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# Protective effect of the methanolic leaf extract of *Eclipta alba* (L.) Hassk. (Asteraceae) against gentamicin-induced nephrotoxicity in Sprague Dawley rats

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ABSTRACT

*Ethnopharmacological relevance:* *Eclipta alba,* also known as *Eclipta prostrata,* is a weed of the family Asteraceae found in tropical and subtropical regions widely used in herbal medicine, including treatment of renal diseases.
*Aim of the study:* This study aims to evaluate the protective effect of the methanolic leaf extract of *Eclipta alba* on gentamicin-induced nephrotoxicity in rats.
*Materials and methods:* Nephrotoxicity was induced in rats by subcutaneous injection of gentamicin (80 mg/kg/day for seven days). Quercetin was used as a positive control. The nephroprotective activity was evaluated by determining blood urea nitrogen, serum creatinine, urinary microprotein, renal catalase and malondialdehyde levels.
*Results:* The extract protected the rat kidneys against gentamicin-induced renal tubular alterations and rises in blood urea nitrogen, serum creatinine, and microprotein levels. Lipid peroxidation and decrement in catalase levels were also ameliorated.
*Conclusion:* The study revealed the protective effect of the methanolic leaf extract of *E. alba* and suggests that the probable mechanism for the nephroprotection by the extract may be due to its good radical scavenging activity and $Fe^{3+}$ ion-reducing ability.



## 1. Introduction

Nephrotoxicity is a frequent complication of drugs and diagnostic agents (Choudhury and Ahmed, 2006). Gentamicin (GM), an aminoglycoside antibiotic, is used for Gram-negative infections; however, nephrotoxicity limits its clinical use (Martinez-Salgado et al., 2007). There is increasing evidence for the role of reactive oxygen species (ROS) in GM toxicity (Martínez-Salgado et al., 2007). The present study was based on the premise that if gentamicin-induced nephrotoxicity is associated to formation of ROS then antioxidant therapy might attenuate nephrotoxicity secondary to gentamicin.

*Eclipta alba,* also known as *Eclipta prostrata,* is a weed of the family Asteraceae found in tropical and subtropical regions and thrives in moist places (Umemoto and Koyama, 2007). It is found throughout the Philippines in the lowlands generally, ascending to 1600 m (Asteraceae, n.d.) where it is known as *tintatintahan*. Various literatures have documented the antiprotozoal (Bapna et al., 2007) and antihyperlipidemic (Kumari et al., 2006) properties of the plant. Moreover, the leaf extract of *E. alba* has been reported to exhibit antioxidant properties which are associated with its flavonoid content (Karthikumar et al., 2007). A study by Sidra et al. (2013) showed its ethnomedicinal use in the treatment of renal diseases. However, there is insufficient evidence that would support this traditional use. Therefore, in this current study we investigated the protective effect of the methanolic leaf extract of *Eclipta alba* on gentamicin-induced nephrotoxicity in rats by administering high subcutaneous doses of the antibiotic for seven days. Elevated levels of serum creatinine, blood urea nitrogen (BUN) and histopathological alterations signified drug-induced nephrotoxicity in test animals. Previous studies on the acute oral toxicity of the methanolic leaf extract of *E. alba* demonstrated its safety with a median lethal dose of more than five grams per kilogram ($LD_{50} > 5$ g/kg) in rats (Kumar et al., 2010) and more than two grams per kilogram ($LD_{50} > 2$ g/kg) in mice (Shaikh et al., 2012).

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## 2. Materials and methods

### 2.1. Standards, reagents and chemicals

Analytical grade solvents (methanol, n-hexane, dichloromethane (DCM), butanol, petroleum ether, acetone, etc.) for extraction and phytochemical analysis were purchased from Belman Laboratories, Inc. in Manila, Philippines. Analytical grade reagents such as quercetin were purchased from Sigma-Aldrich, Singapore through Belman Laboratories, Inc. Standard kits used for the biochemical assays particularly the Creatinine Liquicolor and Urea Liquicolor were from Human GmBh in Germany and were acquired through Biocare Health Resources, Philippines.

### 2.2. Plant material extraction

The plant sample, *Eclipta alba* with voucher specimen number 121840, was collected at Buhi, Camarines Sur in Bicol in April 2014 between 1 and 3 pm and authenticated by Dr. Wilfredo F. Vendivil, curator at the Botany Division of the National Museum. *Eclipta alba* is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes with leaves that are opposite, sessile, lanceolate, (Chokotia et al., 2013) subentire, acute or subacute sparsely strigose with appressed hairs on both sides and with a tapering base; a short, brown stem that is either flat or round and small white flowers arranged in clusters (Sharma et al., 2012) on a long stalk that could reach a height of three inches (Peraman et al., 2011). The plant material was air-dried and ground into powder. The extraction procedure was carried out according to Liu et al. (2012) with minor modifications. The powdered plant material was subjected to extraction with methanol (sample to solvent ratio of 1:5 w/v) at room temperature (22–25 °C) for 24 h in a percolator to obtain the methanolic leaf extract. The percolate was collected every 24 h for four days and was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator.

