



Investigation of Nephroprotective Activity of Thespesia Populnea

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ABSTRACT

Thespesia populnea Linn (Family-Malvaceae) is one of the important drugs used in herbal medicine. It is known by the name Paras-pipal. It is cultivated for ornament and shade and it blooms throughout the year in the tropics. Various parts of this plant are found to have useful medicinal properties such as antibacterial, antiinflammatory, antioxidant, purgative and hepatoprotective activities. The yellow juice of fruits is used in treating certain hepatic diseases. A decoction of the bark is given internally in the diseases of skin and that of fruits as an antidote for poisoning. The seed has purgative properties. The plant has also been effective in treating malaria. A wide range of biologically active compounds such as flavonoids, sesquiterpenoids, tannin, saponins, alkanes, essential oils, sugars, fatty acids and anti-oxidants are present in the plant. This article provides a comprehensive review of its ethano-medical uses, chemical constituents and pharmacological profile as medicinal plants.

Key words: *Thespesia populnea*, Nephroprotective.

INTRODUCTION

Nephrotoxicity is a poisoning effect of some substances both toxic chemical and medication on kidney. There are various forms of toxicity. Nephrotoxicity should not be confused with the fact that some medication have a predominantly renal excretion and need their dose adjusted for the decreased renal function for e.g. heparine. The kidney plays important roles of the maintenance of our endocrine and acid base balance, blood pressure, erythropoiesis etc.

Nephrotoxicity has been recognised as a major complication of aminoglycoside antibiotics for many years. The human beings are exposed to environmental occupational and xenobiotics challenges due to modern life style. Gentamycin is an antibiotic that exhibits a broad spectrum of activity and is valuable in several sepsis. Its use is, however, restricted because of the development of ototoxicity and nephrotoxicity. Nephrotoxicity has been related to a selective accumulation of gentamycin in the renal cortex. Nephrotoxicity is a common complication of aminoglycoside antibiotics therapy in man. The recent years many researchers have examined the effect of plants used traditionally by indigenous healers and herbalists to support kidney function and treat diseases of kidney. Several hundred plants have been examined for use in a wide variety of kidney disorders.

The aim of the research is to find out new nephroprotective drugs from indigenous plants which are potent and non-toxic agents. Normally herbal plants are free from side effects / adverse effects and they are low cost medicines, which will be beneficial for the people. Keeping in this view, we have selected *Thespesia populnea* based on ethnopharmacology information which is traditionally used in various disorders including kidney diseases. The present study aimed at evaluating nephroprotective of *Thespesia populnea* against Gentamicin and Cisplatin induced nephrotoxicity in rats with special reference to biochemical, antioxidant parameters and histopathological studies.

MATERIALS AND METHOD

Collection and authentication of plant materials.

The leaves of *Thespesia populnea* were collected from Science College Nanded and authenticated by Dept of Pharmacognosy, Life Science SRTMU Nanded.

Extraction : The leaves of *Thespesia populnea*.

The authenticated leaves were shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. Coarse powder of the leaves (1 Kg) was Soxhlet extracted with 90% ethanol. The aqueous extract was prepared using the same marc by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanolic (4.2%) and the aqueous extracts (2.4%). The powdered extract was used for study.

Preliminary Phytochemical screening test:

All the preliminary phytochemical tests of *Thespesia populnea* were performed.

1) Detection of alkaloids

Methanol extracts were dissolved individually in dil. hydrochloric acid (10 ml) and then filtered and referred as test solution.

- **Mayer's test:** To 1 ml of test solution of methanolic extract added few drops of Mayer's reagent (Potassium Mercuric Iodide Solution). Cream precipitate indicated the presence of alkaloids.
- **Wagner's test:** To 1 ml of test solution of methanolic extract added equal volumes of Wagner's reagent (Iodine in Potassium Iodide). Reddish precipitate indicated the presence of alkaloids.
- **Hager's test:** To 2 ml of test solution of methanolic extract added few drops of Hager's reagent (Saturated Picric Acid Solution). Bright yellow precipitate indicated the presence of alkaloids.
- **Dragendroff's test:** To 1 ml of test solution of methanolic extract added few drops of Dragendroff's reagent (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids.
- **2) Detection of glycosides:** Methanol extracts were treated individually with dil. hydrochloric acid (10 ml), and then filtered and referred as test solution.
 - **Modified Borntrager's test:** Methanol extract were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicated the presence of anthranol glycosides.
 - **Legal's test:** Methanol extract were treated with sodium nitropruside in pyridine and NaOH. Formation of pink to blood red color indicated the presence of cardiac glycosides.
 - **Keller-killani test:** Methanol extract (50 mg) were treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface is the feature of cardenolide sugar. Appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycoside.

3) Detection of Saponins

- **Froth test:** Methanol extract were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.
- **Foam test:** 0.5 gm of Methanol extract were shaken with 2 ml of water. If foam produced and persists for 10 minutes it indicates the presence of saponins.

4) Detection of phenols

- **Ferric chloride test:** Methanolic extract were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

5) Detection of flavonoids

- **Alkaline reagent test:** Methanol extract were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.
- **Lead acetate test:** Methanol extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

6) Detection of proteins and amino acids

- **Xanthoproteic test:** Methanol extract were treated with few drops of conc. nitric acid. Formation of yellow color indicated the presence of proteins.
- **Ninhydrin test:** To Methanol extract 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated the presence of amino acid.

7) Detection of phytosterols

- **Salkowski's test:** Methanol extract were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes.
- **Libermann Burchard's test:** Methanol extract were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride then boiled and cooled. After that conc. Sulphuric acid (0.5 ml) was added. Formation of brown ring at the junction indicated the presence of phytosterols.

8) Detection of tannins

- **Ferric chloride test:** Methanol extract were dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. Bluish black color indicated the presence of tannins.
- **9) Detection of carbohydrates**

Methanol extract were dissolved individually in 5 ml of distilled water then filtered and referred as test solution.

