International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.15, No.02, pp 83-88, 2022

Nephro-protective Activity of hydro alcoholic extract of D*esmodium triflorum* (L.) DC in Gentamicin induced Albino rats

Jeba Singh[1], Jaslin Edward[2], Danish[1], Dhanya[1], Linda Augustin[1], Nazrin[1], Roshmin Paul[1], Amjad[1], Bino Kingsley[1]*

[1]ELIMS College of Pharmacy, Thrissur-680631, Kerala, India.
[2]Cape Biolab and Research Centre, Marthandam, Tamil Nadu, India.

***Corres. Author: binokin1975@gmail.com**

Abstract: Aim of the study is to scientifically validate traditional nephro protective claim of *Desmodium triflorum* and to determine the therapeutic effect. The cold macerated hydro-alcoholic extract of whole plant of *Desmodium triflorum* was evaluated in gentamicin induced rats. The hydro - alcoholic extract showed promising nephro- protective activity at dose of 100, 200, 400 mg/kg. The hydro- alcoholic extract was devoid of any acute toxic symptoms even at 2000mg/kg dose. The hydro- alcoholic extract of *Desmodium triflorum* is an attractive material for further studies leading to the development of nephro-protective phyto-medicine or conventional pure entity medicine.
Keywords : *Desmodium triflorum* (L.) DC, Nephro-protective, Gentamicin induced rats.

INTRODUCTION

Nephrotoxicity is a renal disease that arises as a result of exposure to industrial or environmental toxic chemicals or medicines and nephro-toxins displaying nephrotoxicity. Some medications can damage kidneys when administered at high concentration or over an extended period of time. In the recent years many researchers have examined the effects of plants traditionally used by indigenous healers and herbalists in treatment of renal diseases[1]. In modern medicine ACE-inhibitors and ARBs are mainly used to induce reno-protection; however, these effects, rather they are mainly effective in nephropathies associated with blood pressure and diabetes etc[2]. Drug-induced nephrotoxicity is an extremely common condition and is responsible for a variety of pathological effects on the kidneys[3]. Nephrotoxicity most commonly affects tubule-interstitial compartment and manifests either acute tubular injury (ATI) or acute interstitial nephritis (AIN). There is a

DOI: http://dx.doi.org/10.20902/IJPTR.2022.150208

growing incidence of drug-induced glomerular disease, including direct cellular injury and immune-mediated injury. However, kidney disease does not develop in all patients exposed to the various potential nephro-toxins. The nephrotoxicity of medications, drugs, or other ingested substances is a complicated process that involves a combination of factors[4]. Potential nephrotoxic effect of the drug, comorbid diseases or conditions (underlying renal dysfunction, cardiovascular disease, diabetes, immunologic diseases, sepsis, etc.), genetic determinants of drug metabolism and transport, immune response genes, drug dose and duration of therapy, drug characteristics (solubility, structure and charge), combinations of potential nephrotoxic drugs, urine pH, metabolic disturbances, older age (>65 year), and female sex are the common risk factors for drug-induced nephrotoxicity agents are neither the drugs of choice for this purpose nor can be used exclusively to produce reno- protective associated with blood pressure and diabetes etc[5].

The important advantage claimed for therapeutic uses of medicinal plants in various ailments is they are safe and economical. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice. Kerala which has a rich culture of practice of traditional system of medicine like Ayurveda and siddha promoted use of herbal drugs even during corona pandemic[6]. Approximately, 19 million adults have chronic kidney disease and an estimated 80,000 persons have chronic kidney failure diagnosed annually in India. Till date for end stage renal failure, renal replacement is the only therapy[7]. The frequency of drug induced nephrotoxicity is approximately 14-26% in adult populations as detailed in previous prospective cohort studies is increasing worldwide[8,9].

MATERIALS AND METHODS

Collection and authentication of plant materials

Whole plant of *Desmodium triflorum* were collected from Thrissur, Kerala. The plant was identified and authenticated by Dr. V. B. Sreekumar, Senior Scientist, Forest Botany Dept., Kerala Forest Research Institute (KFRI), Thrissur, Kerala and a voucher specimen was deposited in the herbarium at KFRI.

