of nephroprotective activity of different extracts of *Biophytum sensitivum*

Parameters	Normal	Gentamicin	Gentamicin + Chloroform extract	Gentamicin + Methanol extract	Gentamicin + Aqueous extract
Serum urea (mg/dl)	60.61±4.768	139.2±1.246 [a]	137.6±2.191[ns]	77.26±2.746***	93.74±4.075***
Serum Creatinine (mg/dl)	1.923±0.2221	8.350±0.02595 [a]	6.153±0.5978*	4.763±0.7592***	5.018±0.5192***
BUN (mg/dl)	35.91±0.1282	132.1±0.4280 [a]	118.2±4.316 [ns]	102.0±12.55*	102.8±5.614*
Uric acid (mg/dl)	2.075±0.007638	16.00±0.02720 [a]	13.09±0.6088**	6.712±0.7926***	13.26±0.8784*

All values are expressed as mean ± SEM, (n = 6 animals) *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = Non-Significant. Superscripts represents a = *** $p < 0.0001$ as compare to control group by One Way Analysis of Variance Test (ANOVA) followed by Dunnett's Multiple Comparison Test.

Table 2: Effect of extracts of *Biophytum sensitivum* on renal LPO, SOD, CAT and GSH

Groups	LPO (U/mg protein)	SOD(U/mg protein)	CAT(U/mg protein)	GSH (U/mg protein)
Normal (Vehicle)	151.4±1.738	1377±13.67	7.523±0.05064	31.64±1.049
Gentamicin	471.5±1.760a	815.1±2.743a	4.002±0.007923a	13.06±0.7854a
Gentamicin + Chloroform extract	394.5±2.490ns	812.7±1.939ns	2.488±0.01682ns	21.97±0.6762ns
Gentamicin + Methanol extract	274.7±1.388***	1008±3.983***	3.208±0.01167**	40.69±0.7559***
Gentamicin + Aqueous extract	202.4±0.9593***	1275±3.897***	4.633±0.1136*	11.19±0.5883ns

All values are expressed as mean ± SEM, (n = 6 animals) *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = Non-Significant. Superscripts represents a = *** $p < 0.0001$ as compare to control group by One Way Analysis of Variance Test (ANOVA) followed by Dunnett's Multiple Comparison Test. LPO-Lipid peroxidation, SOD-Super oxide dismutase, CAT-Catalase, GSH-Glutathione.