- **Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars.
- **Molisch's test:** Filtrates were treated with 2 drops of alcoholic a-naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of carbohydrates.

Ethanol and aqueous extracts of *Thespesia populnea* at doses of 200 and 400 mg/kg b.w. were used to evaluate nephroprotective activity. Stock solution of the extract was prepared in the range of 200-400 mg/ml in water according to the need of study. 4.2.4 Acute toxicity studies"

Acute toxicity studies for aqueous and ethanolic extracts of *Thespesia populnea* were conducted as per OECD guidelines 423 using albino Wistar rats. Each animal was administered the aqueous solution of the extract by oral route. The animals were observed for any changes continuously for the first 2h and up to 24 h for mortality.

NEPHROPROTECTIVE ACTIVITY

Nephroprotective activity of *Thespesia populnea* carried out using Gentamicin and Cisplatin induced nephrotoxicity models in rats.

Gentamicin induced nephrotoxicity in rats"

Albino rats (150-200 gm) of either sex were used for the study. Animals were divided into six groups, each group containing six animals. The study was carried out for nine days and treatment was given for eight days.

Group I served as normal group and received distilled water p.o. for eight days. Group II served as gentamicin group. The gentamicin treated group received 100 mg/kg/day.

gentamicin by the intraperitoneal (i.p.) route.

Group III and IV received 200 and 400mg/kg b.w. of MTA respectively. Group V and VI received 200 and 400 mg/kg b.w.

Animals of group III to VI were administered 100 mg/kg b.w. of gentamicin i.p. along with extracts p.o. for 8 days. After dosing on the day 8, individual rats were placed in separate metabolic cages for 24h for urine collection to determine urine output and urine creatinine content. Blood samples were collected via retro-orbital puncture at the end of these 24h, the serum was rapidly separated and processed for determination of blood urea nitrogen (BUN), serum urea and serum creatinine using of Span Diagnostic kits. Body weight of animal was also recorded. Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination.

Cisplatin induced nephrotoxicity in rats?

Albino rats (150-200 gm) of either sex were used for study. Animals were divided into six groups, each group containing six animals. Study was carried out for eight days and treatment was given for seven days. Group served as normal group and received distilled water p.o. for seven days. Group II served as cisplatin group. Groups III and IV received 200 and 400 mg/kg bw. of MTA (p.o.) for 7 days. Groups V and VI received 200 and 400 mg/kg b.w. of MTE (p.o.) for 7 days. On day 2 cisplatin 5 mg/kg b.w. was administered i.p. to all the animals of groups II.

to VI. After dosing on the day 7, individual rats were placed in separate metabolic cages for 24h for urine collection to determine urine output and urine creatinine content. Blood samples were collected via retro-orbital puncture at the end of these 24 h the serum was rapidly separated and processed for determination of blood urea nitrogen (BUN) and serum creatinine using of Span Diagnostic kits. Changes in body weight were recorded. Six rats per group were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination.

Creatinine Estimation:

Creatinine reacts with picric acid in an alkaline medium to form an orange coloured complex. The rate of formation of this complex is measured by reading the absorbance at 505 nm in selected interval of time and is proportional to the concentration of creatinine. The reaction time and the concentration of picric acid and Sodium hydroxide have been optimized to avoid interference from ketoacids.

Creatinine + picric acid alkaline medium Orange coloured complex

Urea Estimation:

Urea is hydrolysed in presence of water and urease to produce ammonia and carbon dioxide. Under alkaline condition, ammonia so formed, react with hypochlorite and phenolic chromogen to form coloured indophenol, which is measured at 578 nm (570-600 nm). Sodium nitroprusside acts as a catalyst. The intensity of colour is proportional to the concentration of Urea in the sample. Urea H:O Urease Ammonia + CO₂ ,Ammonia + Phenolic Chromogen + Hypochlorite Sodium Nitroprusside Indophenol Blood urea nitrogen was calculated by this formula.

BUN concentration in mg/dl Urea concentration in mg/dl X 0,467

Histopathological study:

Processing of isolated kidneys:

The isolated kidneys were cut into small pieces and preserved in formalin (10% solution) for at least two days. The kidney pieces were washed in running water for about 12 h. This was followed by dehydration with alcohol of increasing strength (70, 80 and 90%) for 12 h each. Then the final dehydration was done using absolute alcohol three times for 12h each. Again the tissue was cleaned by using xylene two times for 15 to 20 min. each. After cleaning, the kidney pieces were subjected to paraffin infiltration in automatic tissues processing unit.

Embedding in paraffin:

Hard paraffin wax was melted and poured into square-shaped blocks. The kidneys pieces were then dropped into the liquid paraffin quickly and allowed to cool.

Staining:

The blocks were cut using microtome to get sections of thickness 4-5 microns. The section was dried completely before staining. Eosin an acidic stain and hematoxylin a basic stain, was used for staining and observed under a photomicroscope.

Observation:

All the slides were observed for changes in histopathological characteristics and photographs were taken. The slides were observed for the following parameters:

- a. Glomerular congestion
- b. Peritubular congestion
- c. Epithelial desquamations
- d. Blood vessel congestion
- e. Interstitial edema
- f. Inflammatory cells
- g. Necrosis
- h. Tubular casts

Statistical analysis:

The data are expressed as mean \pm SD. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by Dunnet and Tukey's test. P-value <0.05 was regarded as statistically significant.

RESULT AND DISCUSSION:

Results of acute toxicity study:

There was no change in normal behavioural pattern of animals and no sign and symptoms of toxicity were observed during the observations which was done continuously for the first two hours and then observed up to twenty four hours for mortality. The extracts were safe up to a maximum dose of 2000 mg/kg b.w. The biological evaluation was carried out at doses of 200 and 400 mg/kg b.w.

