

Nephroprotective and Cardioprotective effect of *Trianthema portulacastrum* linn in drug induced experimental animals

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Abstract- *Trianthema portulacastrum* Linn used as traditional medicine, is a plant of the family Aizoaceae is a prostrate, glabrous, succulent annual herb. The plant is found almost throughout India as a weed in cultivated and wastelands. The plant has a remarkable protection against the drug induced diseases due to the presence of active phytoconstituents. In this present study, we discussed about the nephroprotective and cardioprotective and antioxidant effect of *Trianthema portulacastrum* Linn in experimental animals. Biochemical parameters and histopathological studies were analysed and the results are discussed to show the potent effect of the Siddha medicinal plant *Trianthema Portulacastrum* Linn.

Key words: *Trianthema Portulacastrum*, nephroprotective activity, cardioprotective activity, antioxidant, biochemical parameters.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The medicinal plants have immensely contributed to the health needs of humans throughout their existence. Even today, almost one quarter of prescribed medicines in the world control ingredients from plant origin. These are used as a major source of drugs for the treatments of various health disorders (Iqbal & Rehman, 2004).

Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects. Nowadays, it is being realized that herbs can protect the heart from heart diseases by their cardio protective action by providing an integrated structure of nutritional substances mainly phytochemicals which help in restoring and maintaining balanced body systems (Dhar *et al.*, 1968; Hertog *et al.*, 1993).

***Trianthemma portulacastrum* Linn**

The principal constituent of *Trianthemma portulacastrum* Linn. is ecdysterone and the other constituents are trianthenol, 3-acetylaeuritic acid, 5,2'-dihydroxy-7-methoxy-6,8-dimethylflavone, leptorumol, 3,4-dimethoxy cinnamic acid, 5-hydroxy-2-methoxybenzaldehyde, p-methoxybenzoic acid, and beta cyanin. The plant is used in the treatment of edema in the liver and spleen (Javed, 2000). The plant is lithotropic for the kidney and bladder. Different parts of *Trianthema portulacastrum* Linn. are traditionally used as analgesic, antipyretic, cardio tonic, anti-inflammatory, CNS depressant and stomachic properties and used in asthma, bronchitis, jaundice and oedemas(Kumar *et al.*,2004).

Drug Induced Nephrotoxicity

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. When kidney damage occurs, body unable to rid of excess urine and wastes from the body and blood electrolytes (such as potassium and magnesium) will all become elevated. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because increasing number of potent therapeutic drugs like aminoglycoside antibiotics, chemotherapeutic agents and NSAIDS have been added to the therapeutic arsenal in recent years. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. (Porter and Bennett ,1981).

Drug Induced Cardiotoxicity

Cardiovascular disease (CVD) has become a universal cause of morbidity and a leading contributor to mortality in both developed and developing country. The identification of major risk factors through epidemiological studies and effective control strategies combining community education and targeted management of high risk individuals have contributed to the fall in CVD mortality rates that has been observed in almost all streamlined countries. Isoproterenol caused severe stress in the myocardium resulting in necrosis of heart muscles which caused cardiac dysfunction, increased lipid peroxidation along with an increase in the level of myocardial lipids, altered activities of the cardiac enzymes and antioxidants (Reddy and Yusuf, 1998).

Effect of *Trianthema portulacastrum* on Antioxidant enzymes

Free radicals are atomic or molecular chemical species with unpaired electrons. These free radicals are highly unstable and can react with other molecules by giving out or accepting single electron. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals (Crastes,1990). In view of this, we selected *Trianthema portulacastrum* Linn, to assess the antioxidant activity.

The objectives of the present study were to evaluate the crude powder of whole parts of *Trianthema portulacastrum* for its anti oxidant activity, nephroprotective activity, cardio protective activity and also to identify the nature of the phytochemicals in the selected plant.

EXPERIMENTAL METHODS

Plant Material

Fresh plant sample *Trianthema portulacastrum* Linn were collected from various parts of Thanjavur district. The whole plant were washed, shade dried, powdered. Crude powder of *Trianthema portulacastrum* Linn are given to the experimental rats at the dose of 100mg/100g body weight.

Experimental Design

Male albino rats of 8 – 10 weeks of age weighing between 120 and 150g for nephro-toxicity and 180-200g for cardio-toxicity were used for the study. The animals were housed in polypropylene cages. Animals were divided into five groups of three animals. The animals were acclimatized for a week under laboratory conditions. All experiments were performed according to the norms of the local ethical committee.

