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Pharmacological Evaluation of *Erythrina variegata* Linn (var. *alba*) for Organ Protective Activity

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Abstract: Organ toxicity and gastric ulcer generation is due to the long use of certain classes of life saving drugs. The conventional drugs used in the treatment of kidneys, liver and diseased conditions are sometimes inadequate and can have serious side effects. Therefore, it is necessary to search for alternative drugs for the treatment of these organ toxic conditions. The plant *Erythrina variegata* Linn var. *alba* (Family: Fabaceae) has not been studied for confirming its role as nephroprotective and anti-ulcer activity. The current research work was performed to evaluate the nephroprotective and anti-ulcer activity of *Erythrina variegata* (EV). In nephroprotective study, 70% methanolic extract of EV was tested against gentamicin induced nephrotoxicity in rat model. The degree of protection was assessed by blood urea nitrogen test (BUN), serum creatinine test (Scr) and initial and final body weight (b wt) of rats. Further, histopathology of rats' kidneys was also studied. Anti-ulcer property of 70% methanolic extract of EV was studied by pylorus ligation method in rats and ranitidine was used as standard drug. Results of nephroprotective study (BUN, Scr and b wt) revealed that 70% methanolic extract of EV (100 mg/kg and 200 mg/kg b wt) has markedly reduced the severity of gentamicin induced nephrotoxicity in dose dependent manner. In histopathology, the tubular necrosis was restored to normal. With 70% methanolic extract of 100 mg/kg b wt of EV treatment, the mild peritubular congestion was persisted and 70% methanolic extract of 200 mg/kg, b wt of EV treated rats have shown good protection against gentamicin induced nephrotoxicity. The anti-ulcer study revealed that 70% methanolic extract of EV (100 mg/kg and 200 mg/kg, b wt) had shown significantly reduced total gastric volume, increased gastric acid pH, decreased ulcer index, and overall reduced total acidity in tested rats as compared to standard group.

Keywords: Nephroprotective, Anti-ulcer, Serum creatinine, Blood urea nitrogen, Gastric pH, Ulcer index

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Introduction

The kidneys are among the most vital organs of the human body. The kidneys produce urine by

removing toxic waste products and excess water from the body. Malfunctioning of kidneys can lead

to serious illness or even death. Gentamicin is an effective aminoglycoside antibiotic that is widely prescribed drug for treating patients with infections, but its associated adverse effects of oxidative stress and kidney injury limits its long-term clinical use. The onset of renal failure is usually slower and the daily rise of serum creatinine tends to be lower than other causes of acute renal failure. Scr, BUN characteristically increase 7-10 days after initiation of aminoglycoside therapy. In more than half of the cases with nephrotoxicity, the decline in renal function occurs after the therapy has been completed for long duration (Udupa and Prakash, 2019).

Peptic ulcer disease is one of the common gastrointestinal disorders in clinical practice. The common forms of peptic ulcer are duodenal ulcer, gastric ulcer, non-steroidal anti-inflammatory drugs (NSAID'S) induced ulcer and stress ulcer. Gastric ulcer occurs commonly at old age and lower socio-economic classes of individuals. Many synthetic drugs are used for the treatment of peptic ulcers which cause various adverse effects. Hence, herbal sources of medicines stand out as being exceptional for its ethnic, ethno botanical and ethno pharmacological use (Bhousmik *et al.*, 2017).

Lipid peroxidation and generation of free radicals may occur in gentamicin administration which is highly toxic to tissues (Ramasammy *et al.*, 1985).

Synthetic antioxidants and free radical scavengers like cyclo-dextrin sulphates (Kanato *et al.*, 1992) and polyaspartic acid (Gilbert *et al.*, 1989) have been found to partially reduce gentamicin induced renal damage.

Search for natural nephroprotective drugs as alternative to synthetic drugs has been found in the form of medicinal plants which are enriched with bioflavonoid having antioxidant property. Many investigators have turned to simpler experimental models for studying drugs protective and response of the organs like kidney and liver

cells by potentially toxic agents. Conventional drugs used in the treatment of kidney, liver and other diseased conditions are sometimes inadequate and can have serious side effects (Bakhtiary *et al.*, 2012).

Therefore, it is necessary to search for alternative safe drugs for the treatment of these organ toxic conditions. In the absence of a reliable organ protective drug in modern medicine, there are a number of medicinal preparations in ayurveda recommended for the treatment due to severe undesirable side effects of synthetic agents. There is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess nephroprotective and antiulcer activity.

The plant EV is distributed throughout India in deciduous forest as well as cultivated. The plant is medium sized quick growing tree approximately 18 m high armed with dark coloured prickles bark, smooth shiny papery leaves with trifoliolate, leaflet 10-15 cm long ovate (Warrier, 1994). The stem bark of the plant EV was reported to possess lignans, phenolic compounds and related flavonoids which are known antioxidants properties (Shahriar *et al.*, 2015). The different parts of EV have been used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. In some experiments, it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds (Kumar *et al.*, 2010). This study was performed to evaluate the protective effect of EV in gentamicin induced nephrotoxicity and pyloric ligation induced gastric ulcer in rats.

Materials and Methods

Plant drug collection and extraction:

The stem bark of EV was collected from the surrounding places of Kalaburagi and authenticated by Post-Graduate Department of Botany, H.K.E Society's VG Women's Degree College, Kalaburagi where a voucher specimen

was deposited. The bark was shade dried at room temperature and pulverized. The bark powder was subjected to Soxhlet extraction using methanol (70%) for 6 h. The solvent extracts were concentrated and stored in desiccators until use in the experiments (Khandelwal, 1996).

Experimental animals:

The study was conducted on Wister albino rats weighing 200-250 g and mice 20-25 g of either sex. The animals were procured from Veterinary College, Bidar, Karnataka and fed with standard feed and water was given *ad libitum* under strict hygienic conditions. CPCSEA ethical clearance for handling the animals was obtained from Institutional Animal's Ethical Committee approved by the IAEC of HKES MTRIPS Gulbarga (1948/PO/Re/S/17/CPCSEA-23-02-2017) certificate dated 24-02-2020 prior to this research work.

Determination of acute toxicity (LD₅₀):

The acute toxicity of EV was studied on mice, which was maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No.420 now 425) method of CPCSEA (now CCSEA) was adopted for toxicity studies. Since no mortality was observed at 2000mg/kg. It was thought that 2000 mg/kg was the cut off dose. Therefore 1/10th and 1/20th i.e. 200 mg/kg and 100 mg/kg were selected for nephroprotective and anti-ulcer activities (Kadam and Gaykar, 2017).

Nephroprotective activity:

Four groups (six rats in each group) were selected for study, the group I was used as control (vehicle treated) and saline was given during course of study, group II animals received daily intraperitoneally (ip) gentamicin (80 mg/kg b wt) for 8 days. This has been shown to produce nephrotoxicity in rats (Gibert *et al.*, 1978). The animals of group-III received 80 mg/kg b wt of gentamicin ip route for 8 days. In addition to this they also received 100 mg/kg b wt of 70% methanolic EV extract per orally (po) which was started three days prior to gentamicin injection

and continued for eight days. The group IV animals were given 80 mg/kg b wt of gentamicin ip route for 8 days, in addition to this they also received 200 mg/kg b wt of 70% methanolic EV extract po which was started three days prior to the gentamicin treatment and continued further 8 days. On day ninth, animals were subjected to mild ether anaesthesia and blood samples were collected. The degree of protection was measured by testing BUN, Scr and initial and final gross b wt of the rats (Kumar *et al.*, 2011).

For the histopathological studies the two rats from each group were sacrificed on the last day and kidneys were isolated and preserved in formalin solution. The tissues were processed through routine paraffin method and the kidney sections were stained with hematoxylin and eosin and observed under microscope (Shirwaikar *et al.*, 2003).