Phytochemicals in Ethanolic leave extract

Sr. No.	Name of the phytochemical test	<i>Merremia Tridentata</i> leaf extract
1	Alkaloid test	+
2	Carbohydrate test	-
3	Saponin test	+
4	Flavonoid test	+
5	Tannins test	+
6	Phenol test	+
7	Glycosides test	+

8	Protein and amino acid test	-
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DRUG INDUCED NEPHROTOXICITY:-

Drug (Gentamicin and Cisplatin) induced nephrotoxicity is associated with marked elevation of serum creatinine, blood urea nitrogen, total protein, serum urea and urine creatinine levels. These parameters were recorded in the present study. The parameters recorded in the extract treated groups were compared with control group to evaluate their nephroprotective activity.

Gentamicin induced nephrotoxicity:

Serum creatinine-

Administration of gentamicin produced significant increase ($P < 0.001$) in serum creatinine level when compared to normal group, indicating nephrotoxicity. Administration of MTA 200, MTA 400, MTE 200 and MTE 400 produced significant ($P < 0.001$) decrease in serum creatinine level when compared to gentamicin group. Increase in serum creatinine level induced by gentamicin was reversed on treatment with both the doses of ethanolic and aqueous extracts.

The serum creatinine level in MTA 200, MTA 400, MTE 200 and MTE 400 treated animals were found to be 1.45 ± 0.53 , 1.11 ± 0.45 , 0.83 ± 0.12 and 0.67 ± 0.10 mg/dl respectively as against 3.17 ± 0.68 mg/dl in gentamicin treated animals. MTE 400 produced maximum decrease in serum creatinine level when compared with all other groups: (Table 9, fig 17).

Serum urea-

Serum urea level was significantly ($P < 0.001$) increased in gentamicin group when compared with the normal group, indicating nephrotoxicity. Administration of MTA 200 produced significant ($P < 0.01$) decrease in serum urea level when compared with gentamicin group. Administration of MTA 400, MTE 200 and MTE 400 produced significant ($P < 1$) decrease in serum urea level when compared with gentamicin group.

The serum urea level in MTA 200, MTA 400, MTE 200 and MTE 400 treated were found to be 104.4 ± 0.44 , 85.27 ± 0.41 , 83.24 ± 1.31 and 58.57 ± 0.95 mg/dl when compared to 177.88 ± 1.46 mg/dl in gentamicin administrated animals. MTE 400 produced maximum decrease in serum urea level when compared to all other groups. (Table 9, fig 18)

Urine creatinine

Significant increase ($P < 0.001$) in urine creatinine level was produced on administration of gentamicin when compared to normal group, indicating nephrotoxicity. The reversal of increase in urine creatinine level induced by gentamicin was observed on treatment with ethanolic and aqueous extracts at both the doses. Administration of MTA 200, MTA 400, MTE 200 and MTE 400 produced significant ($P < 1$) decrease in urine creatinine level when compared to gentamicin group. The decrease in urine creatinine level by MTA 200, MTA 400, MTE 200 and MTE 400 were found to be 203.18 ± 0.81 , 110.36 ± 0.96 , 142.69 ± 0.84 mg/dl and 103.63 ± 1.11 mg/dl respectively as compared to 286.62 ± 2.07 mg/dl in gentamicin treated animals. MTA 400 produced maximum decrease in urine creatinine level when compared with all other groups. (Table 9, fig 19)

Blood urea nitrogen-

Blood urea nitrogen (BUN) level was significantly ($P < 0.001$) increased in gentamicin group when compared with the normal group, indicating nephrotoxicity. Administration of MTE 200 produced significant ($P < 0.01$) decrease in blood urea nitrogen level when compared with gentamicin group. Administration of MTA 400, MTE 200 and MTE 400 produced significant ($P < 0.001$) decrease in BUN level when compared with gentamicin group.

BUN level in MTA 200, MTA 400, MTE 200 and MTE 400 treated animals were found to be 48.73 ± 1.61 , 36.88 ± 0.66 , 38.87 ± 1.36 and 27.11 ± 0.80 mg/dl as compared to 83.06 ± 2.0 mg/dl level in gentamicin treated animals. MTE 400 produced maximum decrease in BUN level when compared with all other groups. (Table 8, fig 20)

Body weight-

Administration of gentamicin produced significant ($P < 0.001$) reduction in body weight when compared to control group. Treatment with extracts significantly reduced in the decrease in body weight induced by gentamicin.

The percentage change in body weight in MTA 200, MTA 400, MTE 200 and MTE

400 treated animals were found to be $-6.97 \pm 0.58\%$, $-6.51 \pm 0.65\%$, $-6.70 \pm 1.01\%$ and $3.84 \pm 10.40\%$ respectively as against $-11.91 \pm 14.39\%$ in gentamicin treated animals, where

(-) negative, indicates decrease in body weight. (Table 2)

Histopathological study in gentamicin induced nephrotoxicity-

Control rats showed normal glomerular and tubular histology whereas gentamicin was found to cause glomerular congestion, peritubular congestion and result in the presence of inflammatory cells in kidney. Gentamicin treated also showed vacuolization and necrosis in the proximal tubular epithelial cells. Concurrent treatment with the extracts of *Thespesia populnea* was found to reduce such changes in the kidney histology induced by gentamicin (Table 10, fig 21).

