Nephrotoxicity is defined as renal dysfunction that arises as result of exposure to external agents such as drugs and environmental chemicals. The present work was undertaken to carry out the phytochemical study and nephroprotective activity of methanolic extract of Casuarina equisetifolia leaves in gentamicin-induced nephrotoxicity in Wistar rats. Flavonoids and phenolic acids were identified and quantified using high performance liquid chromatography. Subcutaneous injection of rats with gentamicin (80 mg/kg body weight/day) for six consecutive days induced marked acute renal toxicity, manifested by a significant increase in serum urea, creatinine and uric acid levels, along with a significant depletion of serum potassium level, compared to normal controls. Also oxidative stress was noticed in renal tissue as evidenced by a significant decrease in glutathione level, superoxide dismutase, glutathione-S-transferase activities, also a significant increase in malondialdehyde and nitric oxide levels when compared to control group. Administration of plant extract at a dose of 300 mg/kg once daily for 4 weeks restored normal renal functions and attenuated oxidative stress. In conclusion, Casuarina equisetifolia leaves extract ameliorates gentamicin-induced nephrotoxicity and oxidative damage by scavenging oxygen free radicals, decreasing lipid peroxidation and improving intracellular antioxidant defense, thus extract may be used as nephroprotective agent.

Key Words:  Casuarina equisetifolia, nephroprotective, gentamicin, oxidative stress, nephrotoxicity.

Chemicals. GM sulfate, available commercially as Epigent (80 mg/2 ml ampoules), was provided by the Egyptian International Pharmaceutical Industries Co. (EIPICO, 10th of Ramadan City, Egypt). 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) was procured from Sigma Aldrich (St. Louis, MO). All other chemicals used throughout this study were of pure analytical grades.

Preparation of the extract. Samples of Casuarina equisetifolia were purchased from El-Orman Garden, Ministry of Agriculture, Egypt. The dried leaves of Casuarina equisetifolia (2 kg) were finely powdered and exhaustively extracted with 100% methanol, by maceration at room temperature. The crude methanolic extract was evaporated to dryness under reduced pressure. The process of maceration and evaporation was repeated till exhaustion of the plants powder, and then the residues were combined and weighed.

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Phytochemical screening of the extracts. Preliminary phytochemical screening for alkaloids, steroids, carbohydrates, tannins, fixed oils, proteins, triterpenoids, deoxy-sugar, flavonoid, cyanogenetic and coumarin glycosides carried out on the extract according to the procedures of Khandelwal.\(^{(13)}\)

Separation and quantification of phenolic compounds. Was conducted on Agilent Technologies 1200 Series Separations Module (GmbH, Germany) equipped with G1322A Vacuum degasser, G1311A Quaternary Pump, G1314B Variable Wavelength Detector (SL), G1328B Manual Injector and G1316A Thermostatted Column Compartment was used for HPLC analysis. The extract was separated at 35°C on a reverse phase HPLC, ACE 5 µm C18 column with dimensions 250 × 4.6 mm, detection at 280 nm. The mobile phase used was a gradient of A (CH₃COOH 2.5%), B (CH₃COOH 8%) and C (acetonitrile). The best separation was obtained with the following gradient: at 0 min, 5% B; at 20 min, 10% B. The solvent flow rate was 1 m/min. The volume injected was 20 µl. Phenolic compounds were quantified by using standard calibration for each compound and expressed as mg/100 g.

Separation and quantification of flavonoids. This was done using the above mentioned HPLC system and the same column with a mobile phase of methanol: water 1:1 (0–10 min) and 7:3 (10–20 min) at a flow-rate of 1 ml/min and detection at 339 nm. Each identified flavonoid was quantified by using standard calibration for each compound and expressed as mg%.

