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Phytochemical and Nephroprotective Activity of *Hibiscus Cannabinus* Against Gentamycin Induced Nephrotoxicity in Rats

KEYWORDS: *Hibiscus cannabinus*, Gentamycin, Nephrotoxicity,Nephroprotectivity.

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ABSTRACT *Protective effect of Hibiscus cannabinus leaves extract on gentamycin induced nephrotoxicity in albino wistar rats were investigated by analyzing various biochemical parameters. Gentamycin induced kidney damage was well manifested by significant increase in renal parameters like serum creatinine, serum uric acid, blood urea nitrogen, serum urea and weight of kidney. The oral administration of ethanolic leaves extract of Hibiscus cannabinus (250mg/kg and 500mg/kg,p.o) along with gentamycin reversed these altered parameters to normal level which indicating the nephroprotective efficacy of Hibiscus cannabinus against gentamycin induced kidney injury. Further extensive studies are required for its potential uses in clinical practice.*

INTRODUCTION

Renal failure is a common clinical syndrome. It is defined as a rapid decline in kidney function. Resulting in abnormal retention of blood urea and serum creatinine, which must be excreted. Renal disease is the ninth leading cause of death. Approximately, 19 million adults have chronic renal disease and an estimated 80,000 persons have chronic kidney failure diagnosed annually in India. Recent literature, have shown a prevalence of chronic renal failure of 0.16% and 0.79% in India. Till date for End Stage Renal Failure, renal replacement is the only therapy. In case, of nonavailability of kidney, dialysis is the only alternative, which unfortunately is severely limited by several constraints including a good amount of expenditure. No exclusive drug has been reported so far, as such in any category of medical treatment. Nephrotoxicity is the third most common problem of the renal system with an estimated lifetime risk of 2-5% in Asia, 8-15% in Europe and America and around 20% in the

Middle East[1].

Hibiscus cannabinus (Malvaceae) is an annual or perennial herbaceous bush and has several forms with varying colors of flowers. It is native to China and grown widely as an ornamental plant throughout India. The flowers are considered emollient, and an infusion of the petals is used as a demulcent. Its decotion is given in bronchial catarrh in India. Previous studies show that the plant possesses anticomplimentary, antidiarrhetic and antiphologistic activities[2]. The leaves and flowers have been found to be effective in the treatment of various disorders[3,4].

MATERIAL AND METHOD

Preparation of plant extract: 100gram of *Hibiscus cannabinus* leaves was powdered, dried and continuously extracted for 48hrs with ethanol in a Soxhlet apparatus. The collected extract was stored at 0-4°C until used. The plant extract was pooled and evaporated to dry at 60°C.

Preliminary Phytochemical Screening

Preliminary phytochemical investigation was carried out on ethanolic leaves extract of *Hibiscus cannabinus* for detection of various phytochemicals by following standard methods described in practical Pharmacognosy by C.K. Kokate and R.K. Khandelwal.

Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the Osmania medical college animal house. Before and during the experiment, rats were fed with standard diet. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, and dark/light cycle and relative humiditya. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

Gentamycin Induced Nephrotoxicity In Rats:

The Wistar albino rats (180-200g) of either sex will be randomly divided into 5 groups of 6 each. The different groups will be assigned as described below.

Group I : Vehicle control

Group II : Nephro toxic control(Gentamycin 100 mg/kg)

Group III : *Hibiscus cannabinus*(250 mg/kg,p.o) + Gentamycin (100 mg/kg)

Group IV : *Hibiscus cannabinus*(500 mg/kg,p.o) + Gentamycin (100 mg/kg)

Group V : Standard polyherbal drug cystone (5 ml/kg,p.o) + Gentamycin (100 mg/kg)

Experimental procedure

The gentamicin-treated groups received 100 mg/kg/ day gentamicin by the intraperitoneal (i.p.) route. Rats in the control group I were given sterile saline solution for 8 days. Group II received 100 mg/kg gentamicin i.p alone for for 8 days. Group III received 100 mg/kg gentamicin i.p. and *Hibiscus cannabinus* 250 mg/kg/ p.o. for eight days and Group IV received 100 mg/kg/ gentamicin i.p. and *Hibiscus cannabinus* 500 mg/kg/p.o. for eight days. Group V received 100 mg/kg/ gentamicin i.p. and standard polyherbal drug cystone (5 ml/kg; p.o) for eight days.

After dosing on the 8th day, blood samples were collected

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via cardiac puncture method at the end of these 24 h. The serum was rapidly separated and processed for determination of serum creatinine, serum uric acid, blood urea nitrogen (BUN) and serum urea using commercially available kits of Span Diagnostics. Changes in kidney weight were recorded. Three rats per group were sacrificed and both kidneys were isolated from each rat. The kidneys were weighed and processed for histopathological examination.