Renal damage was induced in rats by induction Gentamicin at a dose of 40mg/ 100kg of body weight and Cardiotoxicity was induced in rats by inducing isoproterenol at a dose of 70 mg / 100kg of body weight intraperatonially.

Experimental animals were distributed randomly, in five groups, containing three animals each.

- The animals in group I served as normal and received rat feed and distilled water *adlibitum*.
- The group II rats served as test and were induced with gentamicin at a dose of 40 mg/ 100g body weight meanwhile,

- Group III rats served as test and were induced with isoproterenol at a dose of 70 mg/ 100g body weight intraperitoneally.
- The animals in group IV provided with rat feed and distilled water along with gentamicin and the animals in group V treated with isoproterenol and Crude powder of *Trianthema portulacastrum* Linn at the dose of 100mg/100g body weight the drug followed by it.
- After the completion of the experimental regimen rats were fasted overnight, anaesthetized with ether, blood was drawn and the serum was separated for various biochemical parameters. Kidney and Heart tissues are also separated for histopathological studies.

BIOCHEMICAL STUDIES

This study carries with different parameters to analyse the nephroprotective, cardioprotective and anti-oxidant effect of the *Trianthema portulacastrum* Linn in drug (Gentamicin and isoproterenol) induced experimental rats.

Serum creatinine level was determined using Creatinine Colorimetric Kit. Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in a short period to avoid interference. (Bartels and Bohmer, 1971; Fabiny and Ertingshausen, 1971). In the estimation of Urea, diacetyl monoxime in the presence of acid hydrolyzes to produce the unstable compound diacetyl reacts with urea to produce a yellow diazine derivative. The colour of this product is intensified by the addition of thiosemicarbazide it was measured at 520nm (Crocker, 1967).

Uric acid reduces phosphotungstic acid in the presence of sodium carbonate to give blue colour which can be measured colorimetrically. Transaminases activities were estimated by Reitman and Frankel method and which was measured spectrometrically. Cystatin C is estimated in serum using the kit and measured at 700/546 nm. Creatine Kinase-MB and Triglycerides were estimated by Friedman and Young method and which was measured by Spectrometrically. Lipid peroxide content was assayed by thio barbituric acid method.

Reduced glutathione was estimated by method of Moron *et al* (1979) and the absorbance was read at 412nm. The activity of mitochondrial glutathione peroxidase was assayed by the method of Rotruck *et al* (1973) and the colour developed was read at 420nm immediately. The Histopathological studies, were carried out in all the groups of normal, control and drug treated rats (Ochei and Kolhatkar., 2000).

Mean values standard were calculated and percentage of inhibition for all the values carried out. (Fisher, 1950).

RESULT AND DISCUSSION

Gentamicin (GM) is an aminoglycoside antibiotic that is very effective in treating life threatening gram negative infection. GM induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule. GM causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure (Sudin, 2001).

The result of this study shows the significant nephrotoxicity induced by gentamicin was evidenced by increase in serum urea, creatinine clearance and urea secretion due to renal tubular necrosis. The administration of crude powder of *Trianthema*

portulacastrum for 30 days was found able to treat and protect renal necrosis against gentamicin induced nephrotoxicity and thereby decreasing the serum urea, creatinine and uric acid.

Cystatin C appears to be a better predictor of glomerular function than serum creatinine. Cystatin C, is a bio-marker, a non-glycosylated 13 kDa protein, has the potential to improve estimates of GFR, because it is thought to be less influenced by muscle mass or diet. Glomerular Filtration rate is estimated assessing cystatin C (Rander *et al.*, 1999 and Newman *et al.*, 1995). In experimental nephrotoxicity studies there was an increase in serum cystatin C due to the impaired glomerular filtration and found decreased in herbal drug treated group (Table:1).

In (Table:2) Aminotransferases (ALT and AST) and Phosphatases are the specific enzymes and are considered to be very sensitive and reliable indicators for measuring hepatotoxic as well as protective effect of various compounds. Renal necrosis induced by gentamicin usually associated with elevated levels of serum enzymes that are indicative of cellular leakage and loss of functional integrity of cell membrane in kidney. (Reitman and Frankel, 1957).

The oral administration of crude powder of Siddha medicinal plant *Trianthema portulacastrum* for 30 days were found to protect the proximal tubular damage induced by lipid peroxidation and activation of antioxidant enzymes. The drug administration was able to protect the renal necrosis and lysosomal latency as evidenced by the inhibitory activity of phosphatases and transaminases. The study also shows the significant efficacy of herbs in the treatment of nephrotoxicity was also evidenced by decrease in urea level and creatinine clearance.

