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Nephroprotective Activity of Ethanolic Extract of *Musa Sapientum* Fruit Peel in Gentamicin Induced Nephrotoxicity.

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ABSTRACT

To evaluate the nephroprotective activity of ethanolic extract of *Musa sapientum* fruit peels in Gentamicin(100mg/kg, p.o. for 7 days) induced nephrotoxicity rats. Rats were treated with ethanolic extract of *Musa sapientum* fruit peel (250 and 500 mg/kg,p.o) for 7 days after gentamicin. The extent of defense was measured using levels of serum and urine parameters like creatinine, urea, uric acid and total protein. Additionally, oxidative stress parameters such as levels of Malonaldehyde (MDA), reduced glutathione (GSH) and activity of superoxide dismutase (SOD) and catalase (CAT) along with histopathology of kidney. The substantially elevated kidney weight, urine volume, serum and urine parameters like creatinine, urea, uric acid, total protein. Oxidative stress parameters such as levels of MDA, GSH, SOD and CAT activities were found to be restored towards normalization by ethanolic extract of Musa *sapientum* fruit peel comparable with standard drug Cystone. In histopathological regenerative changes were observed in kidney section of rats received ethanolic extract of *Musa sapientum* fruit peel. The results suggest that the ethanolic extract of *Musa sapientum* fruit peels possess significant nephroprotective activity.

Keywords: Nephroprotective activity, *Musa sapientum*, Gentamicin induced nephrotoxicity etc.

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INTRODUCTION

Nephrotoxicity is known to be one of the most common kidney problems worldwide. It can be defined as renal dysfunction that arise as a direct result if exposed to external agents such as drugs and environmental chemicals many therapeutic agents like aminoglycoside antibiotics, NSAID's, chemotherapeutic agents have been shown to induced clinically significant nephrotoxicity. Kidney dysfunction is characterized by increased level of serum creatinine,urea,uric acid and imbalance of blood electrolytes such as potassium and magnesium etc. Aminoglycoside antibiotics are commonly used in the treatment of bacterial infections. They have potent antibacterial activity against infections produced by gram-negative bacteria.[1]. Among several aminoglycosides, the graded of nephrotoxicity has been reported to be in following order neomycin>gentamicin>tobramycin. Gentamicin nephrotoxicity which occur in about 30 to 35% for more than 7 days treated patients.[2-3].

Plants have been reported for their protective abilities against several side effects from various synthetic drugs. Such synthetic drugs have serious implication in the functioning of liver and kidney whose diseases are major health concern of modern day. These diseases are caused by exposure to highly toxic chemicals, medications, xenobiotics, etc which occurs as a result of oxidative stress due to release of high quantities of free radicals. Natural products have play an important role throughout the world in treating and preventing human diseases.[4] Hence to overcome the side effects of modern medicine; the herbal drugs have been used for nephroprotective activity [5].

The active constituents present in *Musa sapientum* fruit peel is flavonoids, phenolic compounds, tannins, alkaloids, glycosides, saponins, dietaryfiber, anthocyanins, tannins, carbohydrates, high source of minerals and vitamins. These compounds have been reported to exert various biological and pharmacological effects such as antibacterial, antihypertensive, antidiabetic, antioxidant and anti-inflammatory activities[6]. Traditionally it is used in the treatment of nephritis[7] and also help in controlling the kidney failure[8]. But use of this *Musa sapientum* fruit peel in the treatment of kidney disorder is scientifically not proved. So the present study was aimed to evaluate nephroprotective activity of aqueous and ethanolic extract of *Musa sapientum* fruit peel in gentamicin induced nephrotoxicity in experimental rats.

MATERIALS AND METHODS

Plant Material: In the present study, the fruit peel of *Musa sapientum* were collected locally from in and around Belagavi district also Plant were authenticated by Dr. Harsh Hegde, taxonomist of regional Medical research Centre (Plant authenticated No: RMRC-1429) Belagavi.

Preparation of extract: The authenticated shade dried fruit peels of Musa sapientum belong to the family Musaceae were reduced to coarse powder (40 size mesh). It was subjected to continuous hot extraction (soxhalation) process by using Ethanol as menstrum continued the process till product becomes colorless or for 7- days. The extract was concentrated by using rotary evaporator. The extract was stored in airtight container in refrigerator for further investigation [9]

Drugs and Chemicals: Cystone were obtained from Himalaya Drug Company Bangalore, India. Gentamicin was procured from Micro Labs Ltd Bangalore. Creatinine, urea, uric acid and total protein kit were obtained from Coral Clinical Systems, Verna Goa, India.