Histopathological examination of Kidney

The kidneys were sectioned longitudinally in two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin and eosin and were observed under a computerized light microscope. The data obtained was analyzed using one-way ANOVA followed by Dunnet's multiple comparison test. $P < 0.01$ was considered significant.

RESULTS

Preliminary phytochemical screening

Results of the preliminary phytochemical investigation on *Hibiscus cannabinus* leaves are shows the presence of alkaloids, flavonoids, saponins, steroids, glycosides etc.

Biochemical parameters

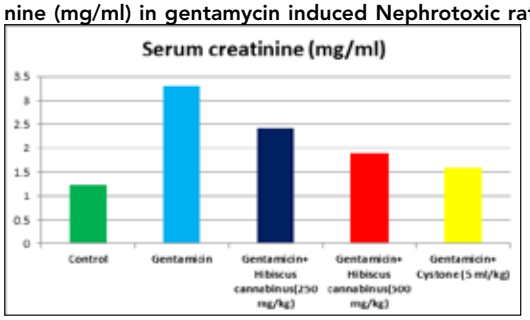
Nephrotoxic animals treated with *Hibiscus cannabinus* showed significant decrease in serum creatinine, serum uric acid, Blood urea nitrogen, serum urea and weight of kidney when compared with Nephrotoxic group II.(Table 1 and Graph I-V)

Table 1: Effect of *Hibiscus cannabinus*on biochemical parameters in gentamycin induced Nephrotoxic rats.

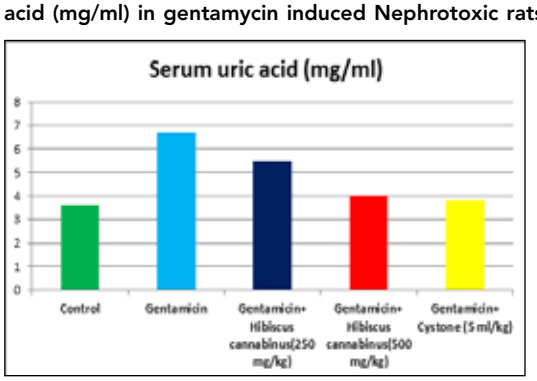
Groups	Serum creatinine (mg/ml)	Serum uric acid (mg/ml)	Blood urea nitrogen (mg/ml)	Serum urea (mg/ml)	Weight of kidney (g)
Control	1.22±0.75	3.62±0.31	15.13±0.87	32.03±1.73	0.91±0.19
Gentamicin	3.32±0.25	6.74 ±0.34	35.12±1.64	68.17±2.63	1.52±0.24
Gentamicin+ *Hibiscus cannabinus*(250 mg/kg)	2.42±0.48*	5.49±1.54*	28.20±1.20*	53.81±3.57*	1.20±0.32*
Gentamicin+ *Hibiscus cannabinus*(500 mg/kg)	1.89±0.64**	4.02±0.35**	21.36±2.64**	40.26±3.36**	1.11±0.25**
Gentamicin+ Cystone (5 ml/kg)	1.59±0.61**	3.80±0.13**	18.02±1.35**	36.36±2.62**	0.99±0.36**

Values are expressed as mean±SEM. n=6 rats in each group.

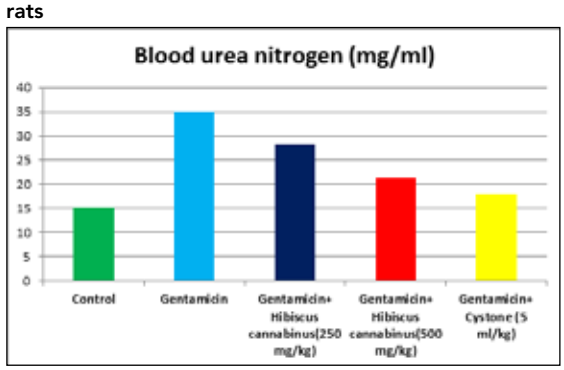
Graph 1: Effect of *Hibiscus cannabinus* on Serum creatinine (mg/ml) in gentamycin induced Nephrotoxic rats



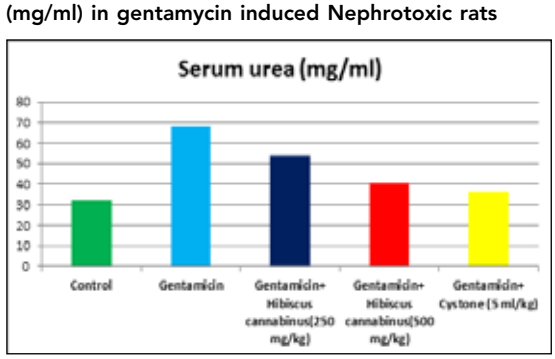
Graph 1I: Effect of *Hibiscus cannabinus* on Serum uric acid (mg/ml) in gentamycin induced Nephrotoxic rats



