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Review Article

Nephroprotective Activity of *Bougainvillea Glabra* Linn. On Gentamicin-Induced Nephrotoxicity in Wistar Albino Rats

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ABSTRACT

Herbal and polyherbal formulations are at boom owing to lesser side effects and higher efficacy and are considered for management of renal complications. To evaluate the Nephroprotective activity of *Bougainvillea glabra* flower extract against Gentamicin - induced nephrotoxicity in rat model. Nephrotoxicity were induced in wistar rats of either sex by intraperitoneal administration of Gentamicin 100mg/kg body weight for eight days. The methanolic extract of *Bougainvillea glabra* (L.) flower (250, 500 mg/kg) administered orally to Gentamicin treated rats for next eight days. Rats were subjected to evaluate biochemical and general parameters and histopathology of renal tissue were performed at the end of 9 days after 8 days treatment. The elevated serum creatinine, blood urea nitrogen levels, urine creatinine, urine volume, kidney weight Gentamicin treated rats were reverted back approximately near to normal after the treatment of *Bougainvillea glabra* (L.) flower at a doses of 250 and 500 mg/kg. *Bougainvillea glabra* (L.) flower possesses protective action against Gentamicin induced nephrotoxicity in wistar rats which may be attributed by its antioxidant effect.

INTRODUCTION

The negative impact of chemicals on renal function is known as nephrotoxicity. Molds and fungus, cancer treatments like cisplatin, antibiotics like amino glycosides, metals like mercury, arsenic, and lead, and illicit drugs like cocaine are a few examples of these materials. Changes in the glomerular filtration rate (GFR), blood urea

nitrogen (BUN), serum creatinine or urine outputs are some indicators of nephrotoxicity however, nephrotoxics can cause kidney damage without altering any recognized clinical marker of renal function.^[1] As kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment such as detoxification and excretion

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of drugs and toxic metabolites it is major target organ for exogenous toxicants. Nephrotoxicity is a kidney specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs. Approximately 20% of nephrotoxicity is induced by drugs, but medication of the elderly increases the incidence of nephrotoxicity up to 66% as the average life span increases. Nephrotoxicity is an adverse side effect of chemotherapy or anticancer medicines [2]. Many drugs, natural products, industrial chemicals and environmental pollutants are mainly responsible for nephrotoxicity. E.g. therapeutic drugs like amino glycoside antibiotics, non-steroidal anti-inflammatory drugs, chemotherapeutic agent's chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic produces acute renal failure. [3] Amino glycosides preferentially affect the proximal tubular cells. These agents are freely filtered by the glomeruli and quickly taken up by the proximal tubular epithelial cells, where they are incorporated into lysosomes after first interacting with phospholipids on the brush border membranes. They exert their main toxic effect within the tubular cell by altering phospholipid metabolism. In addition to their direct effect on cells, amino glycosides also cause renal vasoconstriction [4]. Thus the present study was undertaken to investigate the Nephroprotective effect of *Bougainvillea glabra* on Gentamicin Induced Nephrotoxicity in Wistar Rats.

MATERIAL AND METHOD

Drugs and Chemicals

Bougainvillea glabra flower were collected from the medicinal garden of vidya Bharti College of pharmacy Amravati Maharashtra. The plant materials were identified, verified and authenticated by Prof .M.S.Ghusde, Botanist,

Vidyabharti Mahavidyalaya, Amravati, and Maharashtra, India.

Standard Drug: silymarin100mg/kg tablet manufactured in India by Micro Labs Ltd pharmaceutical and brought from pharmacy store Amravati.

Inducing Drug: Gentamicin100mg/kg manufactured in India by Abbott pharmaceutical and brought from pharmacy store Amravati.