Determination of flavonoid content. Total flavonoid content was determined by a pharmacoepia method (State Pharmacopeia of USSR, using rutin as a reference compound. One ml of plant extract in methanol (10 g/L) was mixed with 1 ml aluminium trichloride in ethanol (20 g/L) and diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min at 20°C. Blank samples were prepared from 1 ml plant extract and 1 drop acetic acid, and diluted to 25 ml. The absorption of rutin solutions was measured under the same conditions. Standard rutin solutions were prepared from 0.05 g rutin. All determinations were carried out in duplicate. The amount of flavonoid in plant extracts in rutin equivalents (RE) was calculated by the following formula (Eq. 1):

\[ X = \frac{(A \times m \times 10)}{(A_0 \times m)} \]  

where: X - flavonoid content, mg/g plant extract in RE; A - the absorption of plant extract solution; A₀ - the absorption of standard rutin solution; m - the weight of plant extract, g; m₀ - the weight of rutin in the solution, g.

Determination of antioxidant activity of Casuarina equisetifolia extract in vitro. DPPH radical scavenging assay. Radical scavenging activity of plant extracts against stable DPPH radical was measured under the same conditions. Standard rutin solutions and corresponding blank solutions.

Assay of reducing power. The reductive capability of the extract was quantified by the method of Oyaizu, 1986.\(^{(16)}\) One ml of Extract (100–1,000 µg) in distilled water was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K₃Fe(CN)₆]. The mixture was incubated at 50°C for 20 min. Then, the reaction was terminated by adding 2.5 ml of 1% trichloracetic acid. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1% FeCl₃. Blank reagent is prepared as above without adding extract. The absorbance was measured at 700 nm in a spectrophotometer against a blank sample. Increased absorbance of the reaction mixture indicated greater reducing power.

Hydroxyl radical (OH) scavenging assay. The reaction mixture (3 ml) containing 1 ml FeSO₄ (1.5 mM), 0.7 ml hydrogen peroxide (6 mM), 0.3 ml sodium salicylate (20 mM) and varying concentrations of the extract (2–10 µg) were taken. After incubation for 1 h at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm.\(^{(17)}\)

Experimental design.

Animals and experimental protocol. Swiss male albino mice (20–25 g) were used for the acute study and 25 g for the chronic study. The extract of the plant under investigation was determined according to Behrens and Karber.\(^{(18)}\)

Acute toxicity study. It was found that the tested extracts were not mortal even at a dose of 3,000 mg/kg and consequently the dose 300 mg/kg was selected for the study.

Thirty six male Wister albino rats weighing (150–200) g were used for this study. The animals were housed in a temperature (25 ± 1°C), humidity controlled room and a 12 h light-dark cycle (lights on at 6:00). Rats were allowed free access to tap water and standard pellet diet. The institutional Animal Ethics Committee approved all experimental protocols. The animals were classified into 6 groups, each of 6 as follows:

- Control group (C): Rats received distilled water.
- GM: Rats received subcutaneous injection of GM (80 mg/kg body weight/day) for 6 consecutive days.
- Curative groups:
  - GM and Casuarina equisetifolia extract treated group, (GM + E): Rats received subcutaneous injection of GM (80 mg/kg body weight/day) for 6 consecutive days, followed by oral administration of Casuarina equisetifolia extract a dose of 300 mg/kg once daily for 4 weeks.
  - GM and Silymarin (Reference drug) treated group, (GM + R): Rats received subcutaneous injection of GM (80 mg/kg body weight/day) for 6 consecutive days, followed by oral administration of Silymarin a dose of 50 mg/kg once daily for 4 weeks.
  - Protective groups:
    - Casuarina equisetifolia extract and GM treated group (E + GM): Rats received oral administration of Casuarina equisetifolia extract at a dose of 300 mg/kg once daily for 4 weeks, followed by subcutaneous injection of GM (80 mg/kg body weight/day) for 6 consecutive days.
    - Silymarin (Reference drug) and GM treated group (R + GM): Rats received oral administration of Silymarin a dose of 50 mg/kg once daily for 4 weeks, followed by subcutaneous injection of GM (80 mg/kg body weight/day) for six consecutive days.