Graph 1II: Effect of *Hibiscus cannabinus* on Blood urea nitrogen (mg/ml) in gentamycin induced Nephrotoxic rats



Graph 1V: Effect of *Hibiscus cannabinus* on Serum urea (mg/ml) in gentamycin induced Nephrotoxic rats



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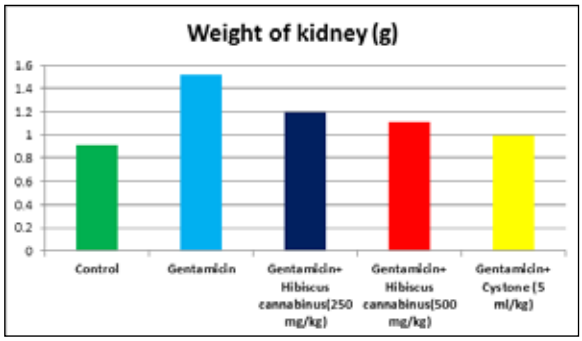
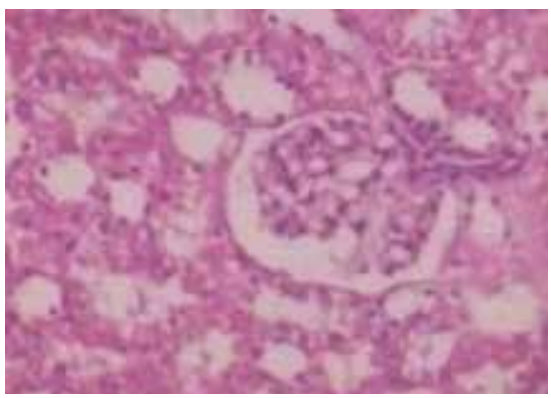
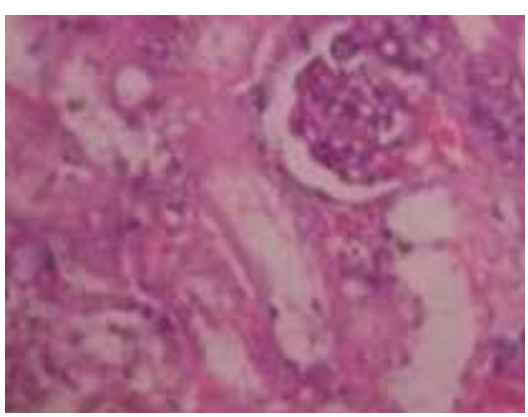


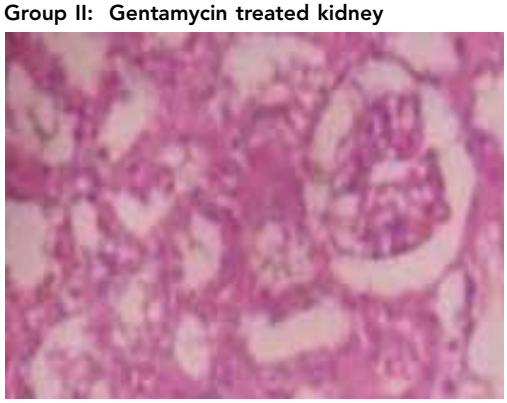
Graph V: Effect of *Hibiscus cannabinus* on Weight of kidney(g) in gentamycin induced Nephrotoxic rats Histopathology of kidney:



