Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 2835-2837
Received: 11-01-2018
Accepted: 12-02-2018

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Nephroprotective activity of *Barleria longiflora* L. (Acanthaceae) against gentamicin induced nephrotoxicity in male albino wistar rats

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Abstract
Now a day's acute renal failures and chronic renal failure are common and serious problems. Number of therapeutic proxies affects kidney ensuing acute renal failure. Herbal medicine has arisen as a skilled approach with serviceable values in treating various diseases and developing an affordable phytotherapy to treat severe kidney diseases. Most of Barleria species reveals a wide range of phytoconstituents and are potent in anti-inflamatory, analgestic, antileukemic, antitumor, antihyperglycemic, anti-amoebic, virudal, antidiabetic, antifertility, hepatoprotective, nephroprotective and antibiotic activities. The objective of this study is to endorse the nephroprotective activity of ethanol extract of *Barleria longiflora* against gentamicin induced nephrotoxicity in rats. Nephroprotective activity was assessed by determination of serum marker levels and kidney antioxidant markers. The drug was administered intraperitonialy at a dose of 100 mg/kg b.w. of gentamicin for 8 consecutive days. These increased levels of serum creatinine, blood urea, uric acid and extent of renal damage were decreased by the ethanol extract of *Barleria longiflora*.

Keywords: *Barleria longiflora*, nephroprotective, kidney, traditional medicine

Introduction
Kidney disease is the ninth leading cause of death. 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 (Cordeiro *et al.*, 2011) [3]. Acute renal failure is reversible loss of kidney function whereas chronic renal failure is irreversible loss of kidney function (Movaliya *et al*., 2011) [8]. Drugs, diagnostic agents, chemical reagents and heavy metals are well known to be nephrotoxic (Mohana Lakshmi *et al*., 2012) [7]. Nephrotoxicity is one of the major kidney problems caused by drug or toxin. The use of herbs as nephroprotective is a major opportunity in Indian perspectives particularly for treating kidney damage (Porter and Bennett, 1981) [10].
Barleria longiflora L. consists of Acanthaceae family. Most of the *Barleria* species are potent anti inflamatory, analgestic, antileukemic, antitumor, antihyperglycemic, anti-amoebic, virudal and antibiotic (Jassim and Naji, 2003; Suba *et al*., 2004; Suba *et al*., 2005) [5, 12-13]. *Barleria longiflora* L. Leaf and root used to treat cough, inflammations, dropsy and kidney stone problems (Madhava Chetty *et al*., 2008). Root given in stricture dropsy and stone (Chopra *et al*., 1999) [2].

Materials and Methods
Mature and healthy plants were collected from Kanyakumari and thirunelveli District after the rainy season (January, February and March). The specimens were authentified by Dr. M. Padma Soma Subramanian, Research Officer (Scientist-II) in Botany, (CCRS, Govt.of India), Mettur Dam. Voucher specimens of the collections are deposited at the Herbarium of Medicinal Plants Garden, Mettur Dam, Tamil Nadu, India.

Botanical Name	: *Barleria longiflora* L.
Family	: Acanthaceae
Collected Locality	: Muppanthal Village, Kanyakumari District, South India.
Altitude	: 75m MSL
Synonym	: *Hygrophila auriculata* (Schumach.) Heine
Common name	: Kannada – Mullanacola, Neeruppigida Marathi – Lamb koranti, Oriya – Suryabbiya, Sanskrit – Adyanda, Telgu – Pinnagoninta.

Preparation of plant leaf extracts
Fresh healthy plants of *Barleria longiflora* (Plate: 1) were collected with root and shade dried

for 8-10 days and grinded into powder. The air-dried and powdered plant materials were taken in different amber coloured bottles, 100g dry powder sample was extracted with 80 % ethanol at 55ºC for 24 hours in soxhlet apparatus. Solvent elimination was done at room temperature and stored.

In vivo Nephroprotective Activity

The experimental protocol was carried out according to the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institutional Ethical Committee (Approval number: ML-EA-CPCSEA/05-2015/17). Throughout the experiments, human care is provided to the animals according to the criteria outlined in the "Guide for the Care and Use of laboratory Animals" prepared by the national Academy of Sciences.

