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EFFECT OF AQUEOUS, ETHANOL AND ACETONE EXTRACT OF *SESBANIA GRANDIFLORA* IN GENTAMICIN - INDUCED NEPHROTOXIC RATS

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ABSTRACT

Phytotherapeutic medicine from medicinal plants has been greatly explored due to its bioactive secondary metabolites production, cost effective, cheaply available and no side effects.In this presentation we studied the nephroprotective effects of aqueous, ethanol and acetone extracts of medicinal plant *Sesbania grandiflora* leaves against gentamicin affected animals. Animals were grouped into six, each groups has six animals (Group I-normal, II- induced, III- standard drug, IV- aqueous extract, V- ethanol extract, VI- acetone extract). Drug and extracts were orally administered and measured urea, uric acid, creatinine and total protein in the control and treated groups. The animal groups I, II, and III shows increased levels of urea, uric acid and creatinine and decreased levels of protein ($p<0.05$) indicates gentamicin induced nephrotoxicity in rats. The animals treated with extracts has shown decreased levels ($p<0.001$) of urea, uric acid and creatinine and increased levels of protein in blood serum compared with controls (Group IV, V, and VI). Further the histopathological examinations also confirm the curative properties of extracts of *Sesbania grandiflora* leaves against nephrotoxicity. Gentamicin affected renal tissue shows severe necrosis and degeneration in tubular compared than control groups animals. These swelling and degenerated tissue in kidney was restored in extracts treated animal groups.

KEYWORDS: *Sesbania grandiflora*, Kidney, Nephrotoxocity,

INTRODUCTION

Kidney is the primary and complex organ of the urinary system which purifies the blood by removing wastes, excretion and maintaining the fluid homeostasis, electrolyte balance, blood pressure, etc.[1] Function of kidney was damaged or injured is called kidney failure or nephrotoxicity. Nephrotoxic injury is the third most common problem of the renal system lead to acute renal failure, in which the kidneys the kidneys suddenly lose their ability to function and chronic renal failure, in which kidney function slowly deteriorates.[2] Nephrotoxic injury occurs in one or both kidneys that results from exposure to toxic materials such as drugs, chemicals, etc. A number of antibiotics including penicillin, cephalosporin, tetracycline, gentamicin and sulfonamides are considered as nephrotoxicants.[3, 4]

Among the antibiotics gentamicin accumulated on human tissue generates reactive oxygen species which reduced the function of renal system, which is otherwise known as aminoglycoside.[5] Gentamicin causes nephrotoxicity by irreversibly binding the 30S subunit of the bacterial ribosome, inhibiting protein synthesis in renal cells, necrosis of cells in the proximal tubule.[6] A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plant solve some of the side effects caused by chemotherapeutic agents.[7] Nephroprotective activity of medicinal plants against kidney injuries studies have reported that are *Andrographis paniculata*[8], *Crotonzambesicus*[9], *Vitisvinifera*[10], *Salix caprea*[11], *Syzygiumcumini*[12], *TinosporaCardifolia*[13], *Ocimumgratissimum* [14], *Kalanchoepinnata*[15]etc.

Sesbania grandiflora belonging to the family,Fabaceae it is known as Agathi. It is a fast-growing tree with rounded leaves and white, red or pink color flowers. The fruits look like flat, long and thin green beans.[16] The leaves used for the treatment of anemia, bronchitis, opthalmia, inflammation, leprosy, gout, and rheumatism.[17] The plant contains phytochemicals like arginine, cysteine, histidine, isolcucine, phenylalanine, tryptophan, valine, threonine, alanine, aspargine, aspartic acid, oleanolic acid, galactose, Rhamnose&glucuronic acid. Fruits are bitter & acrid, laxative, fever, pain, bronchitis, anemia, tumors, colic, jaundice, poisoning. Root is used for treatment of Rheumatism, Expectorant, Painful swelling, Catarrh.[18] In the present study effect of*Sesbania grandiflora*leaves extract in gentamicin induced nephrotoxicity in albino rats was evaluated by using biochemical and histopatological examinations.

MATERIALS AND METHODS

Preparation of plant leaf extracts: *Sesbania grandiflora*leaves were collected and dried at room temperature for 3-5 days and grinded into powder. About 100 g of dried powder was mixed with 100 ml double distilled water for preparation of aqueous leaf extract and filter the solution through whatmann No 1 filter paper and collect the supernatant. For ethanol and acetone extract, 100g of dried leaf powder was mixed with 80% ethanol and 80% acetone respectively, extracted using soxhlet apparatus which is maintained at 55° C for 24 hr. After 24 hr ethanol and acetone solvents were eliminated at room temperatureand stored. The resultants (aqueous, ethanol and acetone extracts) were appeared dark greenish color and then used for assay of nephroprotective activity.

Experimental Design for nephroprotective Activity of *Sesbaniagrandifolia*

Adult male Wister albino rats maintained in animal caging system weighing between 150g-170g were used for the in vivo determination of nephroprotectiveactivity. Animals were divided into six groups insix rats each:

Group I (Normal): Animals were **o**rally receiveddistilled water for 10 days.

Group II (Induced): Orallyadministeredwith gentamicin (80 mg/kg body weight) only for 10 days to induce nephrotoxicity.

Group III (Standard): Orally administered with Cystone (20 mg/kg body weight) along with gentamicin (80mg/kg body weight) for 10 days.

Group IV (Treatment): Animals were orally administered with aqueous leaf extract (300 mg/kg body weight) along with Gentamicin (80mg/kg body weight) for 10 days.

Group V (Treatment): Orally administeredwith ethanol leaf extract (300mg/kg body weight) along with Gentamicin (80 mg/kg body weight) for 10 days.

Group VI (Treatment): Animals orally administeredacetone leafextract (300mg/kg body weight) along with Gentamicin (80 mg/kg body weight) for 10 days.

Cystone was used as positive control for comparing nephroprotective potential of different leaves extract of *S. grandiflora*. Gentamicin is act as nephrotoxin which induces the kidney damage.

Biochemical and histopathologicalstudies

Biochemical analysis: At the end of the experiment all the group of rats were anesthetized with chloroform. Blood sample was collected from jugular veinin plain plastic tubes and

centrifuged to separate serum. Serum was analyzed for urea[19], uric acid[20], creatinine[21] and total protein.[22]

Histopathological studies

After blood sampling, all animals from every group were sacrificed and separated the small slices of kidneys by dissection procedure and fixed in 10% formalin solution. The fixed formalin fixed kidneys were embedded in paraffin wax and serial section were made. Sections are stained with haemotoxylin and eosin then examined using light microscope.Serum was separated from the blood for the analysis of the parameters like Blood Urea

Statistical analysis

Data were analyzed using one way Analysis of Variance (ANOVA) and expressed as mean± S.E.M. value of $p< 0.05$ is considered as Statistical significant.

RESULTS AND DISCUSSION

Biochemical studies

S. grandiflora leaf extract has significant nephroprotective activity was confirmed by estimating biomarkers. Serum samples from treated and untreated animals were subjected to biochemical assays like urea, uric acid, creatinine and total protein level. Group I animals showed normal level of presence of these parameters are 30.16±1.72mg/dl, 5.08 ±0.21 mg/dl, and 0.79 ± 0.02 mg/dl, respectively (Figure 1). The parameters urea, uric acid and creatinine were shows increased levels with significant decreases ($p<0.05$)in gentamicin treated group II animals when compared with group I, whereas aqueous, ethanol and acetone extract of *S. grandiflora* leaves treated groups IV (34.16 ± 2.48 mg/dl, 5.82 ± 0.33 mg/dl and 0.91± 0.13 mg/dl), V (31.28 ±0.54 mg/dl, 5.21 ± 0.12 mg/dl and 0.83± 0.54mg/dl) and VI (32.08 ± 0.21 mg/dl, 5.56 ± 0.13 mg/dl and 0.87±0.21mg/dl) showed the recovery from raised level (Table 1 and 2).