Preparation of plant Extract (Methanolic extract)

The dried flowers were grounded into a fine powder by using a mixer grinder. 100 cc of methyl alcohol and 10 g of powder were macerated together for 2 days at room temperature. A rotary evaporator operating at 40°C condensed, after filtering the solution through Whatmann filter paper. Weighed, placed at 4 °C until use, the dried crude extract. [5]

Experimental animals:

The experiment was performed on Wister rats, 170-200g which was obtained from the animal house of Department of Pharmacology, Vidyabharti college of Pharmacy, Amravati. All the animals were acclimatized to animal house prior to use. They are kept in cage to animal house with 12h light: 12h dark cycle. Animals were fed on pellets and tap water ad libitum. The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals (CPCSEA). Permission Registration number - 1504/PO/RE/S/11/CPCSEA and approval for animal studies were obtained from Institutional Animal Committee (LAEC) of Vidyabharti College of Pharmacy, Amravati, and SGB Amravati University.



Experimental Method:

Thirty male wistar rats were selected and divided in to five groups, with six animals in each group. Protocols to assess the protective effects of *B. glabra* and its isolated Flavonoids and phenolic compound against Gentamicin-induced nephrotoxicity.

Group I (Normal control)

Rats received oral administration of normal saline for 8 days. This group served as a negative control, indicating baseline health without any induced nephrotoxicity or treatment effects.

Group II (Toxicant control)

Rats were administered with inducing agent Gentamicin intraperitoneal 100 mg /kg for 8 days.

Group III (standard control)

Rats were administered 100 mg/kg body weight Silymarin per oral for 8 days with Gentamicin IP 100mg/kg for 8 days.

Group IV (Low dose test group)

Rats were administered 250 mg/kg body weight *Bougainvillea glabra* flower extract orally for 8 days.

Group V (High dose test group)

The rats were administered 500mg/kg body weight *Bougainvillea glabra* flower extract orally for 8 days.

Gentamicin, 100 mg/kg body weight, was administered intraperitoneally daily to rats in groups 2-6 concomitantly with the above treatment for 8 days. Twenty-four hours after the last administration, animals were weighed again and sacrificed under light diethyl ether vapour. [6]

Collection of blood samples and organs:

After 8 days of treatment (24 hours after the last treatment) the rats were weighed again and sacrificed under light diethyl ether vapor. Blood samples were collected by cardiac puncture and used immediately. Blood was collected into plain centrifuge tubes bottles, and were centrifuged immediately at 2500 rpm for 15 minutes to separate the serum at room temperature to avoid haemolysis and used for biochemical assays. The kidneys were surgically removed, weighed and fixed in 10% formaldehyde for histological process. [7]

Biochemical measurements:

Biochemical measurements in the serum Serum creatinine (Cr) and blood urea nitrogen (BUN) level were determined according to Bartels and Fawcett and Scott, 22 respectively. Serum Cr and BUN were spectrophotometrically measured at 550 nm and expressed as milligrams per deciliter (mg/dl). In addition, lactate dehydrogenase (LDH) activity and albumin level were assessed according to the method of Henry et.al.23 and Doumas et al., 24 respectively, and expressed as a unit per liter and grams per deciliter, respectively.

Histopathological examination of kidney:

The isolated renal tissue was fixed in 10% formalin solution, embedded in paraffin and cut into semi-thin sections (2 μ m). The tissue sections were stained with hematoxylin and eosin(H &E) and observed under light microscopy (40 X).

Statistical analysis

All data are expressed as mean $\pm$ SEM (n = 6) and analysed with one-way ANOVA followed by Dunnet's test (* p < 0.0001) vs. GM group.



RESULTS:

Preliminary phytochemical screening:

The extracted materials underwent phytochemical analysis and pharmacological assessment. The extracts underwent first phytochemical analysis to see whether different secondary metabolites including phenolic, alkaloids, Flavonoids, phenols, terpenoids, saponins, tannins, and glycosides reagent solvents of analytical quality.

Acute oral toxicity study:

In acute toxicity investigations, male and female healthy rats were employed by OECD norms -425. After an overnight fast, the rats were split up into three groups, each consisting of five rats. The dosages of extracts were 100, 500, and 2000 mg/kg.p.o. Body weight. For two hours, the rats' behaviour and autonomic profiles were monitored constantly, and for up to 48 hours, any indications of toxicity or mortality were noted. (OECD-425, 2001). The drug under test was taken

orally. The vehicle was administered to the control group's rats at the same dosage as the other treatment groups' animals. Oral administration used a 10 ml/kg dose volume. Based on each animal's body weight on the day of treatment, the amount of test material was provided to each one. Therefore 500mg/Kg and 250mg/kg were selected for further study.

