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Nephroprotective Potential of the Fruit Pulp of *Cassia fistula* in Cisplatin-Induced Renal Injury in Albino Rats

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ABSTRACT

The ethanolic extract of the fruit pulp of *Cassia fistula* was evaluated for its nephroprotective activity in cisplatin-induced renal injury in albino rats. In the study, the fruit pulp extract at a dose of 500 mg/kg body weight reduced elevated blood urea and serum creatinine levels and normalized the histopathological changes. The extract had a marked nitric oxide free radical scavenging effect. The findings suggest that the probable mechanism of nephroprotection by fruit pulp of *Cassia fistula* against cisplatin-induced renal injury could be due to its antioxidant and free radical scavenging activity.

INTRODUCTION

Cisplatin (cis - diamminedichloroplatinum or CDDP) is a potent anticancer drug [9]. It is used intensively in man, being effective in ovarian and bladder carcinoma, neuroblastoma and head and neck carcinoma, Hodgkin's diseases, and lymphoma as well as thyroid endometrial neoplasm. However, the most significant activity is observed in testicular cancer [7]. The clinical use of cisplatin is often complicated by nephrotoxicity [3], Ototoxicity, gastrointestinal disturbances like nausea, vomiting, and myelosuppression.

Nephrotoxicity may also be referred to as renal toxicity.Nephrotoxicity is one of the most common kidney problems and occurs when our body is exposed to a drug or toxin that causes damage to our kidneys. When kidney damage occurs, we are unable to rid our body of excess urine, and wastes. Our blood electrolytes such as potassium and magnesium will all become elevated. Nephrotoxicity occurs when kidney-specific detoxification and excretion do not work properly due to the damage or destruction of kidney function by exogenous or endogenous toxicants. Mechanisms for drug-induced nephrotoxicity include changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, and thrombotic microangiopathy [15].

Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances. Ancient literature has prescribed various herbs for the cure of kidney disease [11]. Co-administration of various medicinal plants possessing nephroprotective activity along with different nephrotoxic agents may attenuate its toxicity. There is a continuous search for agents which provide nephroprotection against the renal impairment induced by cisplatin for which there was no remedy in allopathic system of medicine.

Cassia fistula is a deciduous, medium-sized tree up to 24m in height and 1.8m in girth, cultivated almost throughout India. The tree is one of the widest spread in the forest in India, usually occurring in deciduous forests throughout the greater part of India, ascending up to an altitude of 1,220 m in the sub-Himalayan tract and the outer Himalayas. It is common throughout Gangetic valley, particularly abundant in Central India and South India [2]. The plant is also seen in the hilly tracts of Srilanka and Burma [13]. The fruit pulp of *Cassia fistula* used by the tribals

for the treatment of renal disorders, but no scientific studies have been undertaken to verify the nephroprotective claims of this plant. The present study is an attempt to screen the ethanolic extract of the fruit pulp of the plant for its nephroprotective activity.

MATERIALS AND METHODS

Plant material

The dried fruits were collected from the campus of Pariyaram medical college hospital, Kannur district of Kerala state and the pulp from the fruits were separated carefully by avoiding seeds.

Preparation of the extract

The fruit pulp was extracted by soxhlet apparatus with ethanol. It was then dried to a syrupy consistency**.**

Animals

Healthy adult male albino rats of Wistar strain weighing between 150 – 200 (g)aged 0 - 70 days were used for the study. The rats were housed, two in a cage and maintained in a temperature regulated and humidity controlled environment. The rats were fed with standard food pellets and water.

The study was conducted after obtaining ethical committee clearance from the institutional animal ethics committee of Academy of Pharmaceutical Sciences, Pariyaram Medical College (APSC/CPCSEA/-02/IAEC/2011).

Acute toxicity studies

Oral acute toxicity studies were carried out with 2 rats per dose group. The WHO has set guidelines for toxicity studies of herbal medicine. It supports appropriate usage of herbal medicines and encourages the remedies, which are proved for safety and efficacy. Acute toxicity study was performed for alcoholic extract according to the acute toxic classic method as per OECD guidelines [10], [12].

Cisplatin-induced renal injury

Five groups of eight rats each was used for the study. The first group was administered gum acacia solution (2%w/v) for 15days. The second group was treated with fruit pulp extract alone (500mg/kg body wt.,p.o) for 10 days. On the 16th day, the blood was withdrawn from the first group and on the 11th day from the second group for estimation of renal function tests.

