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Full Length Article

Wheat germ and vitamin E decrease BAX/BCL-2 ratio in rat kidney treated with gentamicin

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ABSTRACT

The intracellular accumulation of gentamicin acts on mitochondria directly (by increasing oxidative stress) and indirectly (by increasing the proapoptotic BAX gene expression levels) and thus activates intrinsic pathway of apoptosis. This study intended to investigate the probable prophylactic effect of wheat germ as a nephro-protective agent in relation to vitamin E as a well-known natural antioxidant on a nephrotoxic model induced by gentamicin. Forty male Sprague Dawley rats were divided randomly into four groups; of 10 rats each: control group, gentamicin group (120 mg/kg/day i.p. for 15 days), vitamin E group (200 mg/kg orally for 45 days) and wheat germ group (20% of the diet for 45 days). Gentamicin injection was started on the 30th day for both groups (3) and (4). It was noticed that wheat germ significantly decreased BAX/BCL-2 ratio. The significant decrement in MDA level and the significant increment in total antioxidant capacity (TAC) level and catalase (CAT) activity of wheat germ treated rats reflecting its antioxidant activity. Additionally, the wheat germ succeeded in improvement of the kidney function of rats of this group which was manifested by amelioration of deteriorated serum creatinine, urea, sodium and potassium levels. In conclusion, the efficacy of wheat germ as anti-apoptotic and antioxidant was higher than that of vitamin E and it effectively protected against the direct and indirect toxic effects of gentamicin on kidney through regulation of BAX-BCL-2 gene expressions and further inhibition of mitochondrial cytochrome *c* translocation into cytosol.

1. Introduction

Nephrotoxicity is defined as a poisonous effect of toxic chemicals and medication on the kidneys. The nephrotoxic consequence of most drugs has been more profound in patients already suffering from renal impairment. Numerous therapeutic agents, which are used in clinical practice, have been stated to produce functional impairment and toxic injuries to the kidney. The cause for this is that the kidney is the chief organ of excretion and is exposed to huge amounts of parent and active metabolites of drugs (Ekor et al., 2010).

Gentamicin is an aminoglycoside broad spectrum antibiotic used against pathogenic gram positive and negative bacteria (Taha, 1993). Administration of gentamicin induced impairment of renal function through the generation of oxygen free radicals (Heibashy et al., 2009). Oxidative stress may occur as a result of either increased oxygen free radical liberation and/or decreased antioxidant enzyme system that protects the cell against cytotoxic free radicals (Khan et al., 2009). The search of protecting against gentamicin nephrotoxicity has concerned much attention and effort during the last decade. This protection may be achieved through decreasing aminoglycosides accumulation by the kidneys, or decreasing the lysosomal phospholipidosis induced by the cell-associated aminoglycosides and the use of antioxidants (Mingeot-Leclercq and Tulkens, 1999).

In the past few years, considerable concern has been focused on the role of naturally occurring dietary constituents for the control and management of various ailments (Connor, 2000). Medicinal plants are effective free radical scavengers. Several studies reported that medicinal plants are less toxic than synthetic antioxidants, such as butylated hydroxyanisol and butylated hydroxytoluene, which are assumed to be carcinogenic and cause cell damage (Ratnam et al., 2006). Natural antioxidants are classified as secondary plant metabolites, such as polyphenols (phenolic acids, flavonoids) and terpenoids (carotenoids). The consumption of nutrients that contain these compounds in large quantities seems to play an important role in prophylaxis against many diseases (Nawirska-Olszanska et al., 2013).

Wheat (*Triticum aestivum L.*) is one of the most vital crops. It has

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been used as a chief constituent for manufacturing of various food products. Wheat germ is the by-product of flour-milling industry. It is a unique source of highly concentrated nutrients. Wheat germ oil is rich in polyunsaturated fatty acids such as oleic, linoleic and α-linolenic acids (Rizzello et al., 2010). Wheat germ is rich in bioactive ingredients such as antioxidants, including tocopherols, phenolics and carotenoids (Gelmez et al., 2009). It is rich source of tocopherols (Schwartz et al., 2008). Wheat antioxidant activities have been widely studied. Extracts rich in antioxidant have been obtained from wheat using several solvents including ethanol, methanol, water, an aqueous ethanol solution and an aqueous methanol solution (Schwartz et al., 2008).

Our work was designed to evaluate the anti-apoptotic and antioxidant activities of wheat germ in relation to vitamin E as a common natural antioxidant on a rat nephrotoxic model induced by gentamicin.

2. Materials and methods

2.1. Chemicals

Gentamicin-sulphate (Garamycin) was obtained from Schering-Plough (U.S.A). Vitamin E® capsules (400 mg) were obtained from Pharco Corporation, Amriya, Alexandria, Egypt. Wheat germ (Golden Green) was purchased from Bon Pharma Company for pharmaceuticals and chemicals, Cairo, Egypt. Malondialdehyde (MDA), catalase (CAT), total antioxidant capacity (TAC), sodium and potassium commercial kits were purchased from Biodiagnostic Company for research kits, Cairo, Egypt. Serum creatinine and urea commercial diagnostic kits were purchased from Spinreact “Girona, Spain” and Diamond diagnostic “London, UK” Companies, respectively. Other non-mentioned chemicals used in the present study were purchased from Sigma, USA.

2.2. Animals and treatments

Forty adult male Sprague Dawley rats, weighing 120–150 g at the beginning of the experiment were used in the present study. The animals were obtained from the Egyptian Organization for Biological Products and Vaccines. The rats were kept at room temperature and exposed to natural daily light–dark cycles. Rats were fed on balanced commercial rat diet with free access of food and water. All experimental procedures were conducted in agreement with the guide for the care and use of laboratory animals and in accordance with the local Animal Care and Use Committee.

One week after acclimatization, the rats were randomly divided into 4 experimental groups of ten rats each:

Group 1 (C-group):
Rats served as control and were given normal saline daily by intraperitoneal injection.

Group 2 (G-treated group):
Rats were administered normal saline by intraperitoneal injection for 30 days, and then followed by intraperitoneal injection of gentamicin for 15 days at a dose of 120 mg/kg/day (Somdaş et al., 2015).

Group 3 (Vit. E-treated group):
Rats were administered vitamin E orally for 45 days at a dose of 200 mg/kg/day (Mehany et al., 2013); the gentamicin “120 mg/kg/day” intraperitoneal injection was started on the 30th day till the end of the experiment.

Group 4 (WG-treated group):
Rats were received wheat germ orally for 45 days at a dose of 20% of the diet (Leenhardt et al., 2008); the gentamicin “120 mg/kg/day” intraperitoneal injection was started on the 30th day till the end of the experiment.

2.3. Sampling and biochemical analysis

2.3.1. Blood sampling

Twenty-four hours after the last dose of treatments, