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Toxicological evaluation and protective effect of ethanolic leaf extract of *Launaea taraxacifolia* on gentamicin induced rat kidney injury

Lydia Enyonam Kuatsienu[1], Charles Ansah[2], Michael Buenor Adinortey[3*]

[1]*Department of Pharmacology, Princefield University College, Ho, Ghana*

[2]*Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[3]*Department of Biochemistry, School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana*

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ABSTRACT

Objective: To evaluate the toxic potential of *Launaea taraxacifolia* leaf extract (LTE) in rats within 14 d of oral administration and also assess the potential of LTE in protecting against kidney injury induced by gentamicin using rat model.

Methods: The protective ability of LTE was done after sub-acute toxicity evaluation has been carried out. Acute Kidney Injury (AKI) was induced by gentamicin at a dose of 160 mg/kg intraperitoneal i.p. Parameters and indicators considered include mortality, clinical signs, body and organ weights, haematological and clinical chemistry parameters. Gross examination and histopathological assessment was also done on selected internal organs.

Results: There were no treatment-related deaths or changes in clinical signs, haematological and clinical chemistry indices during sub-acute toxicity studies with the exception of creatinine levels. This was confirmed by micrographs obtained from histopathological analysis. Co-administration of LTE with 160 mg/kg of gentamicin (i.p) markedly decreased the levels of urea and creatinine when compared to negative control group. Histological studies of kidney tissues showed an insignificant change in tubular epithelium in LTE plus gentamicin treated group compared to LTE treated only.

Conclusions: Data obtained shows that ethanolic leaf extract of *Launaea taraxacifolia* is non-toxic within a 14 d administration at a maximum dose of 1 000 mg/kg bwt and also possesses the ability to protect against gentamicin-induced kidney damage in rats at a dose of 300 mg/kg bwt.

1. Introduction

Gentamicin is amongst the most common antibiotic in the aminoglycoside class that has been approved by many countries for clinical use in managing serious infections caused by aerobic gram-negative bacilli [1]. Nephrotoxicity is a major problem with its usage [2]. Discovering a drug that could be used in the reduction of this toxic effect could enhance its clinical use. Though medications such as deferoxamine, methimazole, vitamin E, vitamin C, diethyldithiocarbamate, L-histidinol and thymoquinon are used for prevention of gentamicin-induced kidney damage [3], none has proven to be clinically efficient at providing all-embracing protection.

Plant medicine research is an area in the drug discovery process that has proven to be successful with managing several conditions. In this report, a plant-commonly referred to as African lettuce that is claimed to possess several medicinal properties [4] was studied. The plant botanically referred to as *Launaea taraxacifolia*, is found in the Tropics and belongs to the family Asteraceae. Previous studies conducted on *Launaea taraxacifolia* leaves showed the presence of flavonoids, tannins, terpenoids, saponins, steroids [5,6].

Though there are reports of bioactivities such as hypolipidemic [7] renoprotective and hepatoprotective in Cisplatin

*Corresponding author: Michael B. Adinortey, Department of Biochemistry, University of Cape Coast, Cape Coast, Ghana

E-mail: michaelbuenor@gmail.com (M.B. Adinortey).

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model [8], to date no report is available on *Launaea taraxacifolia* leaf extracts' ability to protect against gentamicin-induced kidney injury, which is major problem with gentamicin usage. Thus the current study sought to examine the in vivo toxic potential of *Launaea taraxacifolia* leaves extract in Sprague-Dawley rats and also assess the ability of the extract in protecting against gentamicin induced kidney damage in rat model.

2. Materials and methods

2.1. Chemicals, reagents and drugs used

Normal saline, ethanol, EDTA were purchased from BDH Chemicals Ltd Poole England, Gentamicin sulphate (Roche Pharmaceutical Ltd, China).

2.2. Collection and preparation of plant material

Fresh tender leaves of *Launaea taraxacifolia* were collected from the Kwame Nkrumah University of Science and Technology (KNUST) Pharmacognosy garden. Samples were identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana, with a voucher specimen # KNUST/HM1/2012/L060. The fresh leaves were washed and air-dried for three weeks. The dried leaf samples were pulverized and macerated (1: 10 w/v) in 70% ethanol for 3 d. The leaf extract was filtered through a whatman No 1. filter paper. The extract was concentrated under reduced pressure at 50 °C and the dried extract stored in airtight container and kept at −20 °C in a freezer till usage. The yield obtained was about 34.5%.