The remaining groups were treated with a single dose of cisplatin (5mg/kg body wt.,i.p) [5]. Blood was withdrawn from the animal through the retro-orbital vein on the 6th day in the third group and on the 16th day in 4th group to assess renal function. The fifth group was studied for the curative activity of the ethanolic extract. This group was treated with the fruit pulp extract 500 mg/kg body wt., p.o), from 6th day onwards. Blood was withdrawn on the 16th day to estimate the blood urea and serum creatinine levels.

Parameters assessed for the renal function

Body weight: The weight (in grams) of the animals were noted on the first and last day of treatment and the percentage change in body weights were calculated.

Blood urea: Urea concentration in the blood was estimated by an enzymatic method using urease enzyme kit by modified Berthelot method [16].

Serum creatinine: Creatinine level in serum was estimated by alkaline picrate method using creatinine kit [16].

Histopathological examination

Two animals from each group were sacrificed on the day of blood withdrawal and their kidneys were isolated. It was washed with saline and preserved in 10% formaldehyde solution for histopathological studies. The kidneys were processed and embedded in paraffin wax. The sections were stained with hematoxylin and eosin and observed under light microscope.

Statistical analysis

The data was analyzed using One-Way ANOVA followed by Post Hoc Dunnett's test using Graph Pad Prism computer software version 4.03. The level of significance was fixed at 0.05.

Phytochemical screening

The phytochemical screening of various extracts of the fruit pulp of *Cassia fistula* was carried out as per the standard procedure [6], [8].

***In vitro* antioxidant study**

Antioxidant studies were carried out by nitric oxide scavenging activity [14].Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates NO_2, N_2O_4, and N_3O_4. It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, which is measured at 546 nm.

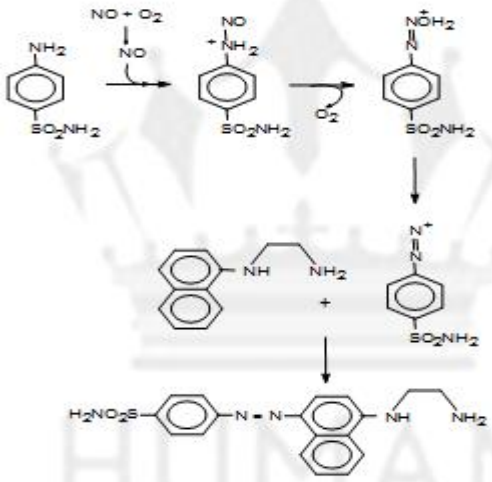

Fig.1: Griess reaction.

RESULTS

Cisplatin-induced renal damage

The cisplatin treated group showed a significant reduction in the body weight on the 6thday and an increase in blood urea and serum creatinine levels as compared to control. Histopathological sections of the kidneys showed marked congestion of the glomeruli. Degeneration of the tubular epithelial cells with casts and inflammatory cells were also observed.

In the curative study, treatment with extract 500mg/kg showed significant reduction of blood urea and serum creatinine levels as compared to the group treated with cisplatin alone, on the 16th day. Histopathological examinations revealed reduced congestion of the glomeruli with the presence of occasional casts.

Antioxidant studies

In vitro evaluation of the fruit pulp of *Cassia fistula* for its antioxidant property revealed a nitric oxide free radical scavenging effect. Percentage inhibition of free radicals increased with the concentration of the plant extract.

Table 1: Effect of ethanolic extract of the fruit pulp of *Cassia fistula.*

Group n=8/group	Treatment regimen	Percentage change in body weight	Blood urea mg/dl	Serum creatinine mg/dl
1	Vehicle	12.21±2.14	33.55±3.97	0.95±0.06
2	Ethanolic extract	22.32±4.56	27.33±2.69	0.88±0.07
3	Cisplatin 6th day	-12.74±1.39[a]	92.39±7.19[a]	1.78±0.08[a]
4	Cisplatin 16th day	-32.79±3.58[a]	87.22±3.25[a]	1.59±0.05[a]
5	Cisplatin +Extract (500mg/kg)	-4.5±1.69[b]	51.39±1.69[b]	1.63±0.07[b]

Values are Mean ±s.e.m (n=8), ap<0.5 vs.control (group 1), bp<0.05 vs. cisplatin 16th day (group 4)

Table 2:Effect of ethanolic extract of *Cassia fistula* fruit pulp on NO scavenging.