The normal protein level in blood is 6.91± 0.59 mg/dl observed in control group I animals which is decreased in gentamicin induced nephrotoxic group II animals is to be 3.02 ± 0.84 mg/dl ($p<0.05$). This decreased levels was recovered in group IV, V and VI animals were orally administered with aqueous, ethanol and acetone extract of *S. grandiflora* leaves to be 7.55± 0.77 mg/dl, 7.61 ± 0.12mg/dl, and 7.46 ± 0.13 mg/dl, respectively. These extract significantly increases ($p<0.001$) the protein level in blood serum. Significant increase in

urea, uric acid and creatinine is observed gentamicin induced nephrotoxic rats whereas no significant increase is observed in different solvent extracts of *S. grandiflora*leaves treated group of rats. Results are shown in Table 2 and Fig 2.The nephroprotective activity of *S. grandiflora*maybe due to the presence of phytochemical constituents such as flavonoids, alkaloid, polyphenol etc.

Histopathological study

Histopathological study showing the protective activity of *S. grandiflora*leaf extracts against changes in renal system induced by gentamicin in the kidney tissue of different experimental groups. The histopathological pattern of normal kidney showing normal tubular brush borders and intact glomeruli and Bowman's capsule (Figure 3A). Fig 3B shows gentamicin affected kidney tissue revealed severe necrosis and degeneration in tubular. Fig 3C shows the repaired kidney tissue from gentamicin toxic affection. Fig 4(A& C)shows the gentamicin treated rats with aqueous and acetone extract of *S. grandiflora* leaves illustrated that normal tubular pattern with a mild degree of swelling and necrosis. Gentamicin induced nephrotoxic rats treated with the ethanol extract showed that improved detoxification in the kidney tissue (Figure 4B). Similarly, Masuda et al[23] reported that *Grifolafrondosa* also possess the nephroprotective activity against cisplatin induced nephrotoxicity, which correlates our results.

Kidney represents the major controlsystem maintaining body homeostatsis. Serum urea, protein, creatinine and uric acid are useful biomarkers in evaluating of nephrotoxicity.[24] Serumbiochemical parameters such as urea andcreatinine were found to be increased significantly after gentamicin administration,clearly indicating renal impairment in kidney. Level of total protein in blood serum islikely to be decreased if there is inhibition ofprotein synthesis or if degradation of protein ispromoted.[25] Hydrogen peroxide and reactive oxygen species (ROS) were generated in rat renal system due to the administration with toxic gentamicin. This abnormal production of ROS may damage and induce cellular injuries and necrosis through the mechanisms includes peroxidation ofmembrane lipids, protein denaturation andDNA damage. The aqueous, ethanol and acetone extract of *S. grandiflora* was found to be normalize the altered blood urea, uric acid, creatinine and total protein bring about a marked recovery in kidneys as evidenced microscopically.

Table 1: Bioactivity of aqueous, ethanol and acetone extract of *S. grandiflora* leaves on blood urea and uric acid in gentamicin induced nephrotoxic rats

Parameters	Blood Urea	Blood Uric acid
Group I (Normal)	30.16±1.72	5.08 ±0.21
Group II (induced)	59.03± 2.19*	8.28 ± 0.54*
Group III (Standard drug)	34.83 ± 3.06***	5.95 ± 0.21***
Group IV (Aqueous)	34.16 ± 2.48**	5.82 ± 0.33**
Group V (Ethanol)	31.28 ±0.54***	5.21 ± 0.12***
Group VI (acetone)	32.08 ± 0.21***	5.56 ± 0.13**

* **p< 0.05,** ** **p < 0.01,*******p < 0.001** value are considered statistically significant (BMRT)