Anti-ulcer activity:

The experimental animals were divided into four groups of six animals in each group and fasted for 48 h with free access to water. Pyloric ligation was performed under light ether anaesthesia to each animal, and then 1% corboxymethyl cellulose solution was given to all animals. The group one animals kept as control; the second group was treated with 50 mg/kg b wt of ranitidine po and the group III and IV animals were treated with 70% methanolic extracts of EV (po) 100 mg/kg and 200 mg/kg b wt, respectively. Then animals were dissected after 4 h, the stomach was carefully removed and gastric contents were collected. The gastric juice was centrifuged at 3000 rpm for 30 min and the volume of the gastric juice was measured. Free and total acidities in the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/L/100 g (Ganachari and Shiv, 2004).

For the histopathological study the two rats from each group were sacrificed, the stomach was cut and opened along the greater curvature and pinned on a soft board for evaluating gastric ulcers spot and calculated ulcer index. The percentage

Table 1: Effect of 70% methanolic extract of EV on gentamicin induced nephrotoxicity in rats

Groups	Treatment regimen	Blood urea nitrogen (BUN) mg/dl	Serum creatinine (Scr) mg/dl	Change in gross Body weight (%)
Group I	Vehicle treated (Control)	31.22±0.61	0.88±0.10	6.20±0.50
Group II	Gentamicin 80 mg/kg, b.w for 8 days in ip	49.10±1.99	1.89±1.20	-14.30±1.42
Group III	Gentamicin 80mg/kg b wt ip for 8 days and 70% methanolic extract of EV drug 100 mg/kg for 8 days	40.54±1.28*	1.79±0.12*	-10.00±1.04*
Group IV	Gentamicin 80mg/kg b wt ip for 7 days and 70% methanolic extract of EV drug 200 mg/kg b wt ip for 8 days simultaneously	34.27±2.3**	1.76±0.17**	-9.01±1.03**

Values are Mean±SEM, n=6, Significance *p<0.01 and **p<0.001 compared to control

inhibition of ulcers was calculated as mean ulcer index of control - mean ulcer index of test divided by mean ulcer index of control x100 (Vogel, 2002).

Statistical analysis:

The values are expressed as mean±SEM. The data were analyzed by using unpaired Students t test. Discrepancies with p<0.001 were considered to be statistically significant.

Results and Discussion

Nephroprotective activity:

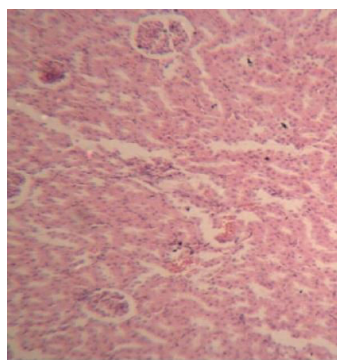
In group I the gross animal's body weight was increased to 6.2 ± 0.51 g, the serum level of BUN 31.22± 0.61 mg/dl and serum Scr 0.88± 0.10 mg/dl was found and kidney histopathology was also observed in normal structural condition. In the group II animals, body weight was reduced to - 14.30 ± 1.42 g, whereas BUN, and Scr levels were raised to 49.10 ± 1.99 mg/dl and 1.89 ± 1.20 mg/dl, respectively. The kidney showed glomerular congestion, infiltration, inflammatory cells, tubular necrosis, peritubular necrosis and presence of casts. The treatment with 70% methanolic extracts of EV at the dosage regimen of 100 (group III) and 200 mg/kg b wt (group IV), caused an increase in b wt and also significantly reduced the BUN as compared to group II (Table 1).

The histopathological study revealed that the gentamicin induced tubular necrosis were reduced and normal cellular structures are maintained in rats treated with 100 mg/kg b wt, of 70% methanolic EV extract, however, mild peritubular congestion were persisted (Fig. 1). In 200 mg/kg b wt of 70% methanolic EV extract treated animals have shown the maximum protection to renal cells damage.

