International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Nephroprotective Activity of *Syzygium Cumini* in Gentamicin Induced Nephrotoxicity in Wistar Rats

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Abstract: ***Objective: The nephroprotective effects of methanolic extract of Syzygium cumini leaf in rats was investigated[1]. Method: Nephrotoxicity was induced by using gentamicin 100mg/kg, intraperitoneal for 7 days. Phytochemical test and acute toxicity test of Syzygium cumini leaf were carried out. The methanolic extract of Syzygium cumini leaf were given to these rats at doses level of 250mg/kg and 500mg/kg, per oral for 14 days. Cystone at the dose of 500mg/kg given per oral as standard drug to separate group of rat for 14 consecutive days for comparing nephroprotective activity. Result: Gentamicin induced nephrotoxicity which was indicated by increased levels of serum uric acid, serum creatinine and decreased serum protein along with increased urine urea, urine uric acid, urine creatinine and total protein[2]. On administration of methanolic extract of Syzygium cumini leaf to albino rats showed significantly decreased in the serum cretinine, serum urea, serum uric acid and increase serum total protein along with decrease urine urea, urine cretinine, urine uric acid, and total protein. These were decrease in malondialdehyde levels and increased levels of catalase, superoxide dismutase, glutathione as compared with gentamicin induced group. In histopathological studies gentamicin induced nephrotoxicity was indicated by degenerative change in kidney tissue, congestion with inflammation. Conclusion: The results showed Syzygium Cumini leaf having nephro protective effect.***

Keywords: Nephroprotective activity, *Syzygium Cumini*, Gentamicin induced nephrotoxicity

1. Introduction

Nephrotoxicity is defined as rapid deterioration of kidney functions. Nephrotoxicity may be due to number of different reason likes polluted environment, contaminated food material, modes, fungi, metal such as mercury, lead, and arsenic and drug of abuse such as cocaine along with certain disorder such as diabetes, dyslipidemia, hypertension and cancer etc. A number of therapeutic agents can adversely affect on kidney functions that may results in acute renal failure of chronic renal damage[3].

Nephrotoxicity is one of the major health issues. According to word health organization kidney disease 12th leading cause of death in the world. The incidence of kidney failure is almost double in the last 15 years. Presently over 1 million people in the world, who are alive either by using number of different medicine, dialysis or kidney graft.

In India approximately, 19 million people are suffering from kidney failure. It is estimated that more than 80, 000 persons are annually diagnosed with kidney failure and about 90% patient of can not afford the high cost of treatment[4].

Increased levels of serum creatinine, urea, and uric acid, as well as other imbalances, are signs of kidney failure. Many therapeutic agents like aminoglycosides antibiotics and NSAIDs can cause kidney failure. Incidence of renal failure increasing day by day, due to various synthetic drugs, uncontrolled diabetes mellitus, dyslipidemia and hypertension. The conventional approach of managements includes dialysis and transplantation.

Nephroprotective substances have protective properties against nephrotoxicity. Some medicinal plants have nephroprotective qualities because they contain a variety of phytochemical components. Flavonoids, phenolic substances, tannins, alkaloids, glycosides, saponins, dietary fiber, anthocyanins, tannins, and carbohydrates are the active components found in Syzygium cumini leaf extract, a significant amount of minerals and vitamins are the main components found in *Syzygium cumini* leaf. These substances have been shown to have a wide range of biological and pharmacological effects, including antibacterial, antihypertensive, antidiabetic, antioxidant, and anti - inflammatory activities. It has historically been used to treat nephritis and to help manage renal failure[5]. However, there is no scientific support for the use of this *Syzygium cumini* leaf to cure kidney disorders. Therefore, the goal of the present research was to evaluate the nephroprotective effects a methanolic extract of *Syzygium cumini* leaf in experimental rats[6].

2. Materials and Methods

Plant Material: *Syzygium cumini* leaves were gathered locally in the Belagavi district for this research. The plant was verified by Dr. Harsh Hegde (Senior Scientist), who gave it the plant authentication number RMRC - 1606. (Belagavi).

Preparation of extract: The authenticated shade dried leaf of *Syzygium cumini* belongs to the family Myrtaceae was reduced to coarse powder which can pass through sieve no.40. It underwent continuous hot extraction (soxhalation) using Mathanol as the solvent. The procedure was continued until the product became colorless, or for seven days. The rotary evaporator was used to condense the extract. For additional research, the extract was kept in a refrigerator in an airtight receptacle. Chemicals and drugs: Cystone were

purchased from the Bangalore, India - based Himalaya Drug Company. From Bangalore's Micro Labs Ltd, gentamicin was purchased. Coral Clinical Systems, Verna Goa, India, provided the creatinine, urea, uric acid, and total protein test[7].

Experimental Procedure: Albino Wistar rats of either sex were used for nephroprotective action while female Wistar rats, usually weighing 200 - 225gm, were used for acute toxicity studies. They were purchased from the Nathajirao G. Halgelakar Institute of Dental Science and Research Center in Belgaum, which belonged to Maratha Mandal. The animals were housed and stabilised for a period of one week. They were kept in standard conditions at a temperature of 22.3 34; relative humidity should be at least 30% and ideally not exceed 70%, with the exception of when cleaning the room, when the goal should be 50 - 60%; and light should be artificial, with the sequence being Twelve hours of light and twelve hours of darkness. Each species is kept separately. Rats were chosen at random, marked to enable personal identification, and maintained in their cage. Institutional Animal Ethical Committee provided their approval for the procedure. (IAEC) [8]

Acute Toxicity Studies: Prior to drug delivery, animals were kept fasting for the entire night. Animals were given a single oral dosage of the methanoloic extract of *Syzygium cumini* leaf (2000 and 5000 mg/kg, bw). Administer the test dosage to one animal at a time. Conduct the primary test to ascertain the LD50 in the event that the animal perishes. If the animal survives, dose four more animals consecutively, resulting in a total of animals tested. In the event that three animals perish, the maximum has been achieved and the main test is carried out. When three or more animals are killed during testing, the LD50 is less than the test dose of 2000 mg/kg, and if five animals are used, the LD50 is higher than the test dose of 2000 mg/kg[9].

Selection of Dose of the extract: A dose of 1/10th of 5000 mg/kg body weight, or 500 mg/kg body weight, was selected for the screening of nephroprotective activity in accordance with oral toxicity studies. To induce dose - dependent activity, a lower dose of 250 mg/kg and a higher dose of 500 mg/kg body weight were chosen[10].

Gentamicin - Induced Nephrotoxicity in Rats: Seven groups, each comprising six Wistar albino rats of either sex (n=6).
Group I: received normal saline was given orally for twenty one days.
Group II: received Gentamicin (100mg/kg) was administered intraperitoneally for seven consecutive days.
Group III: received Gentamicin (100mg/kg i. p) for 7 days +standard drug Cystone (500mg/kg p. o) for fourteen consecutive days.
Group IV: Received Gentamicin (100mg/kg i. p) for 7 days+ methanolic extract (250mg/kg p. o) for fourteen consecutive days.
Group V: Received Gentamicin (100mg/kg i. p) for 7 days + methanolic extract (500 mg/kg p. o) for fourteen consecutive days.

After 24 hours since the last dose, blood was drawn under mild ether anesthesia via a retro - orbital puncture, and serum was then separated by using centrifugation. Rats were slain while under the effects of ether anesthesia, and the kidneys were removed, cleaned in saline, rinsed once more, and then preserved in 10% formalin for histopathological examination[12].

Histopathology Studies
After the animal was killed, the kidney tissues were separated, rinsing them in sterile water, and cutting them into tiny pieces. In 10% formalin solution, the tissue from the slice was retained. Fixation, dehydration, cleaning, impregnation, embedding, section cutting, flattening and mounting of sections, staining of tissue, and mounting are some of the procedures used to prepare tissue for histological research[13].

Statistical Analysis
A mean and SEM were used to describe the findings. Using the software GraphPad Prism 5.0, ANOVA and Tukey's multiple comparison test, were used for the analyze experiment results. The P values were ****P0.0001, ***P0.001, **P0.01, and *P0.05 when compared to the nephrotoxic reference[14].

3. Results and Discussion

Preliminary phytochemical screening: According to a preliminary phytochemical analysis of the methanolic extract of *Syzygium cumini* leaf, there are reducing sugar, carbohydrates, alkaloids, cardiac glycosides, anthraquinone glycosides, saponins, flavonoids, saponins, terpenoids, tannins, and phenolic compounds among other substances.

Acute Toxicity Study: Studies on acute poisoning have shown that methanolic extracts of *Syzygium cumini* leaves are safe. The dose (5000mg/kg body weight) did not cause any mortility or morbidity, nor was there any negative impact noticed

Gentamicin induced nephrotoxicity in rats
General Parameters: Significant increases in body weight and urine volume were observed in nephrotoxic rats treated with a methanolic extract of *Syzygium Cumini* leaf. (Table 1).
Expressions for values include (n=6), meanSD, ****P0.0001, ***P0.001, **P0.01, *P0.05. Nephroprotective (Statistical analysis by one - way ANOVA and Tukey's multiple testing).

Table 1: Effect of Syzygium Cumini leaf extract on Body Weight and Urine Volume in a model of Nephrotoxicity Caused by Gentamicin

Group	Treatment	Dose	No of animals	Body weight (gm)	Urine volume
I	Control (Saline)	2ml	6	233.4±4.74	10.68±1.02
II	Nephrotoxic control (Gentamicin)	100mg/kg	6	194.2±4.45	5.67±0.95***
III	Gentamicin+ Cystone	500mg/kg	6	208.2±2.23***	7.75±1.02**
IV	Gentamicin+ Methanolic extract	250mg/kg	6	205.3±6.18**	6.20±0.95
V	Gentamicin + Methanolic extract	500mg/kg	6	208±5.41***	7.96±0.78**

Biochemical Parameters: When compared to a Gentamicin control, nephrotoxic animals treated with a methanolic extract of *Syzygium cumini* exhibited a significant reduction in serum creatinine, serum uric acid, serum urea, and an increase in serum total protein. (Table2). However, urinary biochemical parameters showed a substantial decline in urine creatinine, urine uric acid, urine urea, and urine total protein when compared to the Gentamicin control. Table 3).

