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EFFECT OF ANTHOCYANIN FRACTION ON CISPLATIN-INDUCED NEPHROTOXICITY

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ABSTRACT
Present study was designed to evaluate the effect of anthocyanin fraction of *Syzygium cumini* on cisplatin-induced nephrotoxicity in male Albino rats. Anthocyanin fraction was administered by gastric intubation at two dose levels. Animals were divided into 5 groups. Group I animals received vehicle. On day 1, Group II animals received cisplatin (6 mg/kg, i,p., single dose). Group III (7.5mg/kg) and IV (15mg/kg) received anthocyanin fraction respectively at 1 hr before, 24 hr and 48 hr after cisplatin injection. Group V received only Anthocyanin fraction. After 72 hr of cisplatin injection, blood and urine were collected and estimated for serum markers level and creatinine clearance. Malonoaldehyde (MDA) levels and histological studies were also conducted. Cisplatin caused renal damage characterized by elevation of Blood Urine Nitrogen, Serum creatinine, Malonoaldehyde level with marked drop in Creatinine clearance, Anthocyanin fraction reversed all the effects induced by cisplatin in dose dependent manner. Histological studies also substantiated above mentioned results. Present study reveals that anthocyanin fraction attenuated the nephrotoxicity induced by cisplatin in rats.
Keywords: Anthocyanin, *Syzygium cumini*, Cisplatin, Lipid peroxidation, Nephroprotector activity

INTRODUCTION

Anthocyanins are the important plant pigments. They belong widespread class i.e., flavonoids, which were reported to exhibit wide spectrum of activities including anti-inflammatory activity[1] and also used to treat diseases resulting from capillary fragility[2-5], prevention of cholesterol induced atherosclerosis[6] and reported to exhibit antioxidant activity[7]. Especially, the plant *Syzygium cumini* has been reported to possess several medicinal properties such as antidiabetic, diuretic and antioxidant. *Syzygium cumini* is rich in anthocyanin; Presence of anthocyanins may partially contribute to the potential medicinal properties of this plant. Hence the present study was undertaken to explore the possibility of anthocyanins fraction to prevent cisplatin mediated renal injury in rats.

MATERIALS AND METHODS

Plant Material

Syzygium cumini fruits was collected from local market and authenticated by botanist, Dr. Madhavachetti, Department of botany, Sri Venkateswara University, Tirupati, A.P., India

Isolation of anthocyanins

Powder (4g) of *Syzygium cumini* fruits, treated for 30min under agitation with 100 ml of 0.12 mol/L HCl in 10% ethanol, then centrifuged for 5 min at 12,000 rpm. Then solvent was evaporated[8] to obtain Anthocyanin fraction.

Chemical test for anthocyanins

When the solution of anthocyanins was made to acidic pH, the solution turned to red and blue color in basic pH.

Pharmocological Studies

Animals

Healthy adult male albino rats (100-150g) of Wister strain aged 60-90 days were used for the study. The rats were housed in polypropylene cages maintained at standard conditions. The animals had free access to standard laboratory chow and tap water. Animals were acclimatized to our laboratory environment for about a week. Animals were handled according to the rules and regulations of Institutional Animal Ethical Committee (IAEC). (Reg no: 930/a/06/CPCSEA;Ref no: SVCP/IAEC/31-0052)

Treatment protocol

Animals were divided into five groups of six animals each.

Group I treated with vehicle orally (2% gum acacia) for three days, and was kept as normal control. Group II injected with single dose of cisplatin i.p (6 mg/kg bd. wt) on day 1. Group III and IV were treated with lower (7.5mg/kg bd. wt) and higher (15 mg/kg bd. wt) dose of anthocyanin fraction of fruits of *Syzygium cumini* by gastric intubation.

The anthocyanin fraction of fruits of *Syzygium cumini* was administered orally one hour before, 24 hours and 48 hours after cisplatin injection (i.p.) to Group III and IV. Group V treated only with high dose of anthocyanin fraction. 72 hours after cisplatin injection animals were sacrificed and blood was directly collected from the heart of each animal for the study of biochemical parameters.

Assessment of renal function

Estimation of serum markers

Blood Urea Nitrogen**:** BUN is determined by DAM method[9]. Absorbance was read from spectrophotometer (Systronics).

Serum Creatinine: Creatinine levels in serum was estimated by the Jaffe's Alkaline Picrate method [9] using a creatinine kit. Absorbance was read from spectrophotometer (Systronics).

Estimation of Urinary functional parameters

Urinary Total Protein (U_{TP}): Urinary total proteins were estimated by Turbidimetry method [9]. Turbidities of urine test and standards were measured against blank at 640nm.

Creatinine clearance (Cl_{cr})**:** Creatinine clearance was estimated by alkaline picrate method[9]. Absorbance of test and standard were measured against blank at 520nm.

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine x Urinary volume/hr}}{\text{Serum creatinine}}$$

Lipid peroxidation (LPO)

The levels of LPO in kidney tissue was determined by Thiobarbituric acid test [10] and the absorbance was measured at 532 nm.