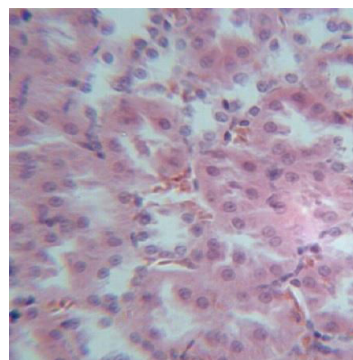
Anti-ulcer activity:

The treatment with 70% methanolic extracts of EV at the dosage of 100 and 200 mg/kg b wt rats showed reduced ulcer index value of 1.10±0.56 and 1.00±0.65, respectively which were statistically significant compared to control (1.20±0.32) and standard (0.49±0.01) group animals (Table 2). The total acidity and free acidity in 100 mg/kg b wt extracts of EV was 10.50±1.51 meq/L/100 g and 10.01±1.14 meq/L/100 g, respectively whereas in 200 mg/kg b wt extracts of EV it was 10.01±1.03 meq/L/100 g and 9.06±1.02 meq/L/100 g, respectively. The gastric pH and gastric volume were also significantly controlled in 100 mg/kg and 200 mg/kg b wt extracts of EV treated animals when compared to control group and standard group (Table 2).

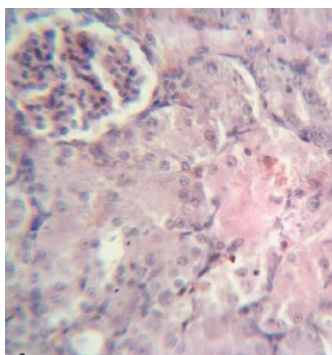
The histopathological observation revealed



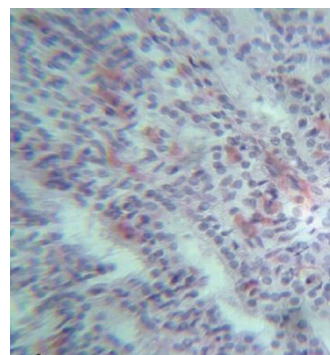
Group I (Normal kidney)



Group III (Gentamicin and EV 100 mg/kg)



Group II (Gentamicin 80 mg/kg)



Group IV (Gentamicin and EV 200 mg/kg)

Fig. 1: Effect of 70% methanolic extract of EV on gentamicin induced nephrotoxicity in rat's kidney.

Table 2: Results of anti-ulcer activity of EV on rats

Treated groups	Volume of gastric juice, in ml/100g b.w	Gastric juice pH	Free acidity meq/L/100g	Total acidity meq/L/100g	Ulcer index
Control (Vehicle treated)	9.41±0.81	4.41±0.24	10.31±0.62	14.12±0.71	1.20±0.32
Standard drug treated (Ranitidine 50 mg/kg b wt, po)	5.38±0.29***	6.52±0.29***	5.98±0.74***	9.06±0.52***	0.49±0.01***
EV drug treated (70%, methanolic 100 mg/kg b wt, po)	6.73±1.76**	5.56±0.92**	10.01±1.14*	10.50±1.51**	1.10±0.56**
EV drug treated (70%, methanolic 200 mg/kg b wt, po)	6.91±1.66*	5.88±1.06**	9.06±1.02*	10.01±1.03**	1.00±0.65**

Values are Mean±SEM, n=6, Significance *p<0.05, **p<0.01 and ***p<0.001 compared to control



Control (Vehicle treated)



Standard drug treated
(Ranitidine 50 mg/kg b wt)



EV drug treated
(70% methanolic 100 mg/kg b wt)



EV drug treated
70% methanolic 200 mg/kg b wt)

Fig. 2: Histopathological observations of rat's stomach.

reduction in ulcer indices including number of lesions and their severity in test drug treated animals as compared to control group (Fig. 2).

