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Original article

Nephro-protective effect of a novel formulation of unopened coconut inflorescence sap powder on gentamicin induced renal damage by modulating oxidative stress and inflammatory markers

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ABSTRACT

Fresh oyster white translucent sap obtained from the tender unopened inflorescence of coconut trees (*Cocos nucifera*) is identified to have great health benefits. Drug induced Nephrotoxicity is one of the major causes of renal damage in present generation. As a therapeutic agent, gentamicin imparts direct toxicity to kidney, resulting in acute tubular necrosis, glomerular and tubulointerstitial injury, haemodynamically mediated damage and obstructive nephropathy.There exists an increasing demand for safe and natural agents for the treatment and/or preventionofchronic nephrotoxicity and pathogenesis of kidney diseases. Our study shows the nephro protective/curing effect of a novel powder formulation of micronutrient enriched, unfermented coconut flower sap (CSP). The study was performed on adult male Wistar rats. The animals were grouped into three and treated separately with vehicle, gentamicin and gentamicin + CSP for 16 days. Initially, gentamicin treatment significantly ($p < 0.05$)reduced thelevels of antioxidant enzymes (SOD, CAT, GPx) and GSH and increased ($p < 0.05$) the levels of creatinine, uric acid, urea, inflammatory markers (nitrite, IL-6, TNF- α, iNOS) and lipid peroxidation. Supplementation of coconut flower sap powder showed significant ($p < 0.05$) reversal of all these biochemical parameters indicating an effective inhibition of the pathogenesis of nephrotoxicity and kidney disease.

1. Introduction

Kidneys are essential for maintaining many aspects of metabolic homeostasis. They perform major functions of the human body –removal of metabolic waste products, regulation of water, electrolyte and acid-base balance, synthesis and regulation of hormones etc. In addition to these, it is one of the principal organs involved in maintaining the nutritional balance of the body. They support activation of vitamin D needed for calcium absorption and produce erythropoietin needed for red-blood-cell synthesis. Renal failure is a complicated disease where the kidney loses its filtering ability, resulting in accumulation of wastes and disturbs the overall chemical balance of thebody. Malnutrition is another major issue in patients with kidney disease, adversely affecting morbidity, mortality, functional activity and patients' quality of life [1].

Aminoglycosides are highly potent, broad-spectrum antibiotics, effective against the treatment of life-threatening infections. Gentamicin remains the most preferred aminoglycoside antibiotic for treatment of many severe bacterial infections (mainly Streptococcal infections). Its therapeutic efficacy, however, is limited by renal impairment associated with the usage of aminoglycoside gentamicin in 24% of patients [2]. Nephrotoxicity induced by gentamicin is clinically known as a nonoliguric renal failure with characterized change in serum creatininelevels and a hypo-osmolar urinary output [3]. The drug may accumulate in epithelial tubular cells causing a range of effects starting with loss of the brush border in epithelial cells and ending in overt tubular necrosis, activation of apoptosis and massive proteolysis.

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Gentamicin also causes cell death by generation of free radicals, phospholipidosis, extracellular calcium-sensing receptor stimulation and energetic catastrophe, reduced renal blood flow and inflammation [4]. Uptake of higher doses results in severe renal damage with inhibition of protein synthesis and suppression of gene expression for the Na^+ and Ca^{2+} exchanger, Na^+-dependent d-glucose transporter and alpha subunit of Na^+/K^+ ATPase [5].

Oxidative stress plays a critical role in the pathophysiology of several kidney diseases. Aminoglycoside induced nephrotoxicity is mainly attributed to induction of Oxidative stress and depletion of antioxidant enzyme activities in kidney [6]. Gentamicin is known to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. The administration of various natural or synthetic antioxidants is known to be beneficial in the prevention and attenuation of various kidney damages. These include vitamins, N-acetylcysteine, lipoic acid, melatonin, dietary flavonoids and phytoestrogens, and many others. Supplementation of antioxidant vitamin C and vitamin E (500 mg/day during 6 months) corrects plasma antioxidant status and attenuates cardiovascular diseases that accompany kidney failure. Tocotrienol (a member of vitamin E family) supplementation exhibited the capacity to reduce proximal tubular injury and renal LPO and increased GSH level and catalase activity. Moreover, it is able to improve the index of NO_2-/NO_3–generation [7]. As far as trace elements are concerned, zinc, selenium and iron are the most likely to be deficient in end-stage kidney failure. Iron deficiency may contribute to the inadequate distribution of zinc in patients with CKD and iron supplementation may decrease the abnormal elevated erythrocyte zinc levels in these patients [8].