2.3. Experimental animals and maintenance

Sprague-Dawley (SD) rats were obtained from the Centre for Plant Medicine Research, Akuapem Mampong, Ghana. All experimental protocols were carried out in accordance with guidelines on the use and care of experimental animals as provided by the Organization for Economic Cooperation and Development (OECD) and approved by the Faculty of Pharmacy and Pharmaceutical Sciences Animal Experimentation Ethics committee of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Animals were fed on rat feed supplied by Ghana Agro Food Company Tema, Ghana and water ad libitum. They were maintained at a 12 h light/dark cycle at (25 ± 2) °C. All animals were allowed to acclimatize to their new environment for two weeks with adequate fresh water and food before the start of any experiment.

2.4. Toxicological evaluation in rat model

2.4.1. Acute toxicity assessment in rat model

In this experiment, acute toxicity study was conducted according to the World Health Organization (WHO) guidelines for the evaluation of safety and efficacy of herbal medicines (WHO, 1993). Thirty male rats between (250–300) g were grouped into six with (n = 5). Group 1 served as the control and received saline. Groups 2–6 received (10, 100, 300, 1 000 and 5 000 mg/kg bwt) of extract orally by gavage. Animals were observed every 30 min for 8 h for any abnormal behavioural.

2.4.2. Sub-acute toxicity assessment in rat model

Twenty-five male rats weighing between (250–300) g were grouped into five with (n = 5). Group 1 served as the control and received normal saline as vehicle. Groups 2–5 orally received (10, 100, 300, 1 000 mg/kg bwt) of extract. The treatments were repeated daily at 10: 00 am GMT for 14 d and observed for any abnormal changes. On day 15, blood samples were taken for haematological and biochemical analysis.

For haematological indices, blood samples were collected into sterile tubes containing EDTA and immediately analysed for TWBC, RBC, HB, PCV, MCV, MCH, MCHC, PLT and LYM using Sysmex Automated Analyser (model KX 21 Kobe, Japan).

Biochemical parameters were performed on blood collected into plain tubes without any anticoagulant. Collected blood samples were centrifuged at 3 000 rpm for 5 min to obtain sera. The following biochemical indices namely total bilirubin (TBIL), direct bilirubin (DBIL), aspartate amino transferase (AST), alanine amino transferase (ALT), total protein (TP), albumin (ALB), globulin (GB), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), urea (URE), creatinine (CR) were measured using Flexor Junior autoanalyzer.

Livers, kidneys, testes, heart and spleen of all rats in experiment were excised, collected and organ weight measured. The body weight of each rat was also assessed during the acclimatization period, once before commencement of dosing, and once on the day of sacrifice after dosing. The relative organ to body weight ratio was then calculated.

2.5. Nephroprotective study in rat model

Male Sprague-Dawley rats weighing (150–200) g were selected for use in this experiment. Animals were randomly put into eight groups, with (n = 5). Group I was given normal saline for 10 days. Group II that was negative control received normal saline from day 1–10, and also gentamicin sulphate 160 mg/kg bwt (i.p.) from day 6–10 simultaneously. Groups III, IV and V were orally given 10, 100 and 300 mg/kg/d (p.o.) extract respectively for 10 d. Groups VI, VII and VIII orally received 10, 100 and 300 mg/kg bwt extract respectively for 10 d and simultaneously with 160 mg/kg bwt gentamicin sulphate (i.p.) from day 6–10.

Experimental animals were all sacrificed after 10 days of treatment and blood samples collected and processed for measurement of serum creatinine (Cr), urea, total protein and plasma electrolytes. The kidneys were removed and processed for histopathology. The tissues were fixed in neutral buffered formalin, embedded in paraffin wax, cut into 3 μm sections and stained with haematoxylin and eosin. They were then viewed under light microscopic (Nikon Eclipse E200, Japan) and captured by Infinity 1 camera microscope under ×10 magnification.

2.6. Data analysis

GraphPad Prism version 5 Software was used for data analysis. One Way ANOVA and Newman – Kuels' post hoc test was used to determine significant difference when compared to vehicle treated control group at 5% level of significance. Data were presented as mean ± SEM (standard error of mean).

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