Nephroprotective effect of *Bougainvillea glabra* flower extract against gentamicin-induced nephrotoxicity study

The results demonstrated significant improvement in blood urea nitrogen, creatinine clearance and serum creatinine levels on treatment with Silymarin, methanolic extract of flower of *bougainvillea glabra* respectively, as these renal markers were increased in animals treated with Gentamicin alone indicating nephrotoxicity.

Effect of treatments on Serum creatinine level blood urea nitrogen and Creatinine clearance.

Table No.1: Effect of treatments on Serum Urea, Creatinine Clearance, and serum creatinine level.

Treatment group	Blood urea nitrogen (mg/dl)	Serum creatinine level (mg/dl)	Urine volume	Creatinine clearance (ml/min)
Normal control	18.832±1.78	0.512±0.07	10.42±2.2	1.636±0.01
Gentamicin (GM) 100mg/kg IP	39.628±4.98	1.25±0.20	5.424±1.2	0.718±0.06
Silymarin 100mg/kg p.o	24.422±2.82*	0.636±0.08*	10.166±1.1*	1.586±0.07*
MEBG 250mg/kg p.o	33.858±1.71*	0.836±0.06*	8.572±1.3*	1.202±0.02*
MEBG 500mg/kg p.o	28.43±1.24*	0.704±0.02*	9.226±0.88*	1.526±0.01*

All data are expressed as mean±SEM (n=6) and analysed with one-way ANOVA followed by Dunnett's test (*p < 0.0001) vs. GM group.

- A: p<0.001* Compared to Normal Control
- B: p<0.001* Compared to Gentamicin Control
- C: p<0.001* Compared to Normal Control



- D: $p < 0.001$ * Compared to Normal Control

Effect on body weight and kidney weight:

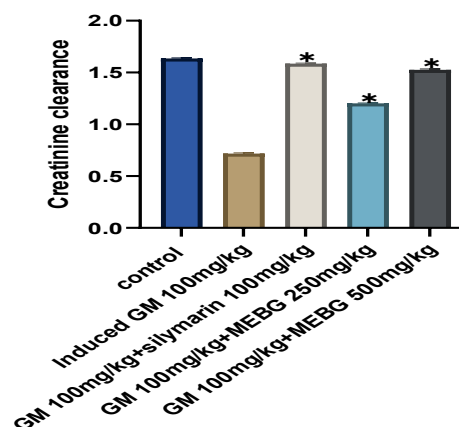
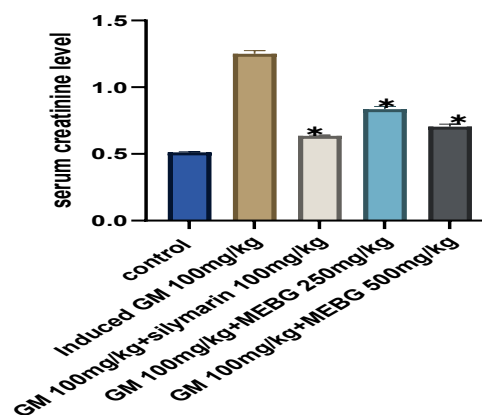
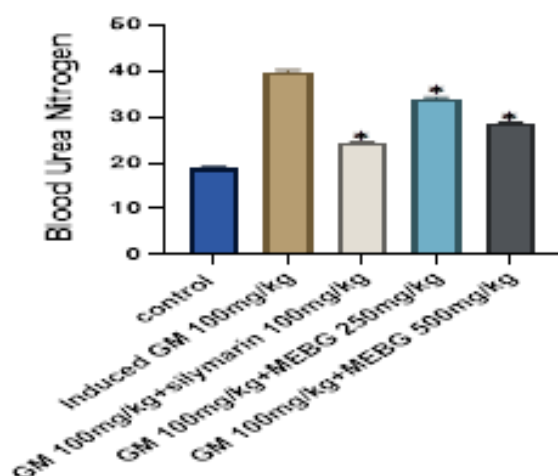
The body weights were decreased in Gentamicin treated and kidney weight were increased animals compared to their control body weights. Treatment