Experimental Procedure: Female Swiss albino mice weighing 20- 25gm were used for acute toxicity study and albino rats (Wistar) of either sex weighing 200-250gm were used for Nephroprotective activity. They were procured from Sri Venkateshwara Enterprises, Bengaluru. The animals were housed, stabilized for 1 week; they were maintained under standard condition at 25^0c ± 2^0c temperature; 60 ± 5% relative humidity & 12-hour light - dark cycle. Rats were fed with standard pellet diet and water ad libitum throughout the course of the study. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC). The experiments were conducted as per the guidelines of CPCSEA[10] and CPCSEA No:263.

Acute Toxicity Studies: Animals were kept overnight fasting prior to drug administration. Animals received a single oral dose (2000 and 5000 mg/kg, bw) of ethanoloic extract of *Musa sapientum* fruit peel. After the administration of *Musa sapientum* fruit peel extract, food was withheld for further 3-4 h. Animals were

observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of two weeks.

Selection of Dose of the extract:

For the assessment of nephroprotective activity $1/10^{th}$ of 5000 mg/kg body weight i.e. (500mg/kg body weight) dose were chosen according to the toxicity studies, to elicit dose dependent activity a lower 250mg/kg and higher dose 500mg/kg body weight were selected [3].

Gentamicin-Induced Nephrotoxicity in Rats: Wistar albino rats of either sex were divided into five groups (n= 6).

Group I: Normal Control: received normal saline by orally for 7 days.
Group II: Toxic Control: received Gentamicin (100mg/kg) by intraperitoneally for 7 consecutive days.
Group III: Standard: received gentamicin (80mg/kg i.p)+standard drug cystone (500mg/kg p.o) for 14 consecutive days.
Group IV: Received gentamicin (100mg/kg i.p) + Ethanolic extract test drug (250mg/kg p.o) for14 consecutive days.
Group V: Received gentamicin (100mg/kg i.p) + Ethanolic extract test drug (500 mg/kg p.o) for 14 consecutive days. After 24h of last dose, the blood sample were collected by puncturing retro-orbital puncture under light ether anaesthesia and serum was separated by centrifugation. Rats were sacrificed by ether anaesthesia kidneys were excised, rinsed clean in saline and preserved in 10% formalin for histopathological study [3].

Histopathology Studies:A portion of the kidney was fixed in formalin (10%) and subject to histopathology studies. The section of the kidney was processed and embedded in paraffin wax section of about 4-6 µm were made and stained with hematoxylin and eosin and photographed [3].

Statistical Analysis: The results will be expressed as a mean ± standard deviation (SD) of six animals in each group. The results will be analyzed statistically using one- way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using Graph Pad prism 5.0 software.

RESULTS

Preliminary phytochemical screening: Results of the preliminary phytochemical investigation on ethanolic extract of *Musa sapientum* fruit peels are shows the presence of carbohydrates, saponins, flavonoids, saponins, Tannins and phenolic compounds etc.

Acute Toxicity Study: There was no morbidity and mortality observed throughout the study. Ethanolic extract of *Musa sapientum* fruit peel was found not toxic to the experimental animals up to the high dose of 500 mg/kg body weight.

Gentamicin induced nephrotoxicity studies

General Parameters: Nephrotoxic animals treated with ethanolic extract of *Musa sapientum* fruit peel showed significant increase in body weight as well as urine volume. (showed in table No: 1).

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Table 1: Effect of fruit peel extract of *Musa sapientum* on Body weight and urine volume in Gentamicin induced nephrotoxic model.

Group	Treatment	Dose	No of animals	Body weight(gm)	Urine volume
I	Control(Saline)	2ml	6	240.47±1.25***	10.85±0.98***
II	Nephrotoxic control (Gentamicin)	100mg/kg	6	201.15±0.96	5.90±0.37
III	Gentamicin + Cystone	500mg/kg	6	235.83±2.87***	9.77±0.92***
IV	Gentamicin +Ethanolic extract	250mg/kg	6	218.51±3.82**	6.08±0.52*
V	Gentamicin +Ethanolic extract	500mg/kg	6	234.08±0.74***	9.81±1.02***

Values are expressed as (n=6), mean±SD, ***P<0.001, **P<0.01. *P<0.05. (Statistically analysed by one-way Anova followed by Dunnet's t-test).

Biochemical Parameters: Nephrotoxic animals treated with ethanolic extract of *Musa sapientum* fruit peel showed significant decrease in serum creatinine, serum uric acid, serum urea and increase in serum total protein when compared to Gentamicin control (showed in table No: 2). Where as urinary biochemical parameters showed significant decrease in urine creatinine, urine uric acid, urine urea and urine total protein when compared to Gentamicin control (showed in table No:3).