Histological Studies

Two animals from each group were sacrificed on day of blood withdrawal and kidneys were isolated. The kidney sections were stained with hematoxylin and eosin and observed under light microscope.

Statistical Analysis

The results are expressed as mean ±SEM and the data was analyzed by one way analysis of variance followed by post hoc Student-Keuls test using SPSS computer software. Statistical significance was considered at $P \leq 0.05$.

RESULTS

Animals which received the Anthocyanin fraction (Group V) for three days exhibited no change in serum markers level and urinary functional parameters. Hence, the Anthocyanin fraction did not show any deteriorative effects on kidney. To assess the neproprotector activity of extract, the data obtained from Anthocyanin fraction treated Groups (III, IV) was compared with Group II (animals which received only cisplatin injection).

Effect on Serum Markers

Table 1 lists the effect of Anthocyanin fraction on cisplatin-induced nephrotoxicity. Intraperitonal administration of cisplatin at 6 mg/kg caused significant elevation of BUN and SC in group II animals, when compared to normal control animals (Group I). On oral administration of anthocyanin fraction in group III and IV, animals exhibited significant reduction in the levels of BUN and SC when compared to group II animals.

Effect on Urinary parameters

The deterioration of renal functions induced by cisplatin and the effect of oral administration of the anthocyanin fraction are given in Table 2. Animals administered with cisplatin excreted high amount of U_{TP} when compared with normal group I animals. Animals which received anthocyanin fraction treatment in group III (7.5mg/kg bd. wt) and group IV animals (15mg/kg bd. wt) reversed the effect caused by cisplatin in dose dependent manner.

The animals received cisplatin alone exhibited decreased levels of Cl_{cr} when compared with normal animals. On oral administration of anthocyanin fraction showed significant increase in Cl_{cr} in group III and IV animals.

Effect on LPO

Kidneys were isolated from the animals to estimate the levels of MDA which was expressed nmol/mg protein. Animals which were treated with cisplatin alone exhibited elevated levels of MDA, when compared to normal control animals. Animals which treated with anthocyanin fraction exhibited dose dependent reduction of MDA levels when compared to group II animals.

Histological studies

The sections of kidneys isolated from rats treated with cisplatin showed desquamation of necrotic epithelial cells, degenerative tubule, glomeruli with areas of hemorrhages, glomerular atropy and picnotic nuclei indicating renal toxicity.

The sections of kidneys isolated from rats treated with low dose of anthocyanin fraction showed moderate degenerative changes i.e., enlarged renal tubule and picnotic nuclei. The sections of kidneys isolated from rats treated with high dose of anthocyanin fraction showed regenerative changes indicating significant but moderate protection.

Table 1: Effect of anthocyanin fraction of *Syzygium cumini* fruits on cisplatin induced nephrotoxicity

Group	Treatment (mg/kg)	BUN (mg/dl)	SC (mg/dl)
I	Normal (2% gum acacia)	26.7±1.5	0.7±0.2
II	Cisplatin (6mg/kg)	62.3±.2.3[a]	2.3±.1.6[a]
III	Anthocyanin fraction (7.5mg/kg)	32.2±2.0[b]	1.4±.0.5[b]
IV	Anthocyanin fraction (15mg/kg)	26.0±1.74[ac]	1.0±0.9[ac]

*Each value represents the mean ± SEM from 6 animals in each group

a P<0.05 when compared with normal group., b: P<0.05 when compared with group II (cisplatin)

c: P<0.01 when compared with normal group I and group II (cisplatin)

Table 2: Effect of anthocyanin fraction of *Syzygium cumini* fruits on Cisplatin-induced nephrotoxicity

Group	Treatment (mg/kg)	LPO (n moles/g)	U_{TP} (mg/24hrs)	Cl_{cr} (ml/hr/100g bd.wt)
I	Normal (2% gum acacia)	11.7±2.16	7.8±0.33	19.8±1.6
II	Cisplatin (6mg/kg)	14.7±0.3[a]	19.9±1.6[a]	5.1±2.6[a]
III	Anthocyanin fraction (7.5mg/kg)	12.6±0.5[b]	12.3±0.3[b]	10.1±1.3[b]
IV	Anthocyanin fraction (15mg/kg)	10.3±1.6[ac]	10.6±1.8[ac]	15.0±0.34[ac]

*Each value represents the mean ± SEM from 6 animals in each group

a P<0.05 when compared with normal group., b: P<0.05 when compared with group II (cisplatin)

c: P<0.01 when compared with normal group I and group II (cisplatin)

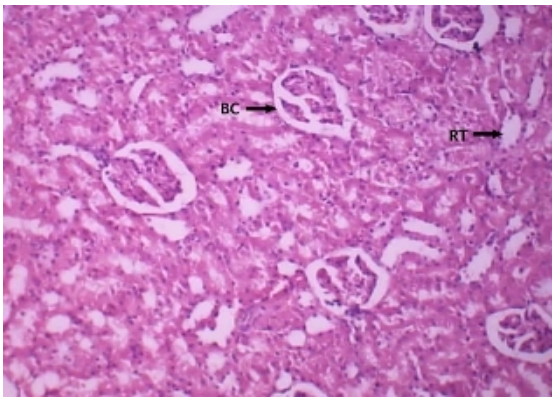

Plate I: Section of normal rat kidney showing normal organization
BC - Normal orchitecture of bowmen's capsule, RT - Normal orchitecture of renal tubule