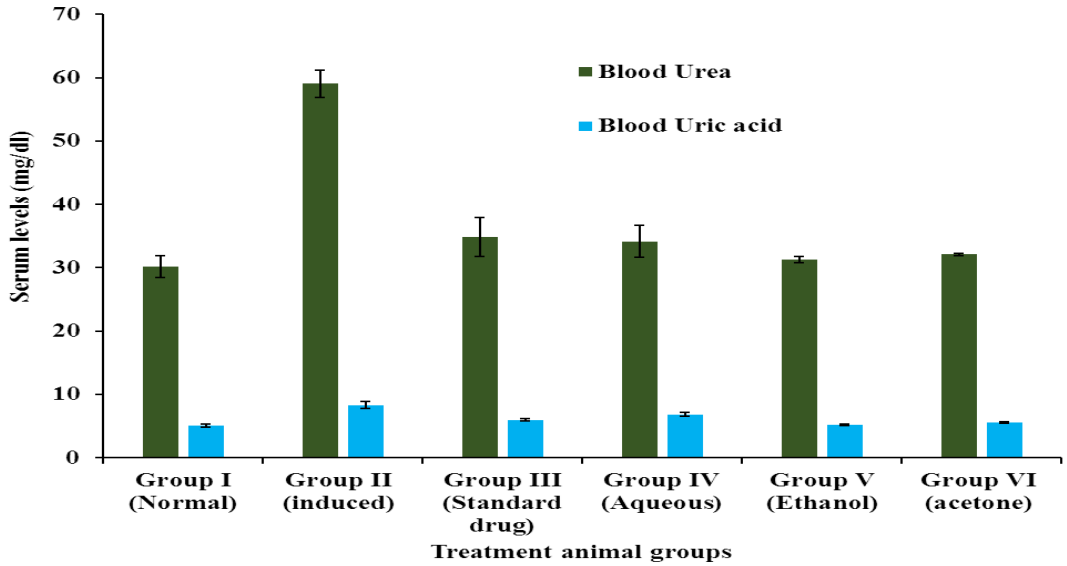

Figure 1: Bioactivity of aqueous, ethanol and acetone *S. grandiflora* leaves extract on the alterations of urea, and uric acid level in blood

Table 2:Bioactivity of aqueous, ethanol and acetone extract of *S. grandiflora* leaves on creatinine and level of total protein in gentamicin induced nephrotoxic rats

Parameters	Creatinine	Total protein
Group I (Normal)	0.79 ± 0.02	6.91± 0.59
Group II (induced)	2.88 ± 0.11*	3.02 ± 0.84*
Group III (Standard drug)	1.01 ± 0.12***	7.55 ± 0.70***
Group IV (Aqueous)	0.91± 0.13**	7.55± 0.77***
Group V (Ethanol)	0.83± 0.54***	7.61 ± 0.12***
Group VI (acetone)	0.87±0.21***	7.46 ± 0.13**

* **p< 0.05,** ** **p < 0.01,*******p < 0.001** value are considered statistically significant (BMRT)

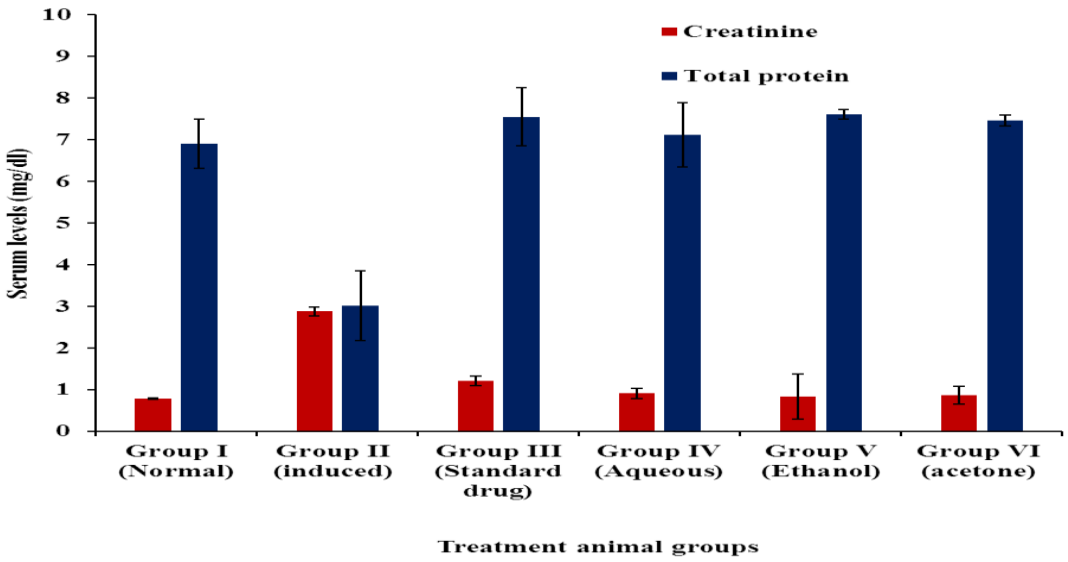

Figure 2:Bioactivity of aqueous, ethanol and acetone *S. grandiflora* leaves extract on the alterations of creatinine, and total protein level in blood