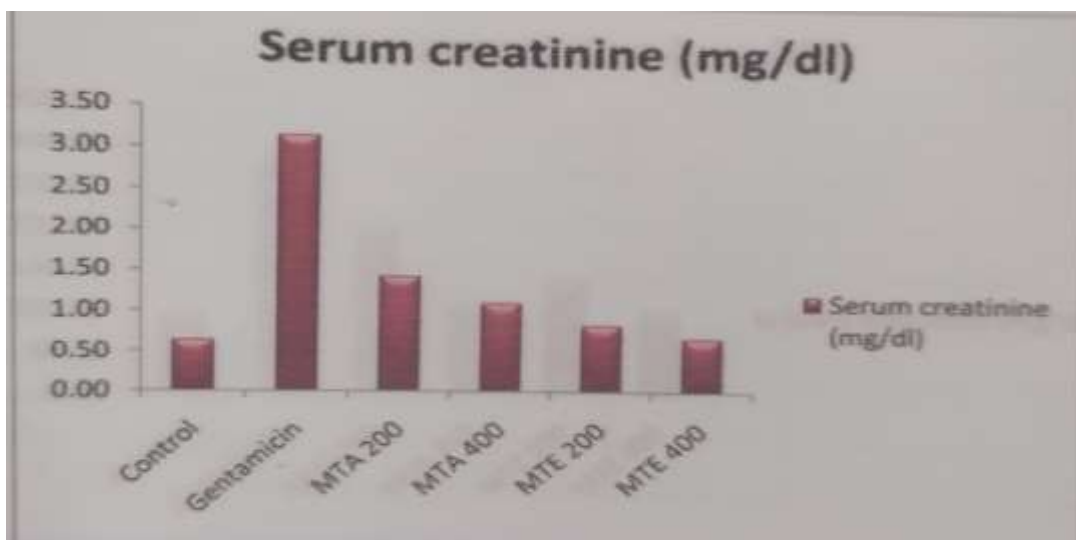
Table 9 Nephroprotective activity of ethanolic and aqueous extract of *Thespesia populnea* in gentamicin induced nephrotoxicity in rats.

Group	Treatment	Serum Creatinine (Mg/dl)	Serum Urea (Mg/dl)	Urine Creatinine (Mg/dl)	BUN (Mg/dl)	Body weight (%change)
I	Control	0.6±0.17	44.03±0.14	98.64 ±1.58	20.21±0.59	3.29 ±0.28
II	Gentamicin	3.17±0.68	177.88±1.46	286.62 ±2.07	83.06±2.0	-11.91±0.39
III	MTA 200	1.44±0.53	104.44±1.25	203.18 ±0.81	48.73 ±1.61	-6.97 ±0.58
IV	MTA 400	1.11±0.45	85.27 ±0.41	110.36±0.96	36.88±0.66	-6.51±0.65
V	MTE 200	0.83±0.12	83.24 ±1.31	142.69±0.84	38.87±1.36	-6.70 ±1.01
VI	MTE 400	0.67±0.10	58.57±0.95	103.63±1.11	27.33±0.80	-3.84 ±0.40

n=6 Values are expressed as means ± S.D.MTA and MTE 200 and 400 indicates *Merremia Tridentata* aqueous and ethanolic extract at 200 and 400 mg/kg

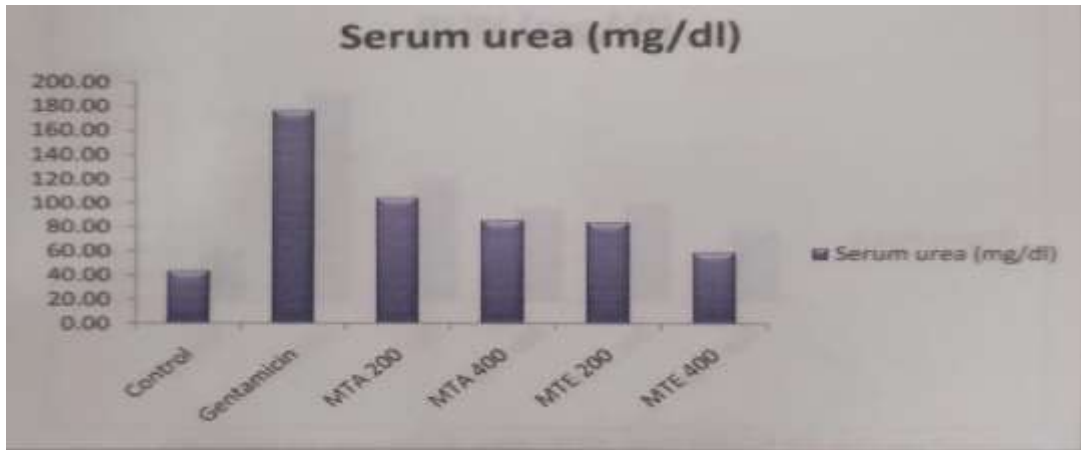
b.w.respectively * $P < 0.05$ ** $P < 0.001$ a-indicates comparison with control ,b-indicates comparison with gentamicin treated group.

Effect of leaves extract of *Thespesia populnea*.on Serum Creatinine level in Gentamicin induced and all extract treated rats .



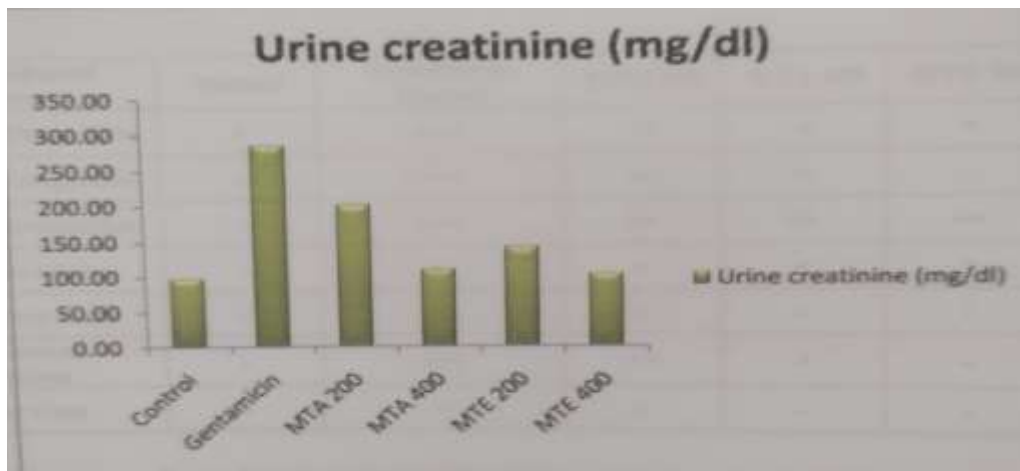
n=6 value are mean ±SEM,where MTA,MTE,MTE 200 and 400indicates *Merremia Tridentata* aqueous and ethanolic extract at 200 and 400 mg/kg.