The sweet, oyster white and translucent sap obtained by tapping the unopened inflorescence of coconut trees (*Cocos nucifera*), was identified as a nutritious natural drink rich in carbohydrates, minerals, vitamins, and amino acids with a low glycemic index. This non- alcoholic beverage has high rejuvenation capacity and is used in traditional medicines for the cure of anaemia, tuberculosis, bronchial suffocation and piles [9]. It possesses a low glycemic index and acts as a natural detoxifying agent. Frequent consumption keeps the human system cool and improves digestion [10].

Herbal medicines are considered to be more effective in treating chronic kidney damages than modern medicines which come with various side effects. Hence, the present study is designed to evaluate the nephroprotective effect of coconut inflorescent sap extracts (CSP) against gentamicin induced rats.

2. Materials and methods

2.1. Materials

Standardized form of unfermented coconut inflorescence sap powder (Patent pending and registered formulation as El-*COCOSEN*™) was provided by M/s Akay Flavours & Aromatics Pvt Ltd, Cochin, India. A detailed analytical test report on various safety parameters including heavy metals, aflatoxins, microbial status and pesticides was also received from the manufacturer. All the chemicals used were of analytical grade from Merck, Bangalore, India. ELISA kits and antibodies were purchased from Sigma-Aldrich, Bangalore, India. RT-PCR kit was purchased from Eppendorf India Ltd, Chennai, India. Kidney function markers were analyzed using respective kits provided by M/s Agappe Diagnostics Pvt Ltd, Bangalore, India.

2.2. Animals

Adult male Wistar rats were used in the study. Selected animals with an average body weight of 150 ± 10 g were acclimatized for a period of 14 days in ventilated cages and housed in an air-conditioned room at 24 ± 2 °C and arelative humidity of 60 ± 5% with a 12 −hour light and dark cycle. All animal experiments were carried out in strict accordance with the ethical norms approved by the Institutional Animal Ethics Committee (IAEC) recognized by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (registration no: CAF/361/2015). Animals were provided a balanced diet in the form of pellets (M/s Amrut Laboratory Animal Feeds, Maharashtra, India) and water *ad libitum*.

2.3. Experimental design for Nephrotoxicity

Twenty four rats were randomly divided into three groups, each containing eight animals, as follows:

Group I: Normal control rats (Con)

Group II: Gentamicin Treated (80 mg/kg) (GM)

Group III: GM + CSP (20 mg/Kg bwt) supplemented (GM + CSP).

Gentamicin was administered intraperitoneally whereas CSP and distilled water were supplemented by oral gavage (intragastrically). Duration of experiment was 16 days and overnight fasted rats were killed by euthanasia.

Blood was collected by direct heart puncture into EDTA coated and non-EDTA vials for analyzing hematological parameters and serum biochemistry.The blood was collected and theserumwas separated for biochemical estimation. Serum was separated from the clotted blood sample by centrifuging at 5000 rpm for 10 min at 4 °C and was stored at −20 °C for analysis.Protein was assayed by the method of Lowry et al. (1951). Kidneys dissected from each group were washedin PBS buffer and kept in 10% formalin for histopathological examinations.

2.4. Measurement of endogenous antioxidants and lipid peroxidation in tissues

2.4.1. Estimation of antioxidant enzymes

The enzyme was assayed by the method of Maehly and Chance [11]. Catalase (CAT) activity was determined by measuring the rate of decomposition of hydrogen peroxide at 240 nm and expressed in terms of *units per mg* protein. The decrease in the absorbance at 230 nm was measured spectrophotometrically. The Superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium (NBT) reduction method described by Kakkar et al. [12]. Glutathione peroxidase (Gpx) activity was analyzed by the method of Lawrence and Burk based on the oxidation of glutathione in the presence of H_2O_2 [13].

2.4.2. Estimation of GSH level

The non-protein thiol, glutathione (GSH) activity was measured using the method reported by Benke et al. (1974) based on the reaction with 5,5-dithiobis-2-nitrobenzoicacid (DTNB) reagent [14].

2.4.3. Estimation of Thiobarbituric Acid Reactive substances (TBARS)

Lipid peroxidation was estimated as Thiobarbituric Acid Reactive substances (TBARS) by following the method of Ohkawa et al. (1979) and expressed as mmol of malondialdehyde produced [15].

2.4.4. Estimation of nitrite levels

Determination of Nitric oxide(NO) concentrations in serum was measured by its breakdown product–nitrite, using the method of Griess et al. (1996). In the presence of H_2O_2, NO is rapidly converted into nitrite and nitrate. Total production of NO, therefore, may be determined by measuring the stable NO metabolite nitrite (NO_2 −). Equal volumes of tissue supernatant and Griess reagent (1% sulphanilamide and 0.1% N-[naphthyl]ethylenediaminedihydrochloride: 1:1) was mixed and absorbance was measured at