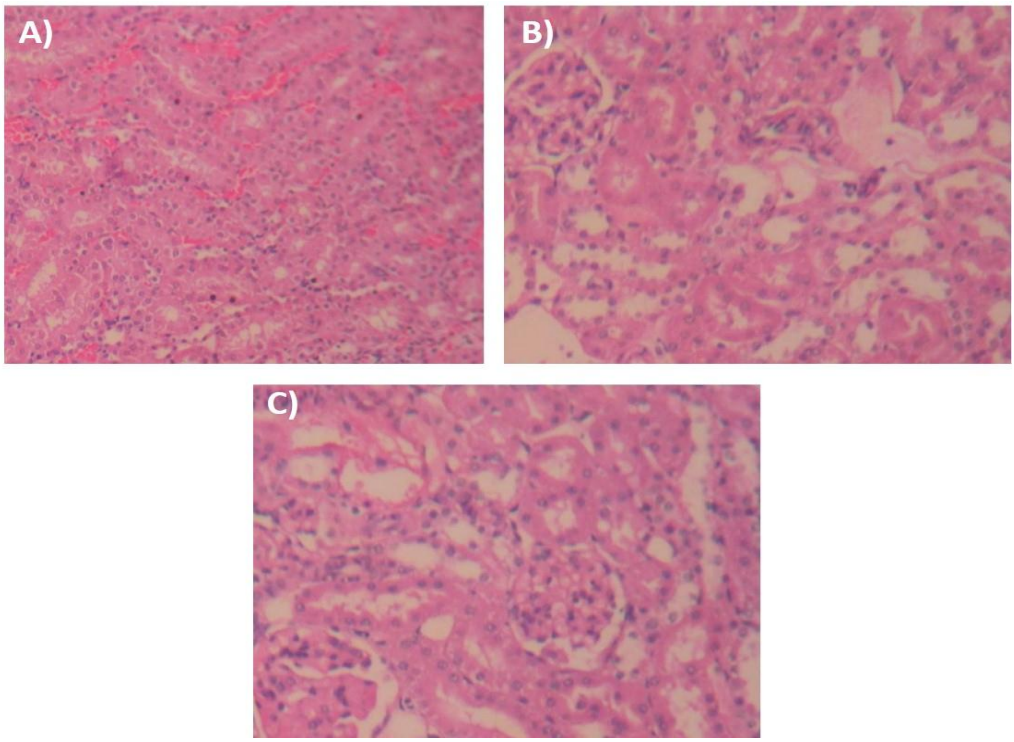

Figure 3: Histological sectioning of (A) normal kidney showing tubular brush borders and intact glomeruli in renal tissues without alterations (B) Representing the tubular necrosis in gentamicin treated animals (C) shows microscopic observation of normalized kidney structure on treated with cystone is a positive control