Blood collection and biochemical assays. Fasting blood samples were withdrawn from the retro-orbital vein of each animal using a glass capillary tube after fasting period of 12 h. The blood samples allowed to coagulate and then centrifuged at 3,000 rpm for 20 min. The separated sera were used for the estimation of serum activities of alanine transaminase (ALT), aspartate transaminase (AST) by using commercial kits (Quimica Clinica Aplicada, Spain). Serum urea and creatinine level was estimated using commercial kits purchased from Stanbio, Boerne, TX. Serum potassium and uric acids concentrations were evaluated using kits from Biodiagnostic, Egypt. Serum vitamin C level was assayed using the method described by Jagota and Dani.\(^{(19)}\)
Preparation of renal homogenate. The whole kidney was accurately weighed and homogenized in ice-saline to prepare a 10% (w/v) tissue homogenate. The homogenate was used for the determination of malondialdehyde level (MDA) and reduced glutathione level (GSH), superoxide dismutase activity (SOD), glutathione-S-transferase activity (GST) and nitric oxide level (NO).

Determination of protein content. The protein content of kidney homogenates was evaluated by following the method of Lowry et al. and using bovine serum albumin as a standard.

Biochemical analysis in kidney homogenate.

Determination of lipid peroxidation. MDA, as lipid peroxidation end product within kidney tissues, was quantified via the established measurement of the level of thiobarbituric acid reactive substances (TBARS). An aliquot of 0.5 ml of 10% homogenate (or standard) was pipetted into a 10 ml centrifuge tube followed by the addition of 3 ml of 1% orthophosphoric acid and 1 ml of 0.6% thiobarbituric acid. After heating for 45 min in a boiling water bath, the mixture was then cooled and 4 ml of n-butanol was added and mixed vigorously. The upper butanol layer was separated by centrifugation and absorbance was measured at 535 nm and 520 nm against a reagent blank.

Determination of GSH level. GSH level in renal tissue homogenates was measured by the method of Ellman. Trichloroacetic acid (5%) was added to adequate dilution of tissue homogenates (0.5 ml) to precipitate the protein content in the samples. This mixture was centrifuged at 10,000 g, 5 min and the supernatant was recovered. Finally, 5,5'-dithiobis(2-nitrobenzoic acid) solution was added to the reaction mixtures, and the absorbance was recorded at 412 nm using spectrophotometer.

Determination of SOD activity. SOD activity in renal tissues was determined by the method of Marklund and Marklund. Pyrogallol (24 mM) was prepared in 10 mM HC1 and kept at 4°C before use. Stock catalase solution (30 µM) was prepared in phosphate buffer (PH 9, 0.1 M), 100 µl of the supernatant was added to Tris HC1 buffer (pH 7.8, 0.1 M) containing 25 µl pyrogallol and 10 µl catalase. The final volume was adjusted to 3 ml using the same buffer solution. Changes in the absorbance at 420 nm were recorded at 1 min. interval for 3 min. Data were expressed as U/mg protein.

Determination of GST activity. GST activity in renal tissues was assayed according to the method of Habig et al. In brief, 2.8 ml of 0.1 M phosphate buffer pH 6.5, glutathione 100 µl and 1-chloro 2,4-dinitrobenzene 100 µl was mixed. The reaction was started by the addition of 25 µl of 10% homogenate fraction. The change in absorbance was observed by continuous recording at 340 nm at 1 min intervals for 3 min, the data were expressed as nmol/min/mg protein.

Determination of NO content. NO level in renal tissue homogenates was determined according to the method of Green et al. The assay is based on the diazotization of sulfanilic acid with nitric oxide at acidic pH and subsequent coupling with N-(10-naphthyl)-ethylenediamine to yield an intensely pink colored product that is measured spectrophotometrically at 540 nm. Sodium nitrite was used as standard.

Statistical analysis. Statistical analysis of differences between means was carried out using ANOVA, followed by the least significant difference (LSD) test for multiple comparisons using SPSS for Windows software, ver. 6.0 (Chicago, IL); p<0.05 was considered statistically significant for all tests.

Results

Phytochemical analysis of methanolic extract of Casuarina equisetifolia leaves extract. HPLC analysis of the total phenolic contents of methanolic extract of the aerial parts of Casuarina equisetifolia revealed the presence of 9 phenolic compounds as gallic acid, protocatechuic acid, para-coumaric acid, chlorogenic acid, salicylic acid, benzoic acid, catechol, pyrogallol and chrys in concentration of (21.41, 13.15, 0.73, 129.42, 3.14, 181.39, 16.17, 1.049.9 and 3.04 mg/100 g plant extract respectively). Pyrogallol represented the highest concentration of the identified phenolic compounds while para-coumaric acid was the lowest concentration (Table 2).