In group II animals, gross body weight was reduced and BUN and Scr levels were raised. In this group there is glomerular congestion, infiltration, inflammatory cells, tubular necrosis, peritubular necrosis and presence of cellular casts. The treatment with EV at the dosage regimen of 100 (group III) and 200 mg/kg b wt (group IV) caused an increase in gross body weight, and a decrease in BUN and Scr level as compared to group II animals. The severity of kidneys' toxicity was controlled and maintained normal structure.

In anti-ulcer activity the EV treated animals showed in significantly reduced total gastric volume, Gastric acid pH value, number of ulcer spots, free acidity and total acidity in tested animals as compared to control group of animals and it was found to be less potential to standard drug treated animals.

Conclusion

In conclusion, the 70% methanolic extract of plant *Erythrina variegata* is nephroprotective and gastroprotective against gentamicin induced toxicity. However, further researches are required to establish and elaborate the molecular level of

mechanisms of action of its nephroprotective and anti-ulcer activity.

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References

- Bhoumik D, Birhanetensay M, Malik A. (2017) Anti-ulcer properties of Herbal drugs: A Review, Int J Biomed Res. 8(3): 116-124.
- Ganachari MS and Shiv K. (2004) Anti-ulcer property of *Ziziphus jujube* Lam leaves extract in rats. J Natural Remedies 4/2; 103-108.
- Gilbert DN, Wood CA and Kohlepp SJ. (1989) Polyaspartic acid prevent experimental aminoglycoside nephrotoxicity. J Infect Disease 159: 945.
- Gilbert DN, Plamp C, Starr P, Bernnet WM, Houghton DC and Porter G. (1978) Comparative nephrotoxicity of gentamicin and tobramycin in rats. Antimicrob Agents Chemotherapy 13(1): 34-40.
- Kadam AB and Gaykar BM. (2017) Assessment of acute oral toxicity of synergistic formulation extract of traditional contraceptive plants. Int J Pharmacogn Phytochem Res. 9(3): 313-318.
- Kanato U, Kajufumi S and Srie T. (1992) Protective effect of cyclodextrin sulphates against gentamicin induced nephrotoxicity in rats. J Pharma Pharmacol. 45: 745.
- Khandelwal RK. (1996) Practical Pharmacology Techniques and Experiments, 3rd edn., Nirali Prakashan.
- Kumar A, Lingadurai S, Jain A and Barman NR. (2010) *Erythrina variegata* Linn: Review on morphology, phytochemistry and pharmacological aspects. Pharmacogn Rev 4(8): 147-152.
- Pramod K, Devala RG, Lakshmayya and Ramachandra SS. (2011) Nephroprotective and nitric oxide scavenging activity of tubers of *Momordica tuberosa* in rats. Avicenna J Med Biotechnol 3(2): 87-93.
- Shahriar M, Nishat ZK, Zara S, Chowdhury SF, Kamruzzaman M, Bakhtiar SIM, Chisty SJ, Syeda NN, Rumana A and Nahia A. (2015) Phytochemical analysis, cytotoxic and in vitro antioxidant activity of *Erythrina variegata* Bark. European J Med Plants 11(3): 1-5.
- Shirwaikar A, Malini S, Chandrika Kumari S. (2003) Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats. Indian J Exp Pharmacol. 41: 58-62.
- Syed Asad Bakhtiyari, Iqbq MM and Ibrahim M. Hepatoprotective and nephroprotective activity of *Phyllanthus amarus* Schum and Thonn. seed extract. Annals Phytomed. 1(2): 97-104.
- Udupa V and Prakash V. (2019) Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. Toxicol Rep. 6: 91-99.
- Vogel Gerhard H. (2002) Drug discovery and evaluation: Pharmacological assay. Springer, ISBN: 9783540423966.
- Warrier PK. (1994) Indian medicinal plants: A compendium of 500 species. Orient Longman Pvt. Ltd, India, pp. 378.