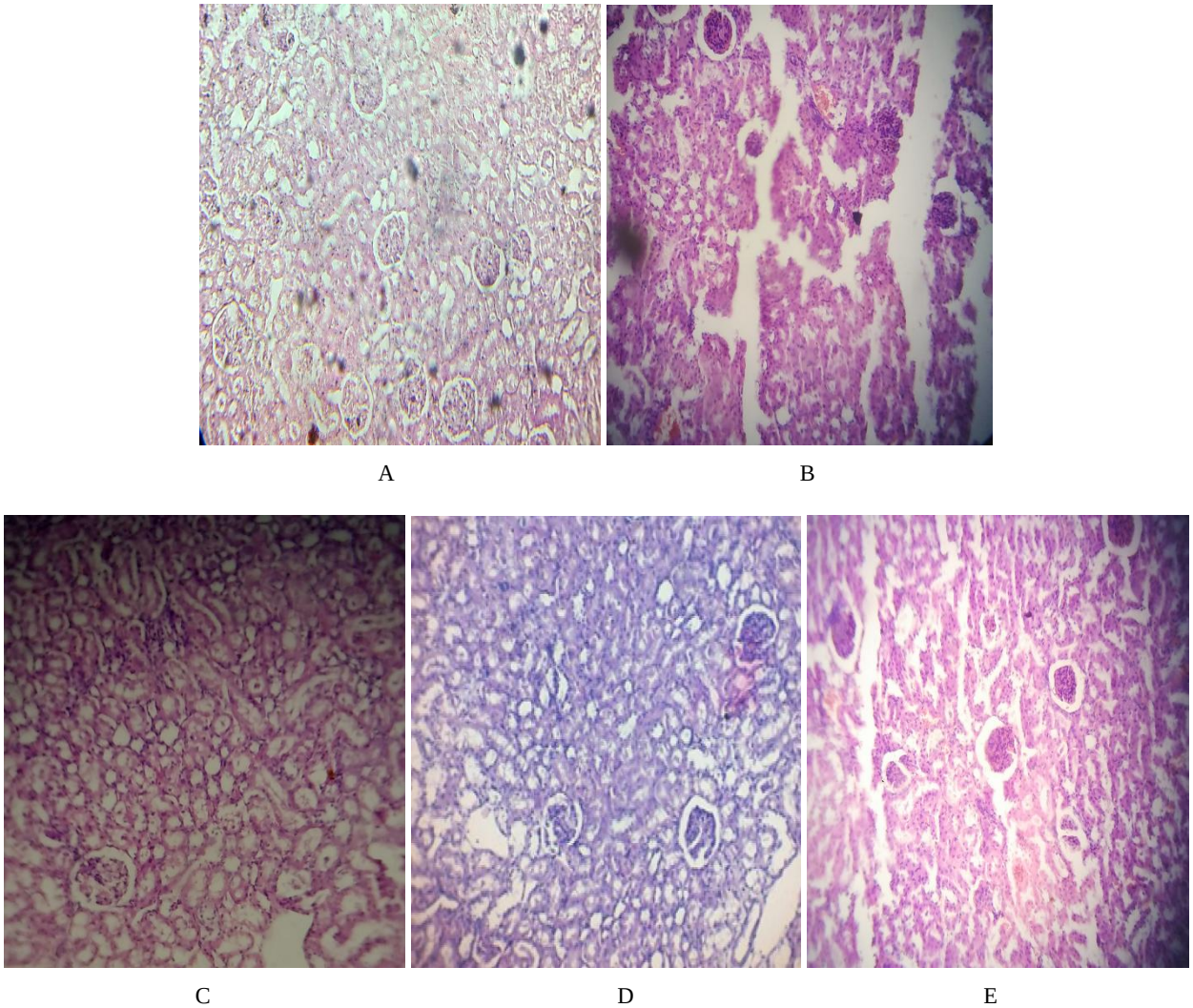

Fig 1: A: Normal Rat show prominent critical tubules and bowman's capsules and showing normal glomerular structure. B: Gentamicin induced rats produced renal tubular damage, tubular dilatation, and glomerular atrophy interruption in the basement membrane around the necrotic tubules and narrowing of the Bowman's space. C, D, and E: Treatment with methanol (C), aqueous (D) and chloroform (E) extracts of *Biophytum sensitivum* showing marked reduce in the renal tubular and intracellular edema membrane, tubular dilatation, and glomerular atrophy when compared to GM induced group.

5. Conclusion

The present study revealed that among the tested extracts of *Biophytum sensitivum* (Linn.) Skeels, aqueous and methanol extracts showed significant nephroprotective activity.

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7. References

1. Singh N, Mani TT. Review on nephroprotective activity of some medicinal plants. Pharmacology online, 2011; 3:1273-1289.
2. Pedraza-Chaverri J *et al*. Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidants. Free Radical Biology and Medicine, 2000; 29: 602-611.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. Edn International Book Distributors, Dehradun, 1999; 1(2):440-441.
4. Arya Vaidya Sala. Indian Medicinal Plants, Orient Longman Limited, Chennai, 2002; 1:271.
5. Vidyarthi RD. A Text book of Zoology. Edn 14, S. Chand and Co., New Delhi, 1967, 45.
6. Agarwal VS. Drug Plants of India. Edn 1, Kalyani Publishers, New Delhi, 1997; 1:222-223.
7. Guruvayoorappan C, Kuttan G. Evaluation of the chemoprotective effect of *Biophytum sensitivum* (L.) DC extract against cyclophosphamide induced toxicity in Swiss albino mice. Drug Metabolism and Drug Interactions, 2007; 22(2-3):131-150.
8. Puri D, Baral N. Hypoglycaemic effect of *Biophytum sensitivum* in the alloxan diabetic rabbits. Indian Journal of Physiology and Pharmacology. 1998; 42(3): 401-406.
9. Guruvayoorappan C, Kuttan G. Protective effect of *Biophytum sensitivum* (L.) DC on radiation-induced damage in mice. Immunopharmacology and Immunotoxicology. 2008; 30(4):815-835.
10. Guruvayoorappan C, Kuttan G. Immunomodulatory and antitumor activity of *Biophytum sensitivum* extract. Asian Pacific Journal of Cancer Prevention. 2007; 8(1):27-32.
11. Natarajan D, Shivakumar MS, Srinivasan R. Antibacterial activity of leaf extracts of *Biophytum sensitivum* (L.) DC. Journal of Pharmaceutical Sciences and Research. 2010; 2(11):717-720.
12. Johnson DB, Dineshkumar C, Arunkanth KR, Giles D, Gopal M, Hubert VG. Antifertility Activity of *Biophytum sensitivum*. Indian Drugs. 2003; 40(9):523-525.
13. Chatterjee TK, Mishra M, Pramanik KC, Bandyopadhyay D. Evaluation of Anti-inflammatory, Antipyretic and Analgesic properties of *Biophytum sensitivum* (L.) DC. Indian Drugs. 2008; 45(2):123-131.
14. Afzal M, Khan NA, Ghufran A, Iqbal A, Inamuddin M. Diuretic and Nephroprotective effect of Jawarish Zarooni Sada - a Poly herbal Unani formulation. Journal of Ethnopharmacology. 2004; 91:219-223.
15. Humes HD, Weinberg JM. Toxic nephropathies. In The kidney, edited by B.M. Brenner and F.C.Rector, Jr. Philadelphia, PA: Saunders, 1986, 1491-1532.
16. Erdem A *et al*. The protective effect of taurine against gentamicin induced acute tubular necrosis in rats. Nephrol Dial Transplant. 2009; 15:1175-1182.
17. Laskshmi BV, Sudhakar M. Protective effect of *Zingiber officinale* on gentimicin-induced nephrotoxicity in rats. International Journal of Pharmacology. 2010; 6(1):58-62.
18. Banday AA, Farooq N, Priyamvada S, Yusufi ANK, Khan F. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. Life Sciences. 2008; 82(9-10):450-459.
19. Yaman I, Balikci E. Protective effects of Nigella sativa against gentamicin-induced nephrotoxicity in rats. Experimental and Toxicologic Pathology. 2010; 62(2):183-190.
20. Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. Clinica Chimica Acta. 2003; 333(1):19-39.
21. Garg NK, Mangal S, Sahu T, Mehta A, Vyas SP, Tyagi RK. Evaluation of anti-apoptotic activity of different dietary antioxidants in renal cell carcinoma against hydrogen peroxide. Asian Pacific Journal of Tropical Biomedicine. 2011; 1(1):57-63.
22. Okokon JE, Nwafor PA, Noah K. Nephroprotective effect of *Croton zambesicus* root extract against gentimicin-induced kidney injury. Asian Pacific Journal of Tropical Biomedicine. 2011; 4(12):969-972.