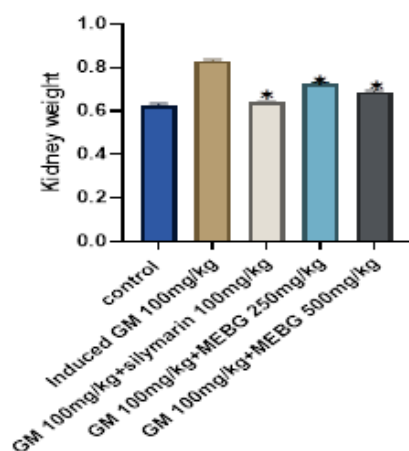
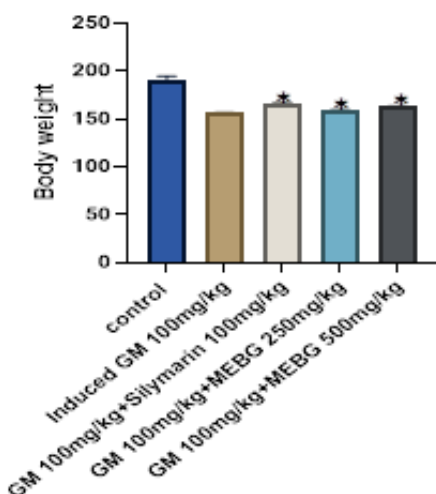
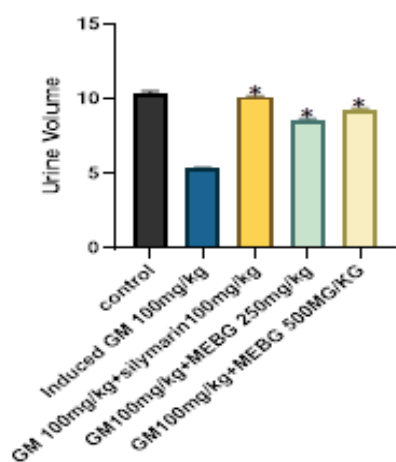
with Silymarin, methanolic extract of flowers of *Bougainvillea glabra* improved the body weights and decreased kidney weight compared to Gentamicin control group but not statistically significant. Comparison of control and inducing, treatment group body weight and kidney weight between the groups is depicted in (Table No: 2)

Groups	Treatment	Body Weight	Kidney weight
I	Control	190.8±1.00	0.624±0.06
II	GM (100mg/kg)	156.2±2.44	0.83±0.07
III	GM (100mg/kg) + Silymarin (100mg/kg)	166±1.78*	0.638±0.11*
IV	GM (100mg/kg) + MEBG (250mg/kg)	159.2±2.56*	0.728±0.09*
V	GM (100mg/kg) + MEBG (500mg/kg)	163.2±2.33*	0.688±0.08*

All data are expressed as mean±SEM (n=6) and analysed with one-way ANOVA followed by Dunnett's test ($*p < 0.0001$) vs. GM group.

- A: $p < 0.001$ * Compared to Normal Control
- B: $p < 0.001$ * Compared to Gentamicin Control





Group I- Treated with normal saline:

The animals of Group I were treated with only normal saline showed normal renal glomeruli with the intact Bowman's capsule. Other structures like brush border cuboidal epithelium which lines the proximal convoluted tubules, simple cuboidal epithelium, which lines the distal convoluted tubules, and macula densa were very prominent.