Preparation of extract

The whole plant of *Desmodium triflorum* was collected, cleaned, dried and powdered. To prepare hydro-alcoholic extract 50g of powder was extracted with 275ml equimolar mixture of water and ethanol by stirring 1hr daily for 72 hrs and extract was filtered using a clean muslin cloth and the solvent was freeze dried in a lyophilizer[10].

Phytochemical and Separation of HAEDT

The hydro-alcoholic extract of *Desmodium triflorum* was subjected to preliminary phytochemical screening[11]. 10µl of HAEDT extract were applied on TLC plate using Camag's ATS4 applicator and developed by Toluene: ethyl acetate: formic acid mobile phase in the ratio (7.5:2.5:0.5v/v/v) up to a distance of 9 cm. Then the plate was dipped in 5% vanillin- sulphuric acid reagent followed by heating at 105℃ After development, the plate was photo documented using Camag's TLC Visualizer under UV 520 nm and then scanned using Camag's Scanner 4 at (D2 LAMP/ Absorption mode, Hg lamp/ Fluorescent mode) finger print profiles of the extract were documented[12,13].

Animals

30 inbred albino rats weighing 150 – 200g reared in Cape Bio lab and Research Centre were used for the experimentation. Animals were caged in uniform hygienic condition and fed with standard pellet diet and water *ad libitum* as per the guidelines of Institutional Animal Ethics Committee (IAEC). IAEC is approved by CPCSEA (Committee for Purpose of Control and Supervision on Experimental Animals).

Acute toxicity study

Healthy female albino rats weighing between 150-200g were taken forthe study and each group has 3 animals. Female albino rats are used for acute toxicity study since females are generally more sensitive between the

sexes. Acute toxicity study was performed according to OECD guidelines 423. Each animal was administered hydro-alcoholic extract of *Desmodium triflorum* orally as a single dose at different doses of 5, 50, 300 and 2000

mg/kg b.w. Animals were observed periodically for the symptoms of toxicity and death for every 2h within 24h and then daily for14 days[14,15].

Determination of efficacy of Hydro- alcoholic extract in Gentamicin induced nephrotoxic rats.

The Gentamicin induced rats were divided into 4 groups. The normal control rats received 1% solution of Sodim Carboxy Methyl Cellulose(0.5ml/day). negative control rats received intraperitoneal injection (i.p) of gentamicin only (100 mg/kg b. wt. /day) for 10 days. Other 3 groups received intraperitoneal injection (i.p) of gentamicin (100 mg/kg b. wt. /day) concurrently with hydro-alcoholic extract of Desmodium triflorum (100, 200 and 400 mg/kg b. wt. /day) orally for 10 days. On 11th day animals were anesthetised for blood collection and sacrificed after blood collection and kidney samples were removed for histopathological studies[16,17,18].

Estimation of serum biochemical parameters

The serum was rapidly separated, processed and subjected to biochemical analysis by ERBA diagnostic kit for the following parameters like urea, uric acid and creatinine.

Histopathological studies

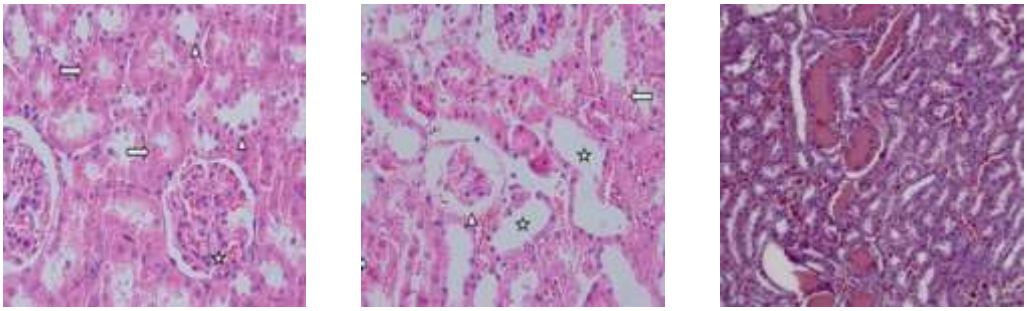
The kidneys were longitudinally sectioned into two halves, after rinsing in normal saline and kept in 10% formalin, dehydrated in gradual ethanol, cleared in xylene, and embedded in paraffin wax. The 5–6 μm sections were prepared using a microtome and stained with hematoxylin and eosin dye for microscopic observation of the histopathological changes.