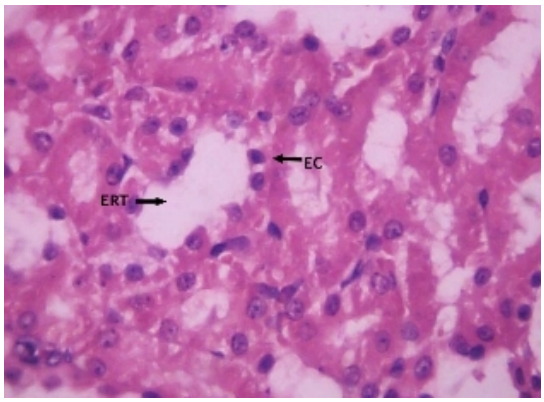

Plate III: Section of rat kidney treated with anthocyanin fraction (7.5 mg+cisplatin) revealing moderate degenerative changes
EC- Epithelial cell, ERT- Enlarged Renal Tubule

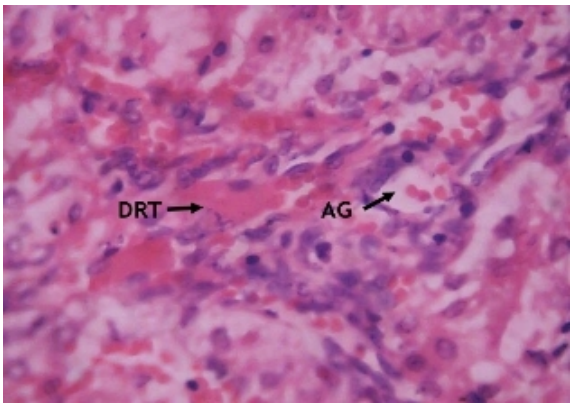

Plate II: Section of rat kidney treated with cisplatin.
DRT - Degenerative renal tubule AG - Atropic Glomeruli

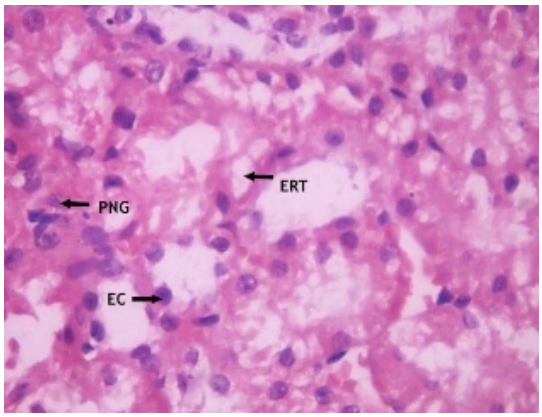

Plate IV: Kidney treated with anthocyanin fraction (15 mg+cisplatin) revealing regenerative changes
EC- Epithelial cell, ERT- Enlarged Renal Tubule, PNG- Picnotic nuclei glomeruli

DISCUSSION

Cisplatin is one of the most important anti tumor agents and is highly effective against a diverse spectrum of malignancies[11]. However, the use of this agent in combating cancer is limited by the development of nephrotoxicity[12, 13], along with various untoward side effects including nausea, vomiting, diarrhea and myelosuppression[14, 15].

The precise mechanism of cisplatin-induced nephrotoxicity has not been elucidated, but it has been suggested that the free radicals of oxygen play an important role[16-18]. Cisplatin-induced nephrotoxocity is related to increase in lipid peroxide levels in kidney[19]. Reports also suggest that there is an involvement of nitric oxide which induces the nephrotoxicity by cisplatin[20, 21]. Previous reports suggested that compounds with antioxidant principles such as Sodium maleate[22], N-acetyl cysteine[23], quercetin[24] exhibited good nephroprotector activity.

Various reports suggested that compounds with antioxidant properties were effectively reduced the nephrotoxicity induced by cisplatin. Anthocyanins also possess good antioxidant activity. Thus efforts are made to exploit the nephroprotector activity of anthocyanins.

In present study, Cisplatin caused renal failure characterized by elevation of serum marker levels, deteriorated the renal functional parameters indicated by increased the urinary protein excretion and decreased the $Cl_{Cr.}$ Cisplatin alone can significantly increase in MDA levels.

Protective effects of anthocyanin fraction isolated from fruits of *Syzygium cumini* were tested at two dose levels *i.e,* 7.5 and 15 mg/kg body weight *p.o.* The doses were selected based on daily intake of anthocyanins i.e, 180-215 mg/day in man [25].

In present study, anthocyanin fraction has exhibited significant activity cisplatin-induced renal damage. These findings correlated with the renal histological examination which revealed the more extensive and marked tubular necrosis with hemorrhages and picnotic nuclei in cisplatin-induced nephrotoxicity.

In conclusion, our result reveals that anthocyanin fraction exhibited significant nephroprotector activity against cisplatin-induced nephrotoxicity.

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