### 2.3. Preliminary phytochemical analysis

A preliminary phytochemical analysis of the plant extract was conducted to evaluate the various groups of phytochemicals that were present (Aguinaldo et al., 2005).

### 2.4. Experimental induction of gentamicin nephrotoxicity and its treatment with the extract

The assay was conducted according to the method of Abdel-Raheem et al. (2010) with minor modifications and as approved by the University of Santo Tomas–Institutional Animal Care and Use Committee with reference number AR-2015-20. Female Sprague Dawley rats (10–12 weeks old) weighing 150–200 g were divided into five groups of six animals. Group I received normal saline solution (NSS) (1 mL/kg BW, subcutaneous (s.c.)); Group II received gentamicin (GM) (80 mg/kg BW, s.c.); Group III received GM (80 mg/kg BW, s.c.) and the methanolic leaf extract of *Eclipta alba* (EA) (300 mg/kg BW, orally (*per orem* (*p.o.*)); Group IV: rats received GM (80 mg/kg BW, s.c.) and EA (600 mg/kg BW, *p.o.*); and Group V received GM (80 mg/kg BW, s.c.) and quercetin (50 mg/kg BW, *p.o.*). Quercetin and EA were administered via oral gavage an hour before the administration of gentamicin for seven days. Blood samples were collected via tail tipping at the start of the experiment (before the administration of any test substance, Day 0) and immediately before the test animals were sacrificed (Day 8). Individual animals were placed in improvised metabolic cages for 16-hour urine collection to determine microprotein levels at Days 0 and 8 of the experiment.

### 2.5. Biochemical analysis

#### 2.5.1. Determination of BUN, serum creatinine, and microprotein levels

Blood samples were collected to determine levels of blood urea nitrogen (BUN) and serum creatinine using Urea Enzymatic Colorimetric Kit (Human Gesellschaft fur Biochemica und Diagnostica mbH, 2003a) and Creatinine Colorimetric Kit (Human Gesellschaft fur Biochemica und Diagnostica mbH, 2003b), respectively, by the methods described by the manufacturer. The microprotein in 16-h urine samples was determined through the Bradford method as described by Marshall and Williams (2000) using bovine serum albumin as a standard.

#### 2.5.2. Preparation of renal homogenate

The method was done according to Khan et al. (2009) with minor modifications. On Day 8, the animals were sacrificed and one kidney was isolated for the preparation of the renal homogenate. The kidneys were washed with phosphate buffer saline solution (pH 7.4) then a 10% w/v homogenate was prepared with the same solution using a tissue homogenizer (IKA Tissue Homogenizer). The homogenate was centrifuged to remove debris and aliquots of the supernatant were stored at −20 °C for biochemical analysis.

#### 2.5.3. Catalase

Catalase activity was measured using the method of Aebi (1974) as cited by Bogdanska et al. (2003). One hundred microliters (100 μL) of freshly prepared hydrogen peroxide (19 mM) solution in phosphate buffer and 195 μL phosphate buffer (50 mM, pH 7.4) was made to react with an aliquot (10 μL) of supernatant. The degradation rate was measured at 240 nm every minute for five minutes and the catalase activity in U/mL was calculated using the molar absorption coefficient (ε) of hydrogen peroxide at 240 nm. Catalase activity was computed.

#### 2.5.4. Lipid peroxidation assay

The extent of lipid peroxidation was estimated through thiobarbituric acid reactive substances (TBARS), the final product in the lipid peroxidation pathway, using the method described by Esterbauer and Cheeseman (1990). Sixty microliters (60 μL) of 1% w/v thiobarbituric acid (TBA) and 75 μL of 10% trichloroacetic acid (TCA) were added to 15 μL of renal homogenate. The mixture was heated in a boiling water bath for 15 min then centrifuged at 10,000 × *g* for five minutes. Absorption was read at 532 nm and level of TBARS was calculated using the extinction coefficient of malondialdehyde (MDA), $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

### 2.6. Histopathological analysis

The animals were euthanized by cervical dislocation. One kidney was isolated from each rat, preserved in 10% neutral buffered formaldehyde solution, and one was submitted to Dr. Cynthia Ochona, a veterinary histopathologist, for histopathological examination. The renal tissue samples were assessed according to the degree of pathological changes based on a 1–4 grading system: 1.0, normal; 1.5, very mild, no remarkable lesions noted; 2.0, mild tubular degeneration on the cortex and medulla; 2.5, mild tubular degeneration on the cortex and medulla with very mild fibroblasts proliferation; 3.0, moderate degeneration on the cortex and medulla with mild fibroblasts proliferation; 3.5, moderate to severe tubular degeneration on the cortex and medulla; 4, severe tubular degeneration on the cortex and medulla. The other kidney was used to prepare the kidney homogenate for antioxidant enzyme assays.