Table 2: Effect of leaves extract of *Syzygium cumini* on Serum in Gentamicin induced

Groups	Treatments	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Total protein (mg/dl)	Serum Uric acid (mg/dl)
I	Control (saline)	0.866±0.080	23.37±1.409	5.203±0.687	3.708±0.60
II	Nephrotoxic control (Gentamicin 100mg/kg)	2.738±0.082***	52.76±1.255***	2.822±0.655***	7.722±0.596***
III	Gentamicin + Cystone (500mg/kg)	1.322±0.069****	31.66±1.356****	4.592±0.536***	4.423±0.649****
IV	Gentamicin + Methanolic extract (250mg/kg)	2.473±0.120***	48.17±2.284****	3.15±0.686	6.12±0.608***
V	Gentamicin+ Methanolic extract (500mg/kg)	1.408±0.085**	32.84±1.398****	4.368±0.348***	4.788±0.601****

nephrotoxic model

Values are expressed as (n=6), mean±SD, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05 Vs nephrotoxic control.

Table 3: Effect of leaves extract of *Syzygium Cumini* on Urine in Gentamicin Induced nephrotoxic model

Treatments	Urine Creatinine (mg/dl)	Urine urea (gm/dl)	Total protein (gm/dl)	Urine Uric acid (mg/dl)
Control (Saline)	11.64±0.67	6.45±0.63	7.42±0.62	1.74±0.08
Nephrotoxic control (Gentamicin 100mg/kg)	33.56±0.67****	16.38±0.64****	17.13±0.62****	4.13±0.08****
Gentamicin + Cystone 500mg/kg	16.22±0.66****	8.90±0.65****	9.70±0.62****	1.86±0.09****
Gentamicin + Methanolic extract (250mg/kg)	29.6±0.71****	15.15±0.61*	15.74±0.44**	3.80±0.18***
Gentamicin + Methanolic extract (500mg/kg)	20.4±0.54****	9.89±0.63****	11.27±0.59****	2.00±0.10****

Values are expressed as (n=6), mean±SD, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05 Vs nephrotoxic control.

Antioxidant Parameters: The following findings were made when observing the amount of oxidative stress brought on by gentamicin toxicity and the impact of leaf extract. Malondialdehyde level elevation and reduction of kidney superoxide dismutase, catalase, and reduced glutathione. *Syzygium cumini* leaf extract substantially increased levels of MDA while restoring the levels of biomarker antioxidant enzymes like SOD, CAT, and GSH that had been depleted. (showed in table No: 4).

Table 4: Effect of various Treatments on Gentamicin induced changes in Antioxidants and Oxidants

Groups	Treatments	SOD (units/mg protein)	GSH (µg/mg protein)	CAT (µmol/mg protein)	MDA (nmol/mg protein)
I	Control (Saline)	13.87±1.47	101.8±1.20	40.35±1.02	15.25±0.82
II	Nephrotoxic control (Gentamicin 100mg/kg)	6.79±1.44****	50.85±1.18****	12.32±1.02****	36.98±0.95****
III	Gentamicin + Cystone (500mg/kg)	11.17±1.47****	89.1±1.62****	35.02±1.01****	24.32±0.81****
IV	Gentamicin + Methanolic extract (250mg/kg)	7.89±1.48	56.19±3.58**	15.25±1.03***	35.19±0.39*
V	Gentamicin + Methanolic extract (500mg/kg)	9.63±0.76**	78.19±1.88****	30.99±1.08****	27.07±1.06****

Values are expressed as (n=6), mean±SD, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05 Vs nephrotoxic control.

Histopathology of Gentamicin induced kidney failure model: Histopathological analysis of the kidney tissue from the normal groups showed a normal glomerulus with tuft of capillaries, Bowman's capsule, tubules surrounded by columnar epithelial cells stained pink for cytoplasm, and basal nuclei blue in color with normal structure. The gentamicin - treated kidney, on the other hand, showed Bowman's capsule - encircled capillary loss and glomerulus degeneration. Significant regenerative changes were seen in the kidney tissue of rats getting *Syzygium cumini* methanolic extract.

4. Discussion and Conclusion

The extract has not shown any toxic symptoms or mortality with oral dose 5000 mg/kg hence, the extracts were safe for study. The both extract of *syzygium cumini* leaf have shown reduction in level of serum (creatinine, urea, uric acid) and increased serum total protein along with decreased urine (creatinine, urea, uric acid and total protein). There was decreased in malondialdehyde level and increased levels of catalase, superoxide dismutase and glutathione as compared with nephrotoxic group. In histopathology study both extracts showed marked regenerative change in kidney

tissue of rats. The nephroprotective activity of leaf extract of *Syzygium cumini* was found significant. The further study is required for extract analysis of chemical constituent which are responsible for nephroprotective activity of the plant.

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