Experimental animals

Male albino Wistar rats (150-200 g) used in the present study were procured from the Small Animals Breeding Station, Mannuthy, Kerala, India. They were housed in polypropylene cages (38 x 23 x 10cm) with not more than six animals per cage and maintained under standard environmental conditions (14h dark /10h light cycles; temp 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water ad libitum. The animals were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted over night before the experimental schedule, but have free access for water *adlibitum*.

Gentamicin induced Nephrotoxicity (Yaman and Balikci, 2010) [15]

Animals were randomly divided into four groups with 6 animals each. Group I animals were normal control group. Groups II-IV was intraperitoneally injected with 100 mg/kg b.wt. of gentamicin for 8 consecutive days. Groups three and four were orally co-administered with ethanol extract of sample *Barleria longiflora* (200 and 400 mg/kg b.w., respectively). Gentamicin was injected exactly one hour after the treatments were given. At the end of the experimental regime, all animals were anaesthetized with ether; blood samples were collected by cardiac puncture and were allowed to clot for 20 min after which it was centrifuged at 2500 rpm for 15 min at 4°C. The separated serum samples were used for measuring the level of urea, uric acid creatinine using commercially available test diagnostic kits and their manual (Beacon Diagnostic P. Ltd., India). The kidneys were immediately removed, weighed and homogenized in Tris Hcl buffer (pH – 7.4) to give a 10% homogenate and used for measuring superoxide dismutase (Marklund and Marklund, 1974) [6], catalase (Sinha, 1972) [11], and lipid peroxidation (Niehius and Samuelsson, 1968) [9].

Results and Discussion

In vivo nephrotoxicity studies

The ethanol extract of *Barleria longiflora* was evaluated for its nephroprotective activity using rat model. Gentamycin is an extensively used aminoglycoside antibiotic. It has been reported to produce nephrotoxicity even at normal therapeutic dose level. Gentamycin Nephrotoxicity occurs in about 15-30% of treated subjects, is manifested clinically as non-oliguric renal failure, with a slow rise in serum creatinine and hypoosmolar urinary output developing after several days of treatment (Abdel-Zaher *et al*., 2008) [1]. Gentamicin is filtered through glomeruli into tubular urine, that binds with anionic phospholipids, such as phosphatidylinositol or phospholipdylserine, in brush border membrane of proximal tubular cells reabsorbed actively via pinocytosis process into tubular cells, taken up by lysosomes and thereafter produces phospholipidosis (Hu *et al*., 1996) [4]. The drug enters into the cells by adsorptive/receptor mediated endocytosis after binding to acidic phospholipids and megalin and is found essentially in 0lysosomes. Animals treated with low, therapeutically relevant doses of aminoglycosides show both lysosomal phospholipidosis and apoptosis in proximal tubular cells (Suzuki *et al*., 1995) [14]. Nephroprotective activity was assessed by determination of serum marker levels and kidney antioxidant markers. This study shows that single injection of gentamycin in rats resulted in deterioration of renal function as indicated by elevation in creatinine, blood urea and uric acid. The drug was administered intraperitonialy at a dose of 100 mg/kg b.w. of gentamicin for 8 consecutive days. These increased levels of serum creatinine, blood urea, uric acid and extent of renal damage were decreased by the ethanol extract of *Barleria longiflora* (Table: 1) at both dose levels that is 200 and 400 mg/kg body weight in rats. This study proposed that the *Barleria longiflora* possess significant nephroprotective activity.