Table 2: Effect of fruit peel extract of *Musa sapientum* on Serum in Gentamicin induced nephrotoxic model.

Groups	Treatment	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Total protein (gm/dl)	Serum Uric acid (mg/dl)
I	Control (Saline)	0.67±0.205***	32.56±0.505***	7.33±0.78***	1.73±0.251***
II	Nephrotoxic control (Gentamicin 100mg/kg)	1.87±0.131	61.96±3.02	5.9±0.965	4.56±0.216
III	Gentamicin + Cystone (500mg/kg)	0.67±0.020***	41.5±2.45***	6.9±0.458***	1.86±0.157***
VI	Gentamicin +Ethanolic extract (250mg/kg)	1.05±0.206	40.66±3.56***	5.6±1.21	3.49±1.25*
V	Gentamicin +Ethanolic extract (500mg/kg)	0.73±0.062***	39.16±0.87***	7.11±0.264***	1.66±0.05***

Values are expressed as (n=6), mean±SD, ***P<0.001, **P<0.01. *P<0.05. (Statistically analysed by one-way Anova followed by Dunnet's t-test).

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Table 3: Effect of fruit peel extract of *Musa sapientum* on Urine in Gentamicin induced nephrotoxic model.

Groups	Treatment	Urine Creatinine (mg/dl)	Urine Urea (mg/dl)	Total protein (gm/dl)	Urine Uric acid(mg/dl)
I	Control(Saline)	12.63±1.45***	18.03±0.94***	7.56±0.81***	1.75±0.33***
II	Nephrotoxic control (Gentamicin 100mg/kg)	34.7±3.011	26.4±5.54	17.8±1.17	4.14±0.417
III	Gentamicin + Cystone (500mg/kg)	15.8±1.82***	15.16±3.09***	7.83±0.15	1.76±0.55
VI	Gentamicin +Ethanolic extract (250mg/kg)	20.73±3.57**	28±3.12	14.8±2.45*	3.4±0.44*
V	Gentamicin +Ethanolic extract (500mg/kg)	16.36±2.02***	17.6±2.5***	7.73±0.75***	1.8±0.30***

Values are expressed as (n=6), mean±SD, ***P<0.001, **P<0.01. *P<0.05. (Statistically analysed by one-way Anova followed by Dunnet's t-test).

Antioxidant Parameters: Oxidative stress parameters of kidney homogenate were measured. A significant increase in MDA while declines in GSH levels SOD and CAT activities were found in Gentamicin treated group as compared to normal control. Treatments with ethanolic extract of *Musa sapientum* fruit peel (250 & 500 mg/kg, p.o) and Cystone (500 mg/kg, p.o) significantly decrease MDA levels while significant elevation in GSH level, SOD and CAT activity as compared to Gentamicin control (showed in table No:4).

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

Gps	Treatment	SOD (units/mg protein)	GSH (µg/mg protein)	CAT (µmol/mg protein)	MDA (nmol/mg protein)
I	Control(Saline)	13.85±1.06***	100.35±2.4***	44.2±0.14***	12.7±1.55***
II	Nephrotoxic control (Gentamicin 100mg/kg)	6.7±0.2	51.4±2.96	15.4±1.89	34.35±2.34
III	Gentamicin + Cystone (500mg/kg)	12.8±2.54***	87.45±3.82**	37.35±2.40***	14.65±0.63***
VI	Gentamicin +Ethanolic extract (250mg/kg)	9.7±0.84**	82.3±1.62**	37.9±2.45***	26.7±1.27*
V	Gentamicin +Ethanolic extract (500mg/kg)	12.85±0.49***	91.95±0.66***	34.3±0.84**	13±0.84***

Values are expressed as (n=6), mean±SD, ***P<0.001, **P<0.01. *P<0.05. (Statistically analysed by one-way Anova followed by Dunnet's t-test).

Histopathology of Gentamicin induced nephrotoxicity model: Histopathological examination of kiney section of the normal kidney (Fig.A) showed a normal glomerulus with tuft of capillaries surrounded by Bowman's capsule with tubules lined by columnar epithelial cell cytoplasm staining pink colour and basal nucleus blue in colour with normal architecture. On the other hand, Gentamicin treated kidney section (Fig.B) showing

glomerular degeneration with loss of capillaries surrounded by Bowman's capsule. The tubules showed nephrotoxicity with severe tubular degeneration and loss of tubular architecture which also evident by accumulation in the centre of the tubule which will result in marked congestion, edema, dialation, necrosis, interstitial and pelvic inflammation. Animals treated with the lower dose (250mg/kg) of ethanolic extract of *Musa sapientum* fruit peels showed (Fig. D) minimal cellular damage when compared to nephrotoxic group and animals treated with the higher dose (500mg/kg) of ethanolic extract of *Musa sapientum* fruit peel showed (Fig. E) almost normal glomerulus with tuft of capillaries surrounded by Bowman's capsule, and tubular arrangements with minimal blood vessel congestion, epithelial cell desquamation, and presence of tubular cast with very few inflammatory cells as like animals treated with standard drug cystone (Fig. C).