Identification of quantification of flavonoids. Fig. 2 represents the separation of flavonoids and flavonols of Casuarina equisetifolia leaves extract. HPLC analysis of the total flavonoidal contents of the same plant extract showed the presence of flavonol constituents as quercetin, rutin and kampferol, flavone constituents as hesperitin and apigenin and flavanone constituent as nareginin in the concentration of (837.9, 834.6, 399.2, 206.2, 59.9 and 384.8 mg/100 g plant extract respectively). Quercetin represented the highest concentration flavonoidal compound while apigenin was the lowest concentration, (Table 3).

The content of flavonoidal compounds (mg %). The flavonoidal content was found to be 11.66 ± 0.2 mg % rutin equivalent.

DPPH' radical scavenging activity. Casuarina equisetifolia extract scavenged DPPH radicals in a dose dependent manner (10–100) µg, with a 50% inhibition (IC50) at a concentration of 18 µg (Fig. 3).

NO radical inhibition. The scavenging of NO by extract was increased in a dose dependent manner as illustrated in Fig. 4. At concentration of 800 µg of extract 50% of NO generated by incubation was scavenged.

Hydroxyl radical (•OH) scavenging activity. The extract of Casuarina equisetifolia exhibited a significant dose dependent inhibition of •OH activity, with a 50% inhibition (IC50) at a concentration of 4 µg (Fig. 5).

Reducing power. The reducing power of extract of Casuarina equisetifolia was very potent and the reducing power of the extract was increased with concentration of sample. The plant extract could reduce the most Fe3+ ions (Fig. 6).

Effect of Casuarina equisetifolia extract on GM-induced renal dysfunction. Subcutaneous injection of normal rats with GM caused a significant increase in serum urea, creatinine, uric acid levels and a significant reduction in serum potassium concentration with respect to normal controls, p<0.05. Whereas in both protective and curative groups the oral administration of Casuarina equisetifolia extract as well as Silymarin (reference drug) to GM-intoxicated rats significantly normalized renal dysfunction, the effect was prominent in the protective groups (Table 4). The treatment of rats with Casuarina equisetifolia extract showed normal serum ALT and serum AST activities indicating its safety.

Effect of Casuarina equisetifolia extract and/or GM treatment on GST, SOD activities and GSH level in renal homogenate. GM intoxication caused a significant decrease in renal GST, SOD activities and GSH level as compared with normal controls, p<0.05. Either treatment with Casuarina equisetifolia ex-

| Chemical group | Identification |
|----------------|----------------|
| Tannins        | ++++           |
| Flavonoids     | +++           |
| Saponins       | -             |
| Alkaloids      | +             |
| Phenolics      | ++++          |
| Terpenoids     | +             |
| Steroids       | +             |

Table 1. Phytochemical analysis of methanolic extract of Casuarina equisetifolia leaves.
Recent evidence indicated that ROS are the potential mediators involved in GM-induced renal dysfunction. GM has been shown to enhance generation of superoxide anion (O$_2^-$), peroxynitrite anion (ONOO$^-$), hydrogen peroxide (H$_2$O$_2$), and (OH) production from renal cortical mitochondria. The interaction between O$_2^-$ and H$_2$O$_2$ in the presence of a metal catalyst (iron) leads to the generation of toxic 'OH, which induces peroxidation of the polyenic lipids of the endoplasmic reticulum and subsequent generation of secondary free radicals derived from these lipids. This destructive lipid peroxidation leads to breakdown of membrane structure and function. Further decomposition of peroxidized lipids yields a wide variety of end products, including MDA. This GM-induced oxidative stress is the central pathway responsible for nephrotoxicity. (29,30)

In our work, oral administration of *Casuarina equisetifolia* leaves extract to GM-intoxicated rats normalized serum urea, Creatinine, uric acid and K$^+$ levels, suggesting that the extract under investigation may introduce protection against GM-induced nephrotoxicity, possibly by attenuating the oxidative stress induced by administration of GM.