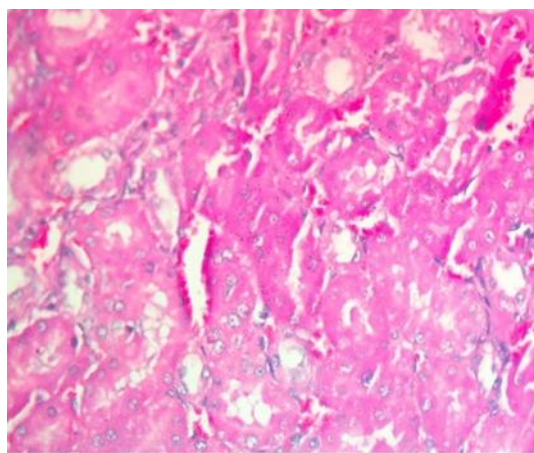


Figure 1. Photomicrograph of kidney of animals treated with normal saline.

Group II- Treated with Gentamicin:

Close examination of the sections at high magnification, revealed the appearance of cells with alterations typical of apoptosis (cell shrinkage and cytoplasm eosinophilia, presence of small and shrunken nucleus with chromatin condensation), breakdown of glomerular capillaries and vacuolar appearance in tubular lumen, glomerular congestion, disruption of glomerular capillaries, vacuolar degeneration of tubular epithelial cell is observed with hyaline cast formation. Atrophic glomeruli are present effecting half of the cortical region.

Results of Histopathology:

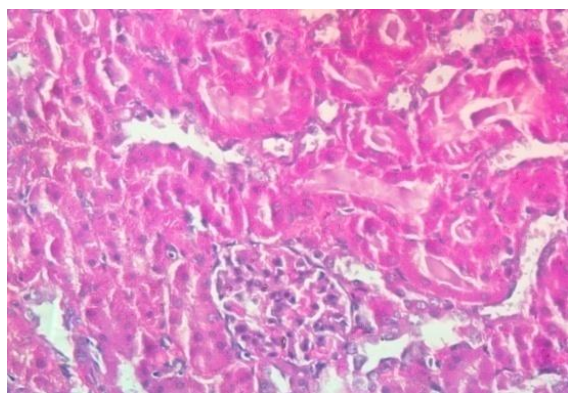


Figure 2. Photomicrograph of kidney of animals treated with Gentamicin

Group III- Treated with Gentamicin+ Silymarin:

Multifocal tubular inflammation in which infiltration of inflammatory cells is noticed. Glomerulus appeared normal, no degeneration nor inflammation was found. Only Moderate tubular degeneration was observed.

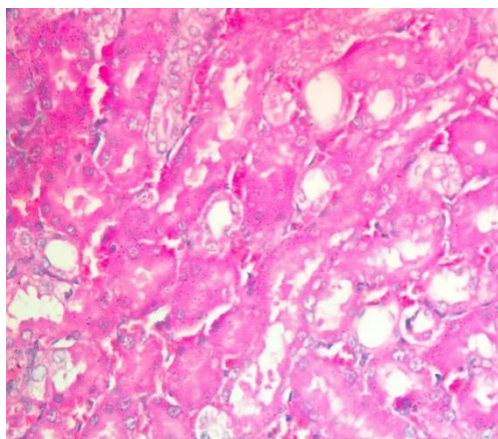


Figure3. Photomicrograph of kidney of animals treated with Silymarin (100mg/kg)

Group IV- Treated with methanolic extract of *Bougainvillea glabra* (250mg/kg) + Gentamicin

Treatment with methanolic extract of *Bougainvillea glabra* 250mg/kg reversed most of the histopathological alterations induced by Gentamicin as seen from the sections from gentamicin treated rats. Moderate tubular interstitial nephritis in which inflammation along with infiltration of inflammatory cells noticed in

the interstitium between tubular regions. Moderate tubular degeneration was also observed.

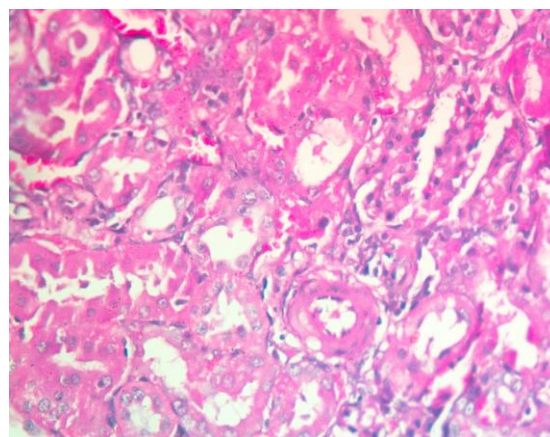


Figure 4. Photomicrograph of kidney of animals treated with *Bougainvillea glabra* (250mg/kg)

Group V- Treated with methanolic extract of *Bougainvillea glabra* (500mg/kg) + Gentamicin.