Effect of leaves extract of *Thespesia populnea*.on Serum Urea level in Gentamicin induced and all extract treated rats .



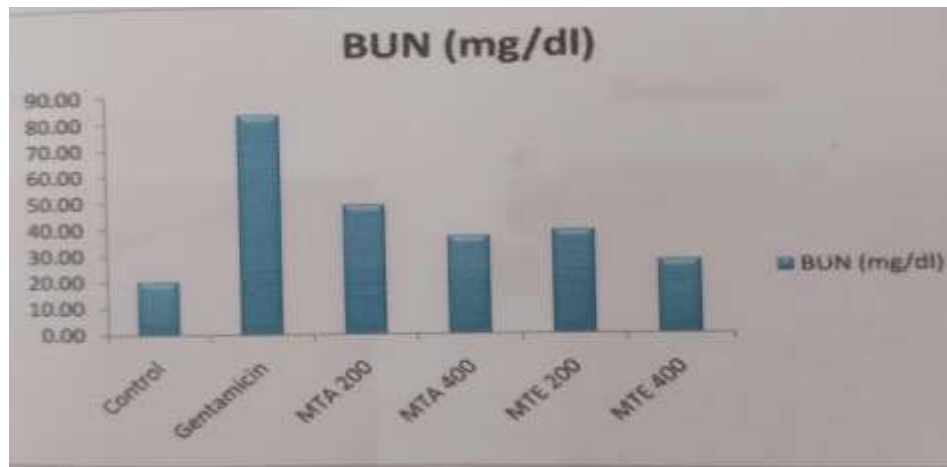
n=6 value are mean \pm SEM, where MTA, MTE, MTE 200 and 400 indicates *Thespesia populnea* aqueous and ethanolic extract at 200 and 400 mg/kg.

Effect of leaves extract of *Thespesia populnea* on Urine Creatinin levels in Gentamicin induced and all extract treated rats .



n=6 value are mean \pm SEM, where MTA, MTE, MTE 200 and 400 indicates *Merremia Tridentata* aqueous and ethanolic extract at 200 and 400 mg/kg.

Effect of leaves extract of *Thespesia populnea* on Blood Urea Nitrogen(BUN) levels in Gentamicin induced and all extract treated rats .



n=6 value are mean \pm SEM, where MTA, MTE, MTE 200 and 400 indicates *Thespesia populnea* aqueous and ethanolic extract at 200 and 400 mg/kg.

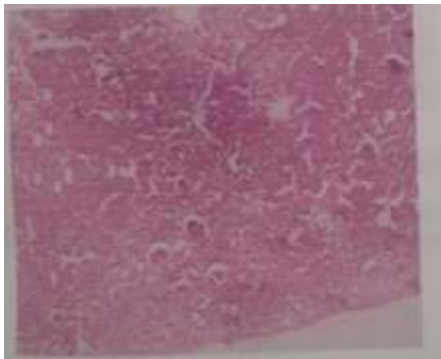
Table 10 Histopathological features of the kidneys of rats of different treatment group in gentamicine induced nephrotoxicity.

Histopathological Features	Normal	Gentamicin Treated	MTA 200	MTA 400	MTA 200	MTA 400
Glomerular Congestion	+	+++	++	+	+	-
Peritubular Congestion	-	+++	++	+	-	-
Blood vessel Congestion	-	+++	++	++	++	+
Interstitial edema	-	++	+	+	+	-
Inflammatory Cells	-	+	+	-	-	-
Mononuclear Infiltration	-	+++	++	+	-	-
Tubular Cast	-	+	+	-	-	-

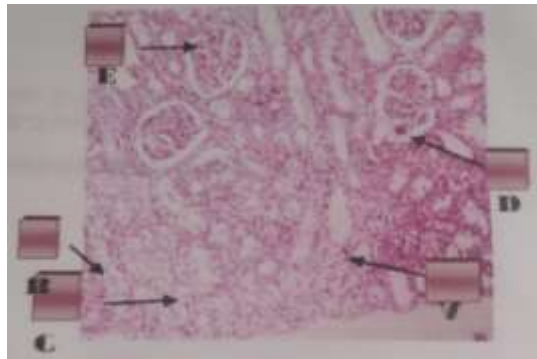
Haematoxyline and eosin stained skin section were scored as mild (+), moderate (++) an severe (+++) for epidermal and /or re-modeling.

Histopathological view of renal section from differents group stained with Matoxyline and eosin.