GSH depletion is a common consequence of increased formation of ROS. An explanation to cellular GSH depletion after GM intoxication is the increased consumption of GSH in non-enzymatic removal of oxygen radicals, or the oxidation of sulfhydryl group existing at the active site of the enzyme molecule. (31)

Also, it has been reported that depletion of cellular GSH level results in concomitant decrease in glutathione-related enzymes activity. (32) Oxidative stress in renal tissue leads to diminished SOD activity. (33)

Most of the antioxidant enzymes become inactive in response to oxidative stress. (34) The decrease of SOD and GST activities in the kidney tissues by GM administration in the present investigation run in parallel to the study of Pedraza-Chaverri et al. (35) However, Free radicals are causative factors for aminoglycosides (GM) induced renal toxicity. (10) It has demonstrated that GM generates ROS that mediate biomolecules oxidation in the kidney. (37) The excessive ROS can damage the protein sensitive thiols. Therefore, GM inhibits the activities of antioxidant enzymes, GST and SOD and depletes thiol cellular content. (18) In our work, *Casuarina equisetifolia* leaves extract consumption improves GSH content and SOD as well as GST activities. These results demonstrated

### Table 2. Identified phenolics in methanolic extract of *Casuarina equisetifolia* leaves

| Test items | Concentration (mg/100 g) |
|------------|--------------------------|
| Gallic     | 21.41                    |
| Protocatechuic | 13.15                   |
| P-Coumaric | 0.73                     |
| Chlorogenic| 129.42                   |
| Catechol   | 16.17                    |
| Pyrogallol | 1049.9                   |
| Salicylic  | 3.14                     |
| Chrysin    | 3.04                     |
| Benzoic    | 181.39                   |

### Fig. 1. HPLC chromatograms of identified phenolic compounds of *Casuarina equisetifolia* leaves extract separated by RP HPLC; Pyrogallol ($t_R = 8.7$ min), Catechol ($t_R = 8.9$ min), Salicylic ($t_R = 11.3$ min), P-Coumaric ($t_R = 12.79$ min), Benzoic ($t_R = 13.54$ min), Chrysin ($t_R = 18.2$ min).
that *Casuarina equisetifolia* leaves extract significantly enhanced antioxidant defense against GM induced oxidative damage in renal tissues. This may be attributed to free radical scavenging property of extract as well as direct antioxidant action.

The findings in the current study is consistent with data of Alqasoumi et al.,

(40) the author investigated the possible protective effect of water spinach *Ipomea aquatica* ethanol extract against GM-induced nephrotoxicity in Wistar albino rats. The author found that concomitant administration of *Ipomea aquatica* extract attenuated the harmful effects of GM both by inhibiting free-radical formation and/or by restoration of the antioxidant systems. The author attributed the protective effect of *Ipomea aquatica* to the presence of flavonoids and tannins that are known to possess potent antioxidant and free radical scavenging properties.

In a previous study was conducted by Shirwaikar et al.,

(41) the author studied the protective activity of ethanolic extract of *Aerva lanata* in cisplatin and GM-induced nephrotoxicity in male Wistar rats using some key parameters such as plasma, liver, and kidney.

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**Table 3.** Identified flavonoids in methanolic extract of *Casuarina equisetifolia* leaves

| Test items     | Concentration (mg/100 g) |
|----------------|--------------------------|
| Rutin          | 834.6                    |
| Rosmarinic     | 384.6                    |
| Quercitin      | 837.9                    |
| Hesperetin     | 206.2                    |
| Narenginin     | 384.8                    |
| Apignen        | 59.9                     |
| Kampferol      | 399.2                    |

**Fig. 2.** HPLC chromatograms of identified flavonoidal compounds of *Casuarina equisetifolia* leaves extract separated by RP HPLC; Rosmarinic (R<sub>t</sub> = 11.24 min), Hesperetin (R<sub>t</sub> = 11.6 min), Rutin (R<sub>t</sub> = 12.06 min, Quercitin (R<sub>t</sub> = 13.09 min), Narenginin (R<sub>t</sub> = 14.1 min), Kampferol (R<sub>t</sub> = 14.75 min), Apignen (R<sub>t</sub> = 15.49 min).

**Fig. 3.** Effect of different concentrations of *Casuarina equisetifolia* extract on scavenging activity of DPPH radicals. Values are expressed as mean ± SEM of three experiments.