The antioxidant activity of the *Trianthema portulacastrum* Linn in gentamicin induced nephrotoxic animals. The result of this study shows that gentamicin produced nephrotoxicity was evidenced by increasing in lipid peroxidation products suggesting the involvement of oxidative stress and suggestive of tubular damage. The drug treated groups exert a protection against oxidative stress and tubular damage against gentamicin induced nephrotoxicity. There was an increased activity in Reduced Glutathione, glutathione peroxidase and Super oxide dismutase activated with produced free radicals and involvement of oxidation stress and finally damage to the proximal tubule. The drug administration was able to treat and protect the proximal tubular damage against gentamicin induced nephrotoxicity, by the activation of antioxidant enzymes (Table:3).

Isoproterenol (ISO) [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride], a synthetic catecholamine and β -adrenergic agonist that causes severe stress in myocardium and infarct-like necrosis of the heart muscles (Suchalatha and Shyamala Devi, 2004). ISO induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes. ISO induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction (Todd *et al.*, 1980).

The oral administration of crude powder of Siddha medicinal plant *Trianthema portulacastrum* against isoproterenol for 30 days were found to protect the cardiac damage induced by lipid peroxidation and activation of antioxidant enzymes. The herbal drug administration was able to protect the cardiac necrosis as evidenced by the inhibitory activity of CK-Mb and TGL (Table:4). The study also shows the significant efficacy of herbs in the treatment of cardiotoxicity was also evidenced by decrease in CK-Mb and TGL level in serum. Elevation of CK is an indication of damage to muscle. It is therefore indicative of injury, rhabdomyolysis, myocardial infarction, myositis and myocarditis.

The observed increase in the body weight in isoproterenol induced rats could be due to the accumulation of water content in the Oedematous intramuscular area in addition with necrosis of cardiac muscle fibres. Decreased activities of these cardiac marker enzymes in the cardiac tissue could be due to the leakage from damaged cardiac tissue into the circulation as a result of necrosis induced by ISO (Kurian *et al.*,2005)

TABLE 1: Nephroprotective effect of *Trianthema portulacastrum* on biochemical parameters- Urea, Uric acid, Creatinine and Cystatin C.

Groups	Dose	Parameters			
		Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Cystatin C mg/l
Normal	Isosaline	2.81±2.24	3.8±3.04	1.4±0.56	1.59±0.79
Control	40mg/kg b.wt	5.66±3.39	6.7±4.69	2.62±1.57	4.82±3.85
Drug Treated	100mg/kg b.wt	3.61±2.16	4.03±2.01	1.59±0.95	2.85±0.85

TABLE 2: Nephroprotective effect of *Trianthema portulacastrum* on Phosphatases and Transaminases

Groups	Dose	Parameters		
		Acid Phosphatase U/l	Alanine Transaminase U/l	Aspartate Transaminase U/l
Normal	Isosaline	15.71±9.42	13.76±9.63	10.75±7.52
Control	40mg/kg b.wt	19.23±3.84	17.21±3.44	18.11±9.05
Drug Treated	100mg/kg b.wt	15.12±4.53	14.11± 5.64	15.28±6.11

TABLE 3: Nephroprotective effect of *Trianthema portulacastrum* on Anti-Oxidants - LPO, Reduced Glutathione, SOD, Glutathione peroxidase.

Groups	Dose	Parameters			
		LPO n moles MDA/mg	Reduced glutathione µg/ mg ptn	SOD Mole/O ₂ decompose/ min/100mg protein	Glutathione peroxide U/mg protein
Normal	Isosaline	2.36±1.41	9.6±5.76	3.3±0.99	2.36±0.16
Control	40mg/kg b.wt	3.46±1.38	5.7±3.99	1.2±0.24	1.60±0.11

Drug Treated	100mg/kg b.wt	1.51±0.90	5.76±4.03	1.78±1.24	2.0±0.14
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TABLE 4: Cardioprotective effect of *Trianthema portulacastrum* CK-MB, TGL, Cholesterol and LPO.

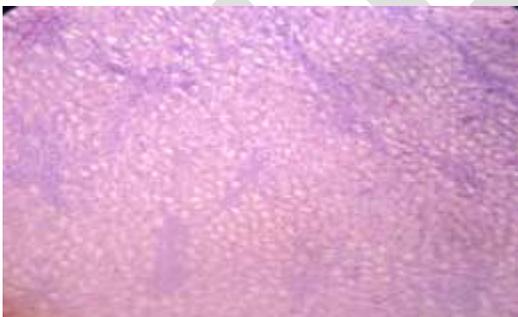
Groups	Dose	Parameters			
		CK-MB U/l	TGL mg/dl	Cholesterol mg/dl	LPO Nmoles MDA/mg
Normal	Isosaline	1.651±0.132	50.0±1.50	45±2.29	2.25±0.157
Control	40mg/kg b.wt	13.2±0.824	133.33±7.31	41±2.40	2.715±0.0962
Drug Treated	100mg/kg b.wt	9.906±0.493	62.5±2.75	45±3.61	1.62±0.0810

Each value is the mean ± SEM of five samples values are significantly different from control and treated rats.