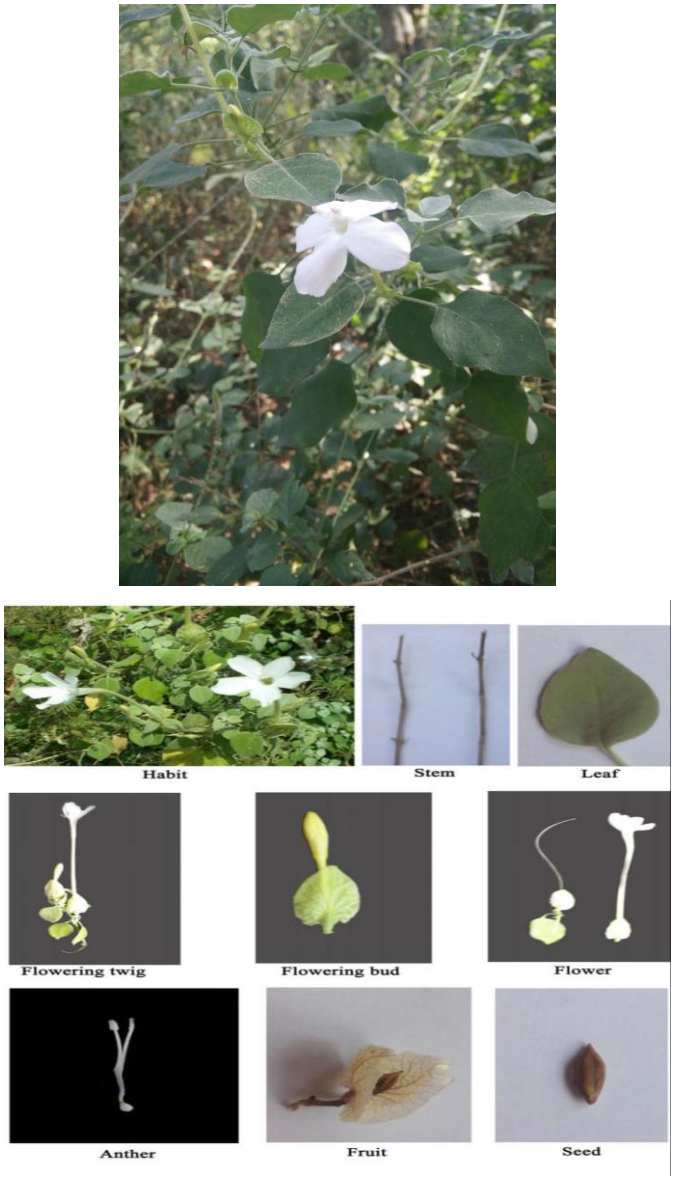

Plate 1: Habit and morphology of *Barleria longiflora*

Table 1: Effect of ethanol extract of *Barleria longiflora* L. sample on serum markers against gentamicin induced nephrotoxicity.

Animals	Parameters	Dose (mg/kg b.wt)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group I	Control	-	28.33 ± 1.80	1.49 ± 0.09	0.15 ± 0.03
Group II	Gentamicin (G)	100 mg/kg	53.66 ± 1.91	3.72 ± 0.25	0.78 ± 0.29
Group III	G + sample low dose	200 mg/kg	50.74 ± 2.99	2.72 ± 0.40	0.65 ± 0.07
Group IV	G + sample High dose	400 mg/kg	37.50 ± 2.96	1.83 ± 0.24	0.28 ± 0.02

Values are expressed as mean ± SEM (n=6).

Table 2: Effect of the *Barleria longiflora* L. on kidney antioxidant markers against gentamicin induced nephrotoxicity.

Animals	Parameters	Dose (mg/kg b.wt)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group I	Control	-	0.81 ± 0.02	19.38 ± 0.92	17.30 ± 1.13
Group II	Gentamicin (G)	100 mg/kg	0.55 ± 0.01	10.92 ± 1.64	32.94 ± 3.68
Group III	G + sample low dose	200 mg/kg	0.60 ± 0.04	13.92 ± 0.86	26.18 ± 3.01
Group IV	G + sample High dose	400 mg/kg	0.73 ± 0.02	16.51 ± 1.46	19.59 ± 2.39

Values are expressed as mean ± SEM (n=6).
SOD – Superoxide dismutase (Units/min/mg protein)
CAT – Catalase (μ moles of H_2O_2 consumed/min/mg protein)
LPO – Lipid Peroxidation (μ moles/mg protein)

Conclusion

The ethanol extract of selected plant protected gentamycin–induced renal damage in rats. From this study, it is clear that the selected medicinal plant play a prominent role against various diseases. The medicinal plant extracts have been reported for its significant nephroprotective activity in animal models. The nephroprotective activity is probably due to the presence of flavanoids in all the few medicinal plants. The results of this study indicate that extracts of plants *Barleria longiflora* have good potentials for use in kidney damage. Hence, this study is concluded that these herbal plants possess nephroprotective activity and it has been proven by different animal models which gives many links to develop the future trials. The results suggest the validity of the traditional claim of this plant.

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