Sl.No.	Concentration µg/ml	Ethanolic extract		Ascorbic acid (Standard)	
		Absorbance	Percentage Scavenging.	Absorbance	Percentage Scavenging.
1	5	0.729	3.77	0.623	9.37
2	10	0.681	7.62	0.613	12.68
3	15	0.611	16.02	0.529	25.34
4	25	0.566	19.62	0.375	56.30
5	50	0.537	29.20	0.213	67.16
6	100	0.459	39.44	0.120	80.49
7	250	0.337	56.12	0.087	89.77
8	500	0.295	61.66	0.064	94.12
9	1000	0.257	65.92	0.008	99.36
10	Control	0.749		0.689	

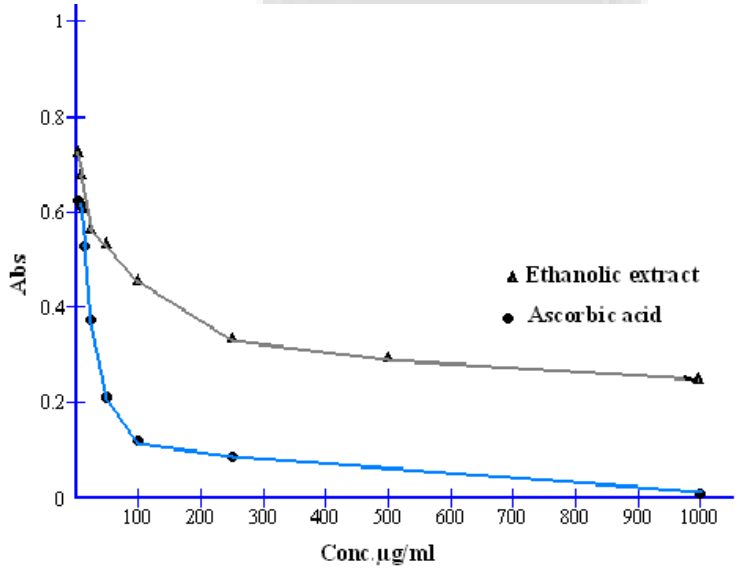

Fig. 2: Effect of ethanolic extract of *Cassia fistula* fruit pulp on NO scavenging

Phytochemical screening

Phytochemical screening of the extracts reveals the presence of carbohydrates, phytosterols, phenolic compounds, proteins, amino acids, and flavonoids.

DISCUSSION

In the present study, cisplatin-induced renal impairment was evidenced by an increase in blood urea, serum creatinine, and acute tubular necrosis. These changes persisted up to the 16th day following administration of a single dose of cisplatin. The ethanolic extract of *Cassia fistula* normalised the raised blood urea and serum creatinine levels. The histopathological features supported the biochemical reports.

In vitro studies of the fruit pulp of *Cassia fistula* was evaluated for its antioxidant property, which reveals a nitric oxide free radical scavenging effect. Nitric oxide has been shown to play a vital role in cisplatin-induced nephrotoxicity [1]. The fruit pulp of *Cassia fistula* has been found to be a rich source of flavonoids. Flavonoids are potent antioxidants and are known to modulate the activities of the enzyme systems, due to their interaction with various biomolecules [4].