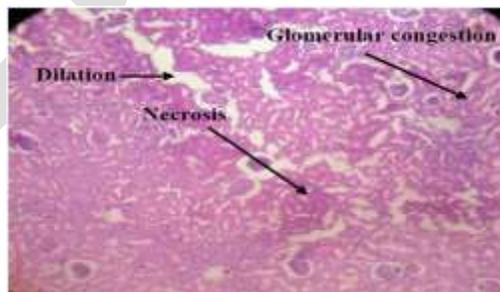
HISTOPATHOLOGY

In histopathological examination of Kidney tissues shows, normal architecture was observed in normal animals whereas renal lesions including marked tubular and focal area necrosis, inflammation and glomerular congestion changes in the kidney of gentamicin treated animals were observed (Group 2- control). The lesions were reduced significantly in animals which were treated with the Siddha medicinal plant *Trianthemna portulacastrum* at the dose of 100 mg/100g.b.wt (doses) to gentamicin treatment.

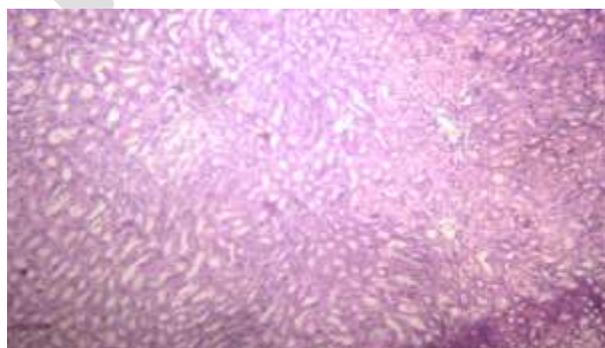
Normal



Control- Gentamicin induced

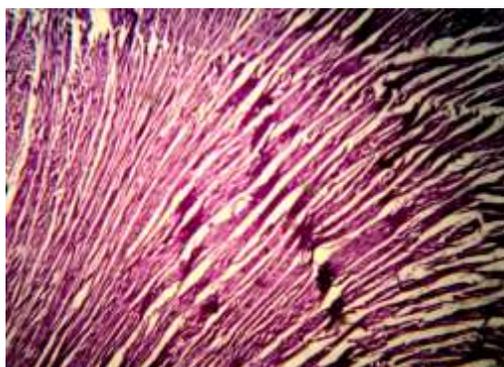


Trianthema portulacastrum treated

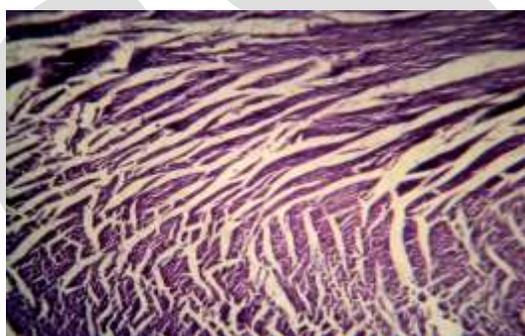


In Heart tissue, Normal group showed normal cardiac architecture and arrangement of myofibril, absence of interfibrillar necrosis, regular and normal multinuclear myofibrils arrangement, vacuolization and macrovesicular changes. In Isoproterenol treated animals exhibited intense interfibrillar necrosis, vacuolization, macroveisicular changes and damage and irregular arrangement and morphological change of myofibrils associated with increased interfibrillar distance. *Trianthema portulacastrum* exhibited significant cardiac remodeling activity against isoproterenol induced rat's heart tissue by normal cardiac architecture, arrangement of myofibrils, and absence of interfibrillar necrosis.

Normal



Control-Isoproterenol induced



Trianthema portulacastrum treated



CONCLUSION

The nephroprotective, anti-oxidant, cardio protective effect of the *Trianthema portulacastrum* be due to the activity of the phytoconstituents present in the Siddha medicinal plant have the nephrotoxic and cardio toxic effect against the aminoglycoside-antibiotic drug gentamicin and isoproterenol. Further studies are suggested on the isolation of active compounds and their protective activity in human.

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