RESULTS

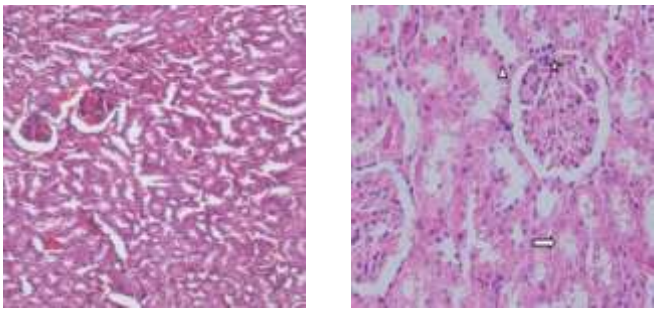
Acute toxicity studies shows no mortality or morbidity observed in animals through the 14 days period following single oral administration at all selected dose levels of hydro-alcoholic extract of *Desmodium triflorum*. The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such as fur, skin, eyes and nose were appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self-mutilation, walking backward and so forth were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal. The elevated levels of urea, creatinine and uric acid seen in gentamicin induced animals were marginally reduced by the hydro-alcoholic extract of *Desmodium triflorum* at a dose of 100, 200 and 400 mg/kg (Table 1). Histopathological studies of kidneys were performed after 10 days of continuous drug treatment and untreated animals (Figure 2. a,b,c,d,e) shows the images of kidney normal control, negative control and animals treated with hydro-alcoholic extract of *Desmodium triflorum*. Normal control group shows normal histological structure of renal parenchyma with renal glomeruli and tubules. Gentamicin treated group shows congestion of renal blood vessels and focal necrosis forming desquamated, degenerated renal glomeruli and tubules. The drug treated group 100 mg/kg shows slight congestion but less degeneration. 200 mg/kg received group shows cells get squamated and protected from degeneration. 400mg/kg dose shows cells get protected from necrosis and desquamation similar to normal kidney. Histopathological study shows nephro- protective effect. Phytochemical analysis shows the presence of Alkaloids, Phenols, Flavonoids, Proteins, Quinones, Saponins, Steroids, Tannin, Tri-terpenoids, Glycosides, Reducing sugar and Coumarins. Hydro-alcoholic extract of *Desmodium triflorum* was resolved into 18 components on TLC and HPTLC (Figure 3 a & b). The Rf value with 0.89 gave the highest % area of 23.16 followed by 0.06 which gave 14.07% area.

Table 1: Effect of HAEDT on Renal parameters

GROUPS	TREATMENT	UREA (mg/dl)	URIC ACID (mg/dl)	CREATININE (mg/dl)
Group I	Solvent control [1% SCMC]	19.2	3.8	0.2
Group II	Negative control [Gentamicin only]	39.86	7.78	7.9
Group III	Gentamicin + 100 mg/kg of HAEDT	28.08	4.23	0.94
Group IV	Gentamicin + 200 mg/kg of HAEDT	24.26	3.94	0.68
Group V	Gentamicin + 400 mg/kg of HAEDT	21.76	3.82	0.38

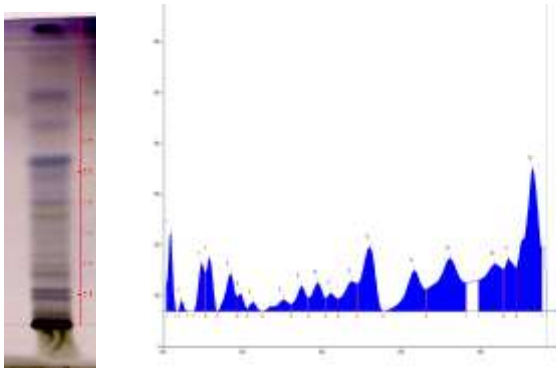


a: control group b: negative control group c: Drug treated (100mg/kg)



d: Drug treated (200 mg /kg) e: Drug treated (400mg/kg)

Figure 2 Histopathology of kidney



(a) (b)

Figure 3 (a & b) TLC and HPTLC Chromatogram at 520 nm:

Figure 3 (a) Thin layer chromatographic separation of hydro alcoholic extract from *D. triflorum* using silica gel 60 F_{254} and the solvent system Toluene:ethyl acetate: formic acid (7.5:2.5:0.5v/v/v). (b) HPTLC profile showing 18 peaks at 520nm