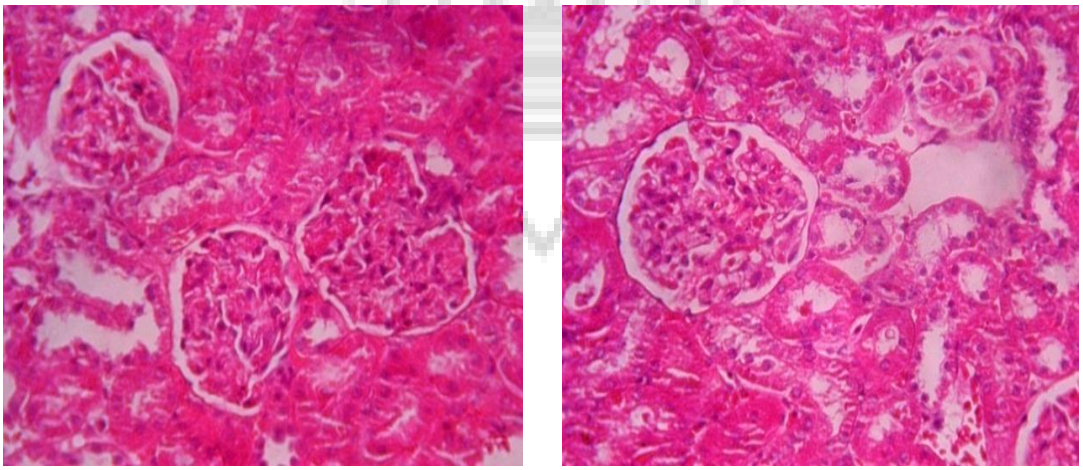

Fig.3:Photomicrograph showing normal glomeruli and normal tubule.

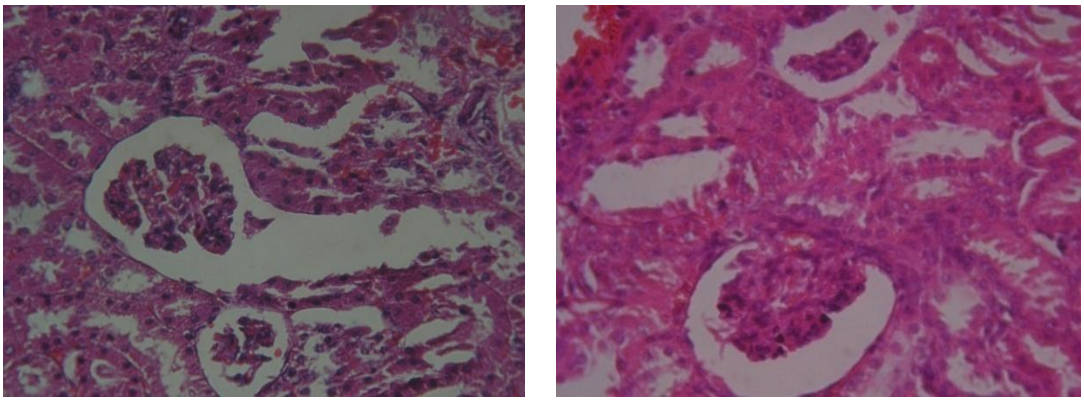

Fig.4:Photomicrograph showing nephrotoxicity with glomerular and peritubular congestion with the presence of occasional tubular casts.

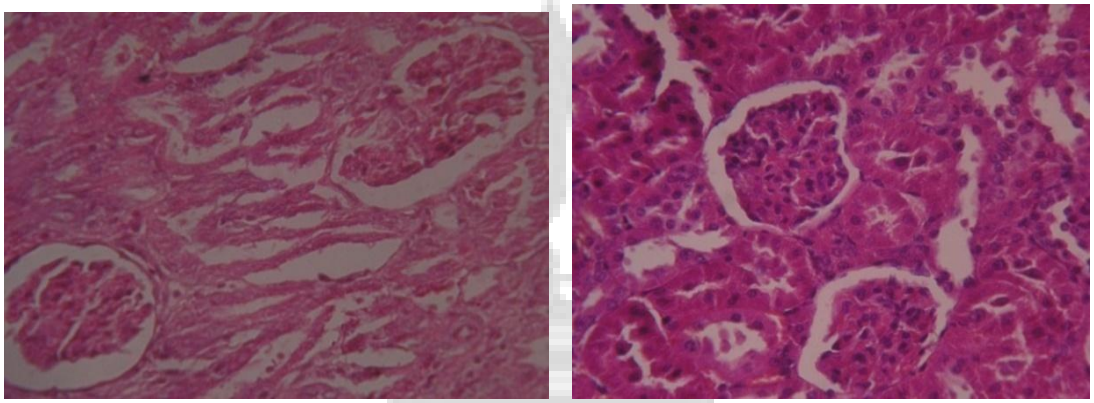

Fig.5: Photomicrograph of the section of rat kidney treated with a 500mg alcoholic extract of the fruit pulp of *Cassia fistula* showing mild glomerular and peritubular congestion and only a few inflammatory cells showing nearly normalization of the kidney.

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