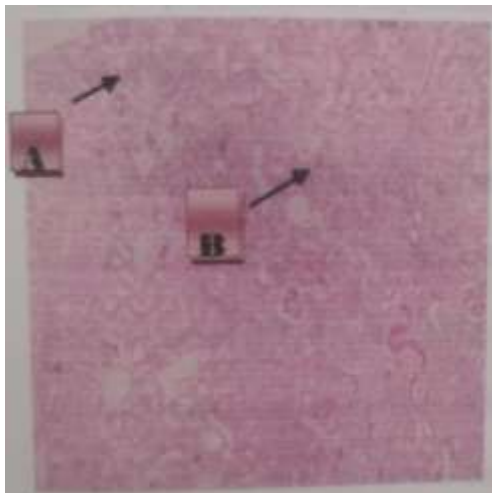
Control



Gentamicine



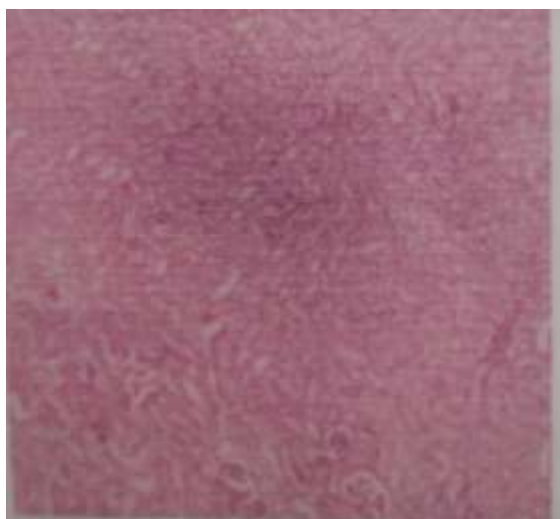
MTA 200



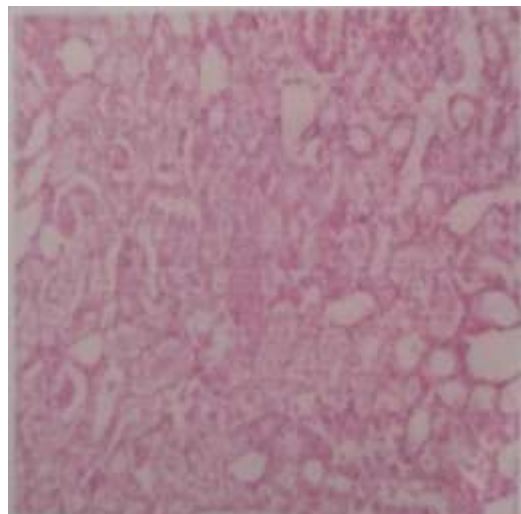
MTA 400



MTA 200



MTA 400



Gentamicine induced nephrotoxicity (kidney section)

A-mono-nuclear infiltrate cells, B-Tubular cast, C-Interstitial edema, D- Blood vessel congestion, E-Glomerular Congestion.

Cisplatin-induced nephrotoxicity:

Serum creatinine:

Administration of cisplatin produced significant increase ($P < 0.001$) in serum creatinine level when compared to normal group, indicating nephrotoxicity. Administration of MTA 200, MTA 400, MTE 200 and MTE 400 produced significant ($P < 0.001$) decrease in serum creatinine level when compared to cisplatin group. The reversal of increase in serum creatinine level by gentamicin was produced on treatment with both doses of ethanolic and aqueous extracts.

The serum creatinine level decreased by MTA 200, MTA 400, MTE 200 and MTE 400 were found to be 1.82 0.29, 0.8440.15, 0.8910.09 and 0.7340.15 respectively as compared to 3.17+0.69 mg/dl in cisplatin treated group. MTE 400 produced maximum decrease in serum creatinine level when compared to all other groups. (Table 11, fig 22)

Serum urea-

Serum urea level was significantly increased ($P < 0.001$) in cisplatin group when compared with the normal group, indicating nephrotoxicity. Administration of MTA 400, MTE 200 and MTE 400 produced significant ($P < 0.001$) decrease in serum urea level when compared with cisplatin group.

The reversal of increased serum urea level induced by cisplatin, seen after treatment with ethanolic and aqueous extracts at both doses

The serum urea level after treatment with MTA 200, MTA 400, MTE200 and MTE

400 were found to be 159.01-1.09, 77.861.32, 85.42+1.45 and 72.891.35mg/dl respectively as compared to 203.591.46 mg/dl in cisplatin treated group. MTE 400 produced maximum decrease in serum urea level when compared with all other group. (Table 11, fig 23)

Urine creatinine

Significant increase ($P < 0.001$) in urine creatinine level was produced on administration of cisplatin when compared to normal group, indicating nephrotoxicity. Administration of MTA 200, MTA 400, MTE 200 and MTE 400 produced significant ($P < 0.001$) decrease in urine creatinine level when compared to cisplatin group.

The decrease in urine creatinine level by MTA 200, MTA 400, MTE 200 and MTE 400 were found to be 176.25±4.28, 118.11 2.66, 146.3745.45 and 108.93+3.43 mg/dl respectively as compared to 260.92+2.07 mg/dl, in cisplatin treated animals. (Table 11, fig 24)

Blood urea nitrogen-

Blood urea nitrogen (BUN) level was significantly ($P < 0.001$) increased in cisplatin group when compared with the normal group. Administration of MTA 400, MTE 200 and MTE 400 produced significant ($P < 0.001$) decrease in blood urea nitrogen level when compared with cisplatin group.

Decrease in BUN level by MTA 200 & 400, MTE200 and MTE 400 were found to be 74.26 1.48, 36.3740.96, 39.8941.55 mg/dl and 34.03+1.08 mg/dl respectively as 95.06 1.48 mg/dl in cisplatin treated group. MTE 400 produced maximum decrease in

blood urea nitrogen level when compared to all other group. (Table 11, fig 25)

Body weight-

Administration of cisplatin produced significant ($P<0.001$) reduction in body weight when compared to normal. Treatment with extracts significantly reduced the decrease in body weight induced by gentamicin.

The percentage change in body weight in MTA 200, MTA 400, MTE 200 and MTE 400 treated animals were found to be $-11.15 \pm 1.18\%$, $-7.56 \pm 1.83\%$, $-10.00 \pm 0.21\%$ and -5.02 ± 0.46 respectively as against $-17.75 \pm 1.12\%$ in cisplatin treated animals, negative (-) indicates decrease in body weight. Administration of ethanolic and aqueous extracts at both the doses reversed in the increase in serum creatinine, serum urea, urine creatinine and BUN level induced by the nephrotoxics in gentamicin and cisplatin induced nephrotoxicity models.