Treatment with methanolic extract of *Bougainvillea glabra* also reversed most of the histopathological alterations induced by Gentamicin. Photomicrographs of kidneys from this group revealed more prominent protection by showing normal glomeruli with absence of atrophy and hypertrophy.

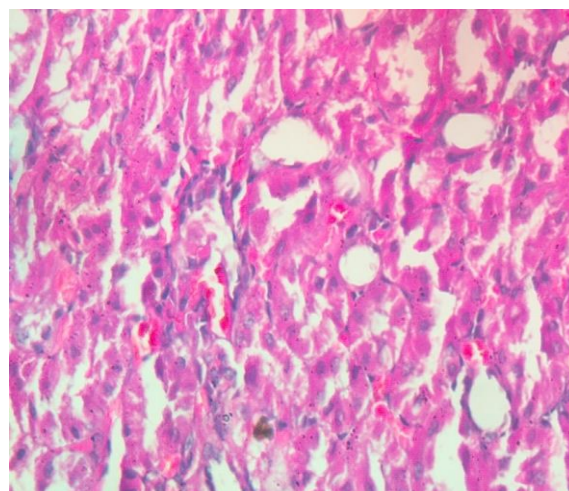


Figure 5. Photomicrograph Kidney animals treated with methanolic extract of *Bougainvillea glabra* (500mg/kg)

DISCUSSION:

Nephrotoxicity is a common clinical syndrome defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted. There are few chemical agents to treat acute renal failure. Studies reveal back synthetic nephroprotective agents have adverse effect besides reduce nephrotoxicity. There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy. Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed, along with the dietary measures, plant preparation formed the basis of treatment of disease until the introduction of allopathic medicine. Ethnomedicinal plants can be used to help forestall the need of dialysis by treating the causes and effect of renal failure, as well as reducing the many adverse effect of dialysis. The phytochemicals found to be present in the roots extract of MEBG are the flavonoids, terpenoids, alkaloids, tannins, and Phenolic compound saponins. Among them tannins, triterpenoids, flavonoids and saponins, phenolic compound could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity. Acute toxicity studies revealed the non-toxic nature of the methanolic extract of *Bougainvillea glabra* Linn. There was no lethality or any toxic reactions found with high dose(2000mg/kg body weight) till the end of the study. According to the OECD 420 guidelines (Acute Oral Toxicity: Fixed Dose Procedure), an LD50 dose of 2000 mg/kg and above was considered as unclassified so the methanolic extract of *Bougainvillea glabra* Linn was found to be safe. GM is used for treatment of serious infections, and its therapeutic effects are significantly improved by doses calation.

However, high-dose therapy with GM is limited by its cumulative risk of nephrotoxicity. Initially the general parameters (Urine volume, Body weight and Kidney weight) are investigated. In GM induced rats observed that the urine output was decreased, and after treatment with silymarin (100mg/kg) and methanolic extract of *Bougainvillea glabra* L.(250mg/kg,500mg/kg) the urine output was increased gradually. The rats treated with GM shown marked decreased of body weight and increased kidney weight as compared to normal group also caused a marked reduction of glomerular filtration rate, which is accompanied by increase in serum creatinine level, BUN and decline in creatinine clearance indicating induction of acute renal failure. With Methanolic extract of *Bougainvillea glabra* L at the dose level of 250 and 500 Mg/kg body weight and kidney weight for 8 days significantly lowered the serum level of creatinine with a significant body and kidney weight Loss, decreased urine output and creatinine clearance when compared with the nephrotoxic control group. Rats that were administered oral MEBG (250mg/kg and 500mg/kg) had decreased serum BUN and CRE levels. This indicated that MEBG decreased the overall damage caused by high-dose GM. This was corroborated by a decrease in kidney damage. Increases in BUN and serum Cr levels in GM-treated rats indicated a reduction in glomerular filtration rate (GFR) and renal failure. Serum Cr levels are more important indicators than BUN for the determination of earlier phases of kidney disease. Conversely, BUN begins to rise only after marked renal parenchymal injury occurs. In the present study, increases in serum Cr and BUN and decrease in creatinine clearance induced by GM were significantly blocked by the administration of MEBG. The success of MEBG in reducing Cr and BUN concentrations and elevated creatinine clearance could be attributed to its anti-oxidant properties because ROS may be involved in the