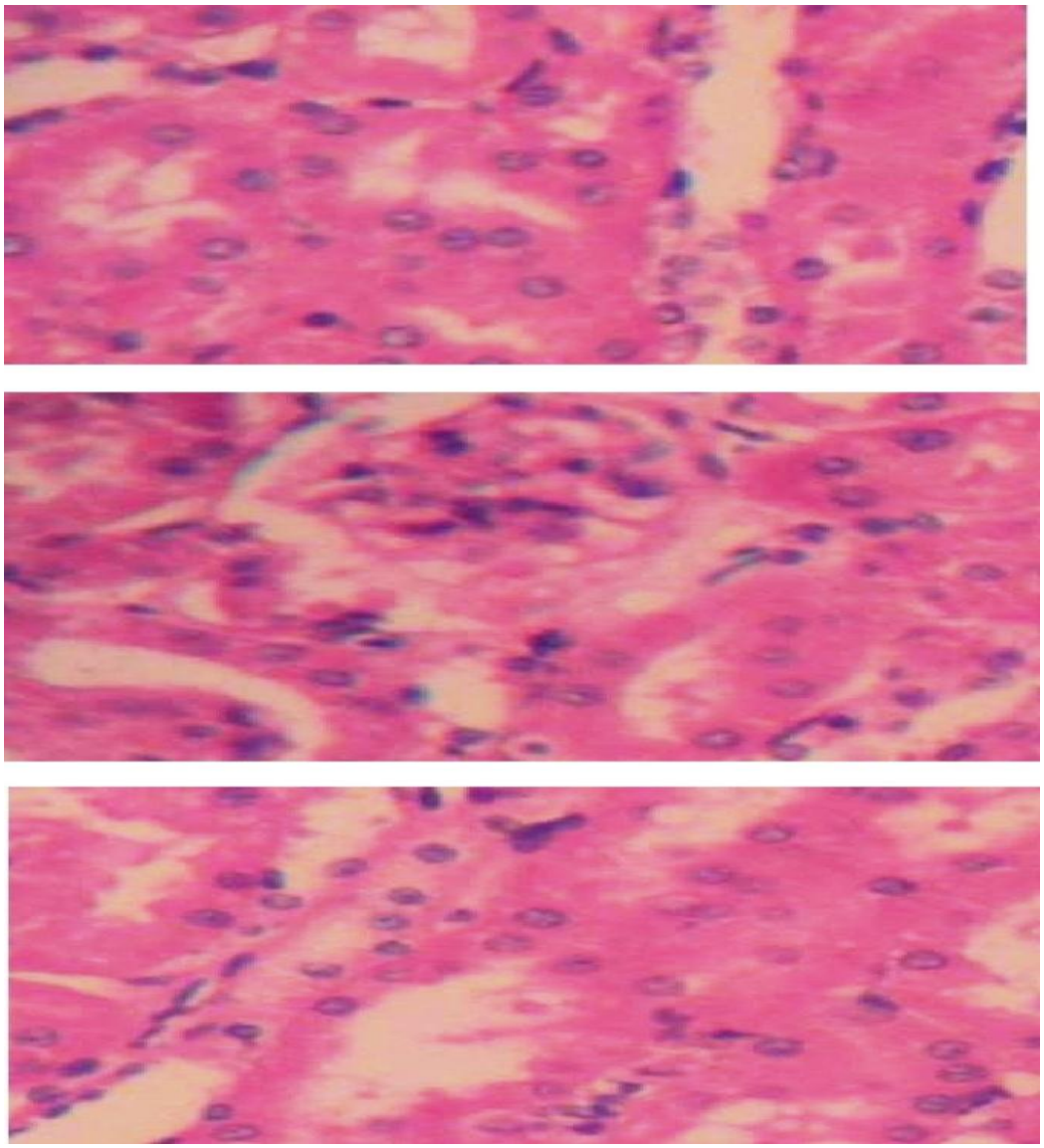

Figure 4: Histological structure of kidney treated with (A) aqueous extract (B) ethanol extract (C) acetone extract of *S. grandiflora* leaves on gentamicin induced nephrotoxicity rat animals revealed normalized and restored function of renal system

CONCLUSION

The results of our present study concluded that extracts of *S. grandiflora* leaves possesses significant nephroprotective activity against gentamicin induced nephrotoxicity rats. Leaves extracts of *S. grandiflora* were prepared by using different solvents like aqueous, ethanol acetone and orally administered to renal failure rats. Gentamicin treated animals (group II) shows increased levels of urea, uric acid and creatinine and decreased levels of protein than control group I & II. Plant extract of *S. grandiflora* possesses almost equipotent nephroprotective activity when compared with standard drug treated animal group III. The extract of *S. grandiflora* at adose level of 300mg/kg body weight was found to normalize the

abnormal levels of urea, uric acid, creatinine and protein and noticed the marked recovery in kidneys was confirmed by histopathological study. Biochemical and histopathological results were indicated that the leaves extract of *S. grandiflora* has profound nephroprotective activity against gentamicin treated rats. The activity elicited by the extract might be due to presence of bioactive compounds like flavonoids, alkaloids, steroids, lipids, triterpenoids etc. These results suggested that potential use of aqueous, ethanol and acetone extract of *S. grandiflora*, a novel useful phytotherapeutic agent for nephrotoxicity.

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