DISCUSSION

Acute toxicity studies show there was no mortality or morbidity up to 2000 mg/kg body weight. The results of nephro-protective study carried out by Gentamicin induction and evaluation of renal parameters like urea, uric acid and creatinine revealed that Group II animals (negative control) challenged only with Gentamicin showed a drastic increase in all the parameters when compared to Group I solvent control animals. In Group III, IV & V animals the renal parameters were very moderately altered. The histopathological evidence also revealed almost a normal architecture like Group I animals, In Group III – V when compared to Group II animals where a significant damage was noted. In conclusion the hydro-alcoholic extract from *Desmodium triflorum* showed nephro-protective activity against Gentamicin induced rats. The hydro-alcoholic extract is very promising for further studies leading to the development of valuable medicine for monotherapy or combination therapy for renal dysfunction.

REFERENCES

1. Hussain T, Gupta RK, Sweety K. Nephroprotective activity of Solanum xanthocarpum fruit extract against gentamicin–induced nephrotoxicity and renal dysfunction in experimental rodents. Asian Pac J Trop Med., 2012, 5; 686-91.
2. Nieren B W. Clinical Pharmacology. McGraw-Hill MedicalPublishing Division, New Delhi., 2000, 4; 79-82
3. Perazella MA. Pharmacology behind common drug nephrotoxicities. Clinical Journal of the American Society of Nephrology., 2018, 13: 1897-1908. DOI: 10.2215/CJN.00150118
4. Dhodi DK. Drug-induced nephrotoxicity. International Journal of Basic & Clinical Pharmacology. 2014, 3; 591-597.
5. Paueksakon P, Fogo AB. Drug-induced nephropathies. Histopathology., 2017, 70; 94-108. DOI: 10.1111/his.13064.
6. Al-Naimi MS, Rasheed HA, Hussien NR. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharma Tech Res., 2019, 10; 95.
7. Sundararajan R, Bharampuram A, Koduru R. A review on phytoconstituents for nephroprotective activity. Pharmacophore. 2014, 5; 160-82.
8. Mansoor M, Brahmini CS, Rao SD. Phytochemical and nephroprotective activity of Ginkgo biloba against gentamicin induced nephrotoxicity in rats. Int. J. Adv. Pharm. Med. Bioallied Sci., 2015, 3; 98-101.

9. Ahmad QZ, Jahan N, Ahmad G. et al., An appraisal of nephroprotection and the scope of natural products in combating renal disorders. J Nephrol Ther., 2014, 4; 170.
10. Karim R, Begum MM, Jui Y. et al., In-vitro cytotoxic and anti-vibrio cholerae activities of alcoholic extracts of *Desmodium triflorum* (L.) whole plant and *Terminalia citrina* (Roxb.) fruits. Clin Phytosci., 2021, 7; 2-10.
11. Sarker, Satyajit D., Zahid Latif, and Alexander I. Gray. "Natural product isolation." *Natural products isolation*. Humana press., 2006, 1-25.
12. Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (airy shaw) from Brunei Darussalam. Journal of King Saud University Science., 2013, 27; 224-232.
13. Wagner H, Baldt S. Plant drug analysis-a thin layer chromatography. Atlas. Second edn. Germany: Springer., 1996.
14. CPCSEA Guidelines, Govt of India.
15. OECD Guidelines for testing of chemicals: Acute Oral Toxicity 2001; 423
16. Azab AE, Fetouh FA, Albasha MO. Nephro-protective effects of curcumin, rosemary and propolis against gentamicin induced toxicity in guinea pigs: Morphological and biochemical study. Am J Clin Exp Med., 2014, 2; 28-35.
17. Aiswarya N, Chandran V, Teerthanath S. Nephroprotective effect of aqueous extract of *Pimpinella anisum* in gentamicin induced nephrotoxicity in wistar rats. Pharmacognosy Journal., 2018, 10.
18. Padmini MP, Kumar JV. An experimental study of biochemical and histopathological study on gentamycin induced renal failure in albino rat and the effectiveness of punarnava (*Boerhaevia Diffusa*) on reversal of renal damage. Nephrol Dial Transplant., 1994, 9; 135-40.

*****