impairment of GFR. Reactive oxygen species have been considered to be important mediators of drug-mediated nephrotoxicity. One of the pathophysiological mechanisms of GM toxicity could also be explained by oxidative stress.

There are some data showing that oxidative damage has an important role in GM-induced nephrotoxicity in rats. Recent studies suggest that oxidative stress-induced lipid peroxidation plays a role in the pathogenesis of GM-induced nephrotoxicity. The latest studies emphasized that many anti-oxidant substances have Reno protective effects due to their ability to decrease lipid peroxidation. Lipid peroxidation leads to the breakdown of membrane structure and function. The present study, demonstrated that the protective effect of MEBG as evidenced by decreased BUN and Cr levels, decreased creatinine clearance and histopathological improvement. Thus, the results indicates that MEBG decreases the nephrotoxic effect of GM. The Renal protective effect of MEBG may also be due to a reduction in lipid peroxidation of renal tubular cells in the rat kidney. In the present study, renal parenchymal damage, such as tubular dilatation and vacuolization, was observed by histopathological examination of GM-treated rat kidney. The mean weight of the kidney tissue increased significantly because of renal parenchymal damage. These findings show that severe degeneration in cortical tubular cells results from GM administration. As a consequence of acute tubular necrosis, GFR decreases, although BUN, Cr levels increased and creatinine clearance increased. We observed that the histopathological changes were significantly lower in GM + MEBG-treated rats. Based on the above results, it may be concluded that *Bougainvillea glabra* Linn exerted potential Nephroprotective activity against Gentamicin induced Nephrotoxicity in rats. To investigate the detailed mechanism of medicinal

plant *Bougainvillea glabra* Linn. Needs further studies as concern with future prospective.

CONCLUSION

In conclusion, the results of the present study infer that the antioxidant activity and medicinal properties of *Bougainvillea glabra* may be responsible for protection against GM-induced renal damage. When compared between the two extracts, moderate dose extract showed minimum effect potent response in comparison to high dose of methanolic extract of *bougainvillea glabra*. These findings also suggest the probable efficacy of *Bougainvillea glabra* extract against oxidative deterioration and can be useful novel nephroprotective agent in the prevention of oxidative stress-related degenerative diseases. Further studies are warranted in pre-clinical and clinical set up, and for studying the structure of phytoconstituents in establishing pharmacological activities.

Future Prospective

Research on the nephroprotective effects of *Bougainvillea glabra* is still in its early stages. Preclinical studies using animal models have shown promising results, indicating that extracts of *Bougainvillea glabra* can mitigate the nephrotoxic effects of Gentamicin. For example; studies have demonstrated reductions in markers of kidney damage, such as serum creatinine and blood urea nitrogen (BUN), and histopathological improvements in renal tissues. However, more research is needed to fully understand the mechanisms involved and to establish the efficacy and safety of *Bougainvillea glabra* in humans. Future research should focus on:

- Conducting well-designed clinical trials to confirm the nephroprotective effects of *Bougainvillea glabra* in patients undergoing Gentamicin therapy.



- Determining the optimal dosage and form of *Bougainvillea glabra* for nephro protection.
- Further elucidating the molecular pathways through which *Bougainvillea glabra* exerts its protective effects.

Exploring the potential synergistic effects of combining *Bougainvillea glabra* with other nephroprotective agents or standard treatments to enhance over all kidney protection

Conflict of Interest

We declare that we have no conflict of interest.

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