The above observation indicates that the extracts exerts nephroprotective activity as the marker of namely serum creatinine, serum urea, urine creatinine and BUN were brought back to normal levels on administration of ethanolic and aqueous of *Thespesia populnea* (Table 11)

Histopathological study in cisplatin induced nephrotoxicity-

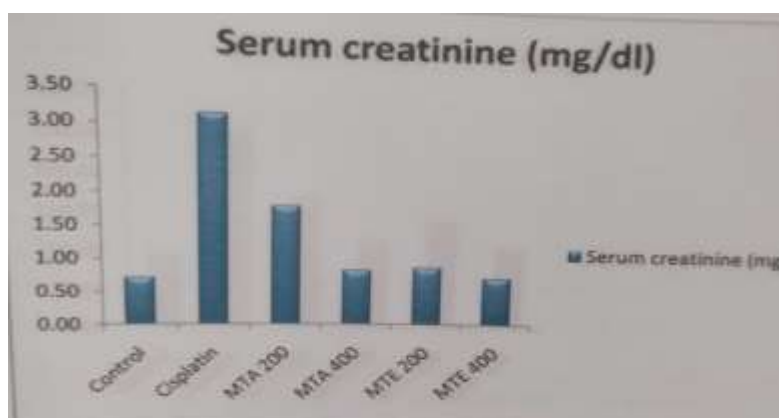
Histopathological changes in kidney section of the control group was found normal glomerular and tubular histology. Cisplatin group showed severe epithelial degeneration with glomerular congestion, peritubular congestion, mononuclear infiltration and blood cell congestion which results in the formation of inflammatory cell in kidney. There was noticeable vacuolization and necrosis in the proximal tubular epithelial cells in cisplatin treated group. Concurrent treatment with the extract of *Thespesia populnea*, was found to reduce such changes in the kidney histology induced by cisplatin. (Table 12, fig 26).

Table 11 Nephroprotective activity of ethanolic and aqueous extracts of *Thespesia populnea*. in cisplatin induced nephrotoxicity in rats.

Group	Treatment	Serum Creatinine (Mg/dl)	Serum Urea (Mg/dl)	Urine Creatinine (Mg/dl)	BUN (Mg/dl)	Body Weight (% change)
I	Control	0.72 ± 0.72	44.03 ± 0.16	98.64 ± 1.56	20.21 ± 0.58	3.29 ± 0.29
II	Cisplatin	3.17 ± 0.69***a	203. ± 1.46***a	260.59 ± 2.07***a	95.06 ± 1.94***a	-17.75 ± 1.12
III	MTA 200	1.82 ± 0.29**a	159.01 ± 1.09***a	176.25 ± 4.28***a,b	74.26 ± 1.48***a	-11.15 ± 1.12
IV	MTA 400	0.84 ± 0.15***b	77.86 ± 1.32***b	118.11 ± 2.66***b	36.37 ± 0.96***b	-7.56 ± 1.83
V	MTE 200	0.89 ± 0.09***b	85.42 ± 1.45***b	146.37 ± 5.45***a,b	39.89 ± 1.55***b	-10.00 ± 0.21
VI	MTE 400	0.73 ± 0.15***b	72.89 ± 1.35***b	108.93 ± 3.43***b	34.03 ± 1.08***b	-5.02 ± 0.46

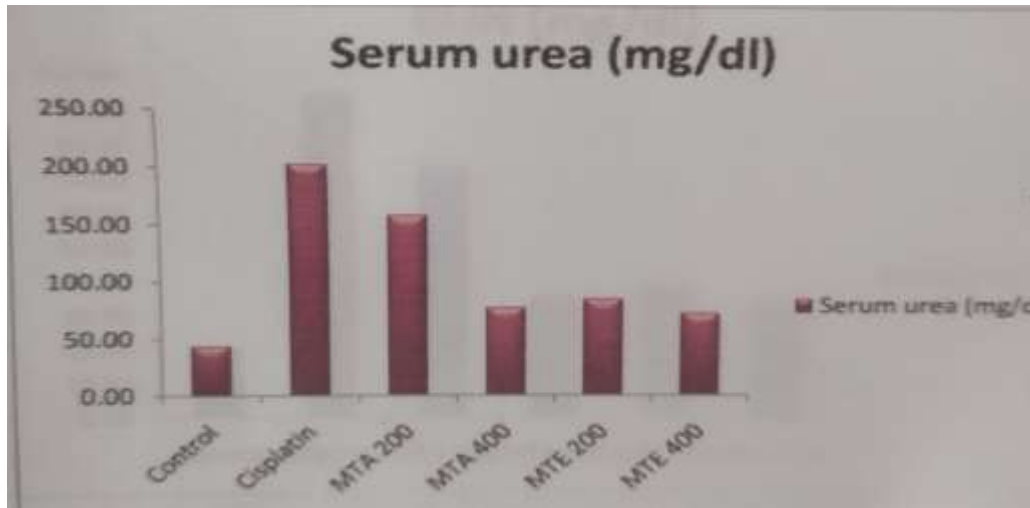
n=6 Values are expressed as means ± S.D. MTA and MTE 200 and 400 indicates *Thespesia populnea* aqueous and ethanolic extract at 200 and 400 mg/kg

b.w.respectively * $P<0.05$ ** $P<0.001$ a-indicates comparison with control ,b-indicates comparison with Cisplatin treated group.

Effect of leaves extract of *Thespesia populnea*.on Serum Creatinine level in Cisplatin induced and all extract treated rats .

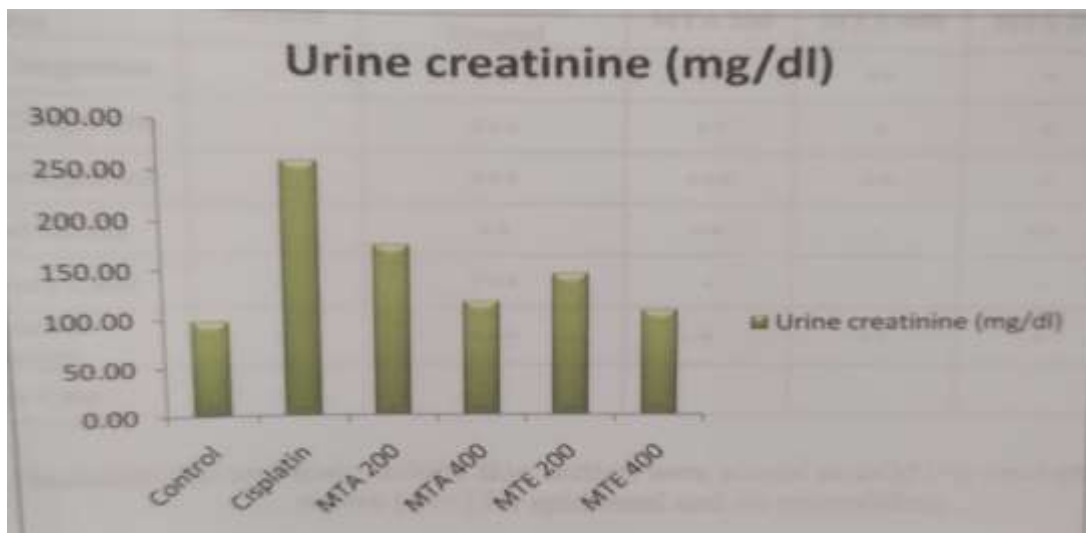
n=6 value are mean ± SEM, where MTA, MTE, MTE 200 and 400 indicates *Thespesia populnea* aqueous and ethanolic extract at 200 and 400 mg/kg.