**Fig. 4.** Effect of different concentrations of *Casuarina equisetifolia* extract on scavenging activity of NO radicals. Values are expressed as mean ± SEM of three experiments.
Natural antioxidants such as flavonoids and polyphenols are believed to possess antioxidant properties due to their reducing and chelating capabilities. Flavonoids and polyphenols are secondary plant metabolites that are widely distributed in fruits, leaves, bark, and other parts in plants with free radical scavenging abilities. In the present study, there was a significant increase in renal NO in GM-treated rats. The rise of renal NO in GM-treated rats has been previously reported. This supported by the finding of Aerva lanata was found to normalize the raised blood urea, serum creatinine and bring about marked recovery in kidneys. The author mentioned that flavonoids such as kaempferol 3-rhamnoside and kaempferol 3-rhamnoglucoside have been reported to be present in Aerva lanata. Flavonoids are well known potent antioxidant and free radical scavengers. Hence, the probable mechanism of nephroprotection by Aerva lanata may be attributed to its antioxidant and free radical scavenging property.

Table 4. Effect of Casuarina equisetifolia extract and/or GM treatment on studied serum biochemical parameters

| Group     | Urea (mg/dl) | Creatinine (mg/dl) | Uric acid (mg/dl) | Potassium (mg/dl) | Vitamin C (mmol/L) | SALT (U/L) | SAST (U/L) |
|-----------|--------------|--------------------|-------------------|-------------------|-------------------|------------|------------|
| C         | 23.43 ± 1.2  | 0.43 ± 0.014       | 1.47 ± 0.094      | 5.29 ± 0.4        | 1.39 ± 0.15       | 26.6 ± 2  | 52.1 ± 4.9 |
| GM        | 119 ± 10.2** | 1.97 ± 0.016*      | 2.53 ± 0.16*      | 4.07 ± 0.03*      | 0.82 ± 0.02*     | 27.8 ± 2.2 | 54.4 ± 3.9 |
| GM + E   | 33 ± 2***    | 0.43 ± 0.011**     | 1.21 ± 0.11**     | 5.86 ± 0.26**     | 1.49 ± 0.12**    | 29.1 ± 1.9 | 53 ± 5     |
| E + GM    | 23.88 ± 1**  | 0.44 ± 0.02**      | 0.92 ± 0.1*       | 4.83 ± 0.04**     | 1.56 ± 0.099**   | 28.2 ± 2.5 | 52 ± 5     |
| GM + R   | 29.4 ± 2.3***| 0.39 ± 0.011**     | 1.0 ± 0.03**      | 4.9 ± 0.15**      | 1.34 ± 0.12**    | 29.3 ± 2.4 | 55.6 ± 4.89 |
| R + GM    | 27.31 ± 1.5**| 0.41 ± 0.007**     | 1.1 ± 0.04**      | 4.55 ± 0.02***    | 1.48 ± 0.04**    | 28.4 ± 2.3 | 51 ± 3.78  |

Results are expressed as mean ± SE. *Significantly different from control group, **Significantly different from GM treated group, †Groups GM + E and GM + R are significantly different from group E + GM, ††Group R + GM is significantly different from group GM + E, †‡Groups E + GM, GM + R and R + GM are significantly different from group GM + E.

Table 5. Effect of Casuarina equisetifolia extract and/or GM treatment on GST, SOD activities, GSH, MDA and NO level in renal homogenate

| Group     | GST (nmol/min/mg protein) | SOD (U/mg protein) | GSH (nmol/g tissue) | MDA (μmol/g tissue) | NO (μmol/g wet tissue) |
|-----------|---------------------------|-------------------|---------------------|---------------------|------------------------|
| C         | 45.6 ± 1.9                | 55.2 ± 1.27       | 20 ± 0.78           | 52.6 ± 1.04         | 125 ± 3.95             |
| GM        | 38.8 ± 1*                 | 46.2 ± 1.4*       | 15.9 ± 0.45*        | 60.4 ± 0.6*         | 140 ± 1.38*            |
| GM + E    | 43.8 ± 0.64**             | 53.1 ± 1.11**     | 19.4 ± 0.49**       | 54 ± 0.75**         | 123 ± 2.75**           |
| E + GM    | 44.3 ± 0.89**             | 52.4 ± 0.76**     | 19.2 ± 0.55**       | 54.6 ± 0.7**        | 126 ± 2.39**           |
| GM + R    | 48.8 ± 0.6**              | 59 ± 1.86**       | 19.9 ± 0.86**       | 52.4 ± 0.8**        | 125 ± 3.2**            |
| R + GM    | 47.2 ± 1.3***             | 58.8 ± 1.8**      | 21 ± 0.8**          | 53.4 ± 0.7**        | 126 ± 3.5**            |