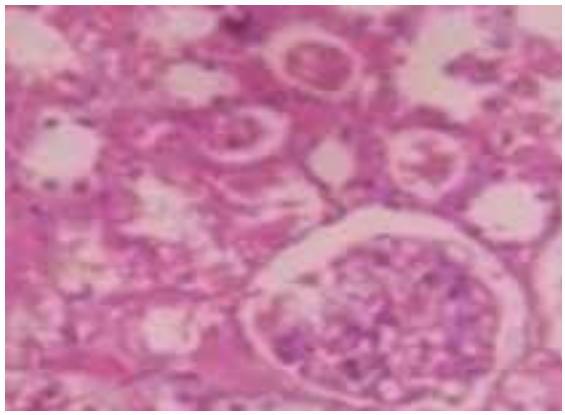
Group I: Normal section of kidney



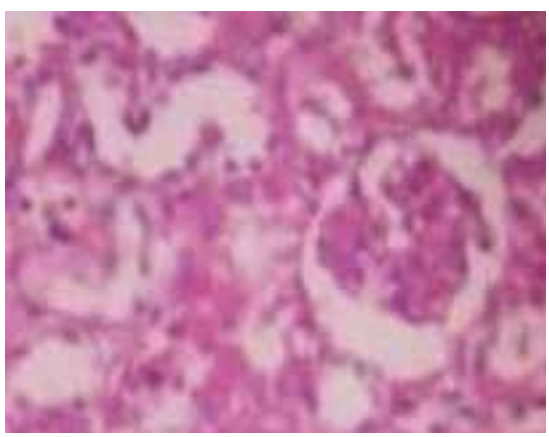
Group II: Gentamycin treated kidney



Group III: Gentamycin+ Extract (200 mg/kg)



Group III: Gentamycin+ Extract (200 mg/kg)



Group III: Gentamycin+ Standard Cystone (5 ml/kg)

Histopathology of kidney: Group I shows kidney section of control group showing normal tubular brush borders, intact glomeruli and Bowman's capsule. Group II shows the severe tubular necrosis and degranulation. Group III shows showed normal tubular pattern with a mild degree of necrosis, swelling and degranulation. Group IV shows showed normal tubular pattern, with no degree of necrosis. Group V shows normal tubular brush borders, intact glomeruli and Bowman's capsule.

DISCUSSION

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin[5]. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because there is an increasing number of potent therapeutic drugs like aminoglycoside antibiotics, NSAID's, chemotherapeutic agents have been added to the therapeutic arsenal in recent years[6]. Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic also induces nephrotoxicity. Prompt recognition of the disease and cessation of responsible drugs are usually the only necessary therapy[7]. 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. Early literatures have prescribed various herbs for the cure of renal disorders. Co-administration of various medicinal plants possessing nephroprotective activity along with different

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nephrotoxic agents which may attenuate its toxicity. The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood[8]. In addition to this, there is a failure of regulation of fluid and electrolyte balance along with endocrine dysfunction. The renal failure is fundamentally categorized into acute and chronic renal failure[9].

The kidney disorders are world problem. Despite its frequent occurance, high morbidity and high mortality, its medical management is currently in adequate, no therapy has successfully prevented the progression of kidney diseases, even though newly developed drugs have been used to treat chronic kidney disorders these drugs have often side effects. Therefore, that is an essential research about suitable herbal drugs, that could replace the chemical ones. Plants extracts have been used by traditional medical practitioners for the treatment of kidney disorders for centuries. Phenylpropnoids or polyphenolic compounds are a large group of herbal chemical compounds with well-known treat mental and protective effects.

Aminoglycoside antibiotics have been widely used for gram-negative bacterial infections. However, their nephrotoxicity and ototoxicity are the major limitations in clinical use. Among several aminoglycoside antibiotics, the grade of nephrotoxicity has been reported to be in the following order as, neomycin > gentamicin > tobramycin[10].

Gentamycin Nephrotoxicity occurs in about 15-30% of treated subjects, is manifested clinically as non-oliguric renal failure, with a slow rise in serum creatinine and hypoosmolar urinary output developing after several days of treatment[11]. Gentamicin is filtered through glomeruli into tubular urine, that binds with anionic phospholipids, such as phosphatidylinositol or phospholipdylserine, in brush border membrane of proximal tubular cells reabsorbed actively via pinocytosis process into tubular cells, taken up by lysosomes and thereafter produces phospholipidosis. The drug enters into the cells by adsorptive/receptor mediated endocytosis after binding to acidic phospholipids and megalin and is found essentially in lysosomes. Animals treated with low, therapeutically relevant doses of aminoglycosides show both lysosomal phospholipidosis and apoptosis in proximal tubular cells[12].

The present thesis entitled "Nephroprotective activity on the leaves of *Hibiscus cannabinus* against chemical induced toxicity in the experimental rats" deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant. As already reported that *Hibiscus cannabinus* is used in the treatment of high blood pressure, menopause-related cognitive decline, tinnitus, post-stroke recovery, peripheral arterial disease, macular degeneration, or altitude sickness.

CONCLUSION

In the present study, it was observed that treatment with gentamycin induced a significant elevation in the levels of serum urea, creatinine, Blood urea nitrogen, serum urea and weight of kidney. However, daily treatment with *Hibiscus cannabinus*(250mg/kg and 500mg/kg) for 8 days conferred nephroprotection on gentamycin induced rats in a dose dependent fashion offered maximum protection. Further investigation on the isolation and identification of active components in the leaves lead to chemical entities with potential for clinical use in the prevention and treatment of nephrotoxicity.

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