Figure 1: Histopathology of Gentamicin induced nephrotoxicity model:

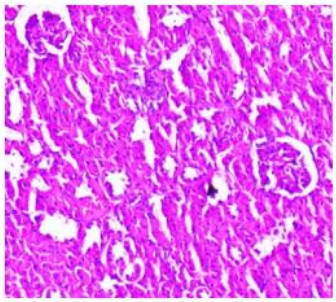


Fig (A) Normal kidney

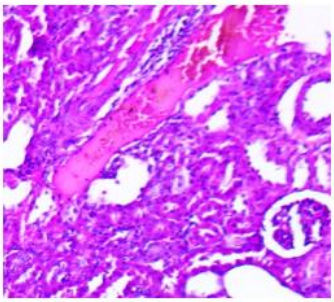


Fig (B) Treated with Gentamicin

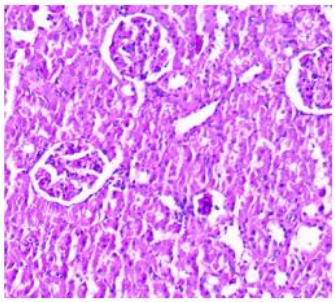
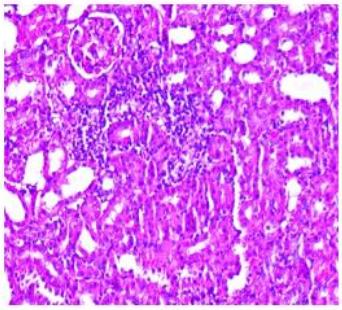
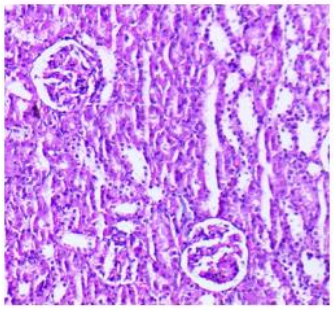


Fig (C) Treated with Cystone



(D) Treated with Ethanolic extract 250mg/kg



(D) Treated with Ethanolic extract 500mg/kg

DISCUSSION AND CONCLUSION

Nephrotoxicity is a poisonous effect due to drugs and its over dosage on the kidney[11]. Gentamicin induced nephrotoxicity is a model of acute renal failure caused by oxidative stress generated through the induction of superoxide. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localised mainly in the proximal tubules. It is complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal tubular necrosis, followed by deterioration and renal failure. The toxicity of gentamicin is believed to relate to generation of reactive oxygen species (ROS) in kidney[12].

A significant increase in Serum creatinine, Urea, Uric acid while declined in Total protein. Administration of with Musa sapientum fruit peel extract(250 & 500 mg/kg, p.o) significantly decrease Serum creatinine, Urea, Uric acid while significant elevation in total protein level and in case of urine biochemical analysis Significant increase in creatinine, Urea, Uric acid and total protein Administration of with *Musa*

sapientum fruit peel extract(250 & 500 mg/kg, p.o) significantly decrease creatinine, Urea, Uric acid and total protein

A significant increase in MDA while declines in GSH content, SOD and CAT activities were found in Gentamicin treated group. Administration of with *Musa sapientum* fruit peel extract(250 & 500 mg/kg, p.o) significantly decrease MDA levels while significant elevation in GSH level, SOD and CAT activity dose dependent manner as compared to Gentamicin control. Histopathological studies on isolated kidney revealed that the both extract, reversed the kidney damage and also restored normal kidney architecture. In summary, the fruit peel of *Musa sapientum* in an ethanolic extract showed statistically significant nephroprotective activity. Therefore, it seemed that Ethanolic fruit peel extract, due to its antioxidant properties, reduced cellular damages in kidneys. This nephroprotective activity of the Musa sapientum fruit peel extract may be due to antioxidant activity which may be due to the presence of flavonoids and phenolic compounds as reported in our study. The results of our study reveal the nephroprotective activity of Musa sapientum fruit peel extract.

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