Results are expressed as mean ± SE. *Significantly different from control group, **Significantly different from (GM) treated group, †Groups GM + R and R + GM are significantly different from group GM + E, ††Group GM + R is significantly different from group GM + E, †‡Groups E + GM, GM + R and R + GM are significantly different from group GM + E.
Abdelaziz and Kandeel. The authors demonstrated that NO was increased by GM induced renal toxicity.

Moreover, Kandeel et al. reported that there are an increase in the levels of oxidative stress biomarkers including NO in response to GM toxicity. NO induce renal cellular damage through formation of peroxynitrite that provoke the damage to the cellular structural molecules. However, Abdelaziz and Kandeel demonstrated that the renal toxic effect of GM mediated by increase of NO is ameliorated by antioxidants. In the present study, the normalization in the level of renal NO could be attributed to antioxidant activity of the Casuarina equisetifolia extract.

Preliminary phytochemical screening of Casuarina equisetifolia extract revealed the presence of alkaloids, terpenoids, flavonoids, polyphenols, steroids and tannins. Tannins are complex polyphenolic compounds widely found in higher plants. Similar to many polyphenols, tannin has been shown to possess antioxidant activity. The antioxidant activity of Casuarina equisetifolia extract could be also attributed to its flavonoidal content. Flavonoids act as scavengers of various oxidizing species i.e., O$_2^−$, OH or peroxyl radicals, they also act as quenchers of singlet oxygen. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity. Phenolic compounds are effective hydrogen donors, making them very good antioxidants. The antioxidant activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Comparative analysis of gene expression between renal cortex and papilla in nediaplatin-induced nephrotoxicity in rats. Environ Toxicol Pharmacol 2005; 19: 473–477.

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The direct contribution of O$_2^−$ in the pathogenesis of nephrotoxicity are also well substantiated. The nephroprotective effect of Casuarina equisetifolia extract may therefore be attributed to its potent nitric oxide and O$_2^−$ scavenging effects. In conclusion, it is proposed that the nephroprotective activities of the Casuarina equisetifolia leaves extract in GM-induced nephrotoxicity may be due to presence of phytochemicals like flavonoids, tannins which may act as antioxidants individually or synergistically. Casuarina equisetifolia leaves extract could constitute a lead to discovering a novel pharmaceutical formulation which will be useful for treatment of drug-induced nephrotoxicity.

**Abbreviations**

ALI Alkaline transaminase
AST Aspartate transaminase
DPPH 2,2-Diphenyl-1-picrylhydrazyl hydrate
GM Gentamicine
GSH Glutathione
GST Glutathione-S-transferase
MDA Malondaldehyde
NO Nitric oxide
RNS Reactive nitrogen species
ROS Reactive oxygen species
SOD Superoxide dismutase

**Conflict of Interest**

No potential conflicts of interest were disclosed.

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Preliminary phytochemical screening of Casuarina equisetifolia extract revealed the presence of alkaloids, terpenoids, flavonoids, polyphenols, steroids and tannins. Tannins are complex polyphenolic compounds widely found in higher plants. Similar to many polyphenols, tannin has been shown to possess antioxidant activity. The antioxidant activity of Casuarina equisetifolia extract could be also attributed to its flavonoidal content. Flavonoids act as scavengers of various oxidizing species i.e., O$_2^−$, OH or peroxyl radicals, they also act as quenchers of singlet oxygen. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity. Phenolic compounds are effective hydrogen donors, making them very good antioxidants. The antioxidant activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Comparative analysis of gene expression between renal cortex and papilla in nediaplatin-induced nephrotoxicity in rats. Environ Toxicol Pharmacol 2005; 19: 473–477.

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