Effect of leaves extract of *Thespesia populnea* on Serum Urea level in Cisplatin induced and all extract treated rats .



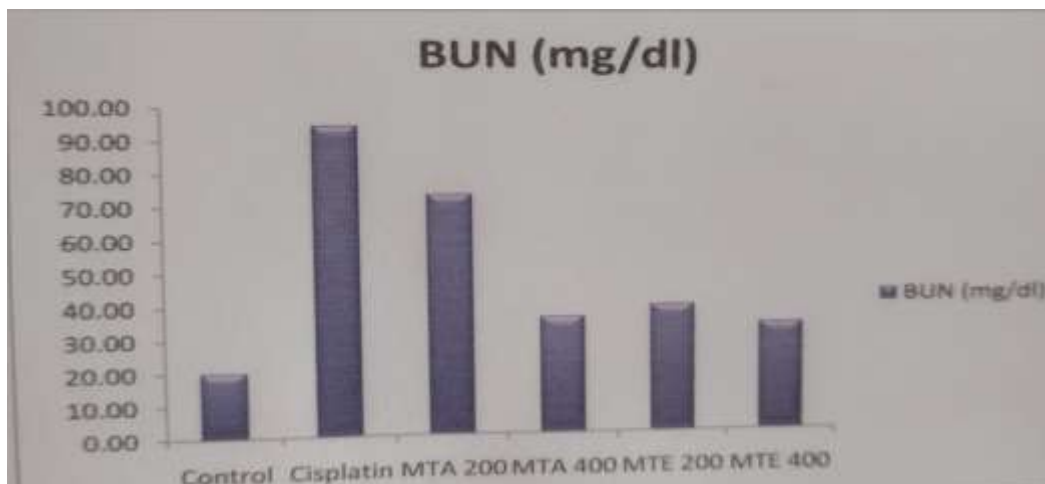
n=6 value are mean ±SEM, where MTA, MTE, MTE 200 and 400 indicates *Thespesia populnea* aqueous and ethanolic extract at 200 and 400 mg/kg.

Effect of leaves extract of *Thespesia populnea* on Urine Creatinine level in Cisplatin induced and all extract treated rats .



n=6 value are mean ±SEM, where MTA, MTE, MTE 200 and 400 indicates *Thespesia populnea* aqueous and ethanolic extract at 200 and 400 mg/kg.

Effect of leaves extract of *Thespesia populnea* on Blood Urea Nitrogen (BUN) level in Cisplatin induced and all extract treated rats .



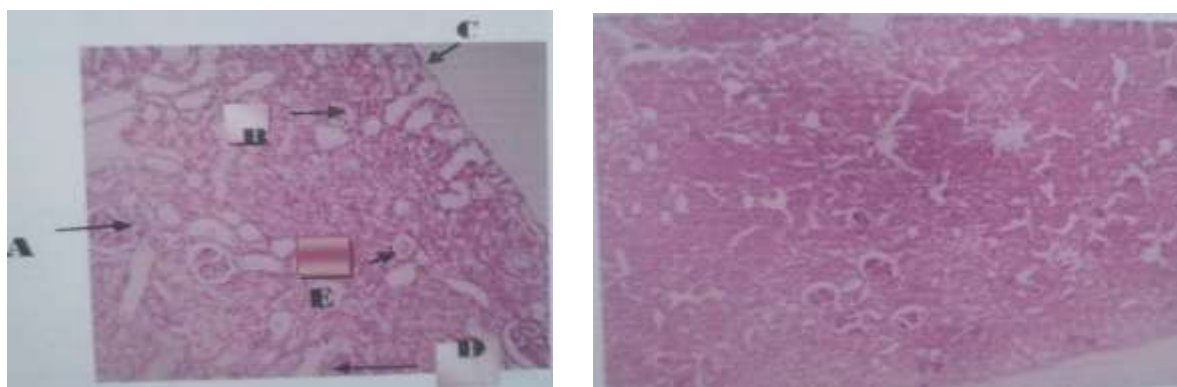
n=6 value are mean ±SEM, where MTA, MTE, MTE 200 and 400 indicates *Thespesia populnea* aqueous and ethanolic extract at 200 and 400 mg/kg.

Table 12 Histopathological features of the kidneys of rats of different treatment group in gentamicine induced nephrotoxicity.

Histopathological Features	Normal	Gentamicin Treated	MTA 200	MTA 400	MTA 200	MTA 400
Glomerular Congestion	-	+++	++	++	+	-
Peritubular Congestion	-	+++	++	+	+	-
Blood vessel Congestion	-	+++	+++	++	+	+
Interstitial edema	-	++	++	-	++	+
Inflammatory Cells	-	+++	-	-	-	-
Mononuclear Infiltration	-	+++	+	++	+	-
Tubular Cast	-					

Haematoxyline and eosin stained skin section were scored as mild (+), moderate (++) an severe (+++) for epidermal and /or re-modeling.

Histopathological view of renal section from differents group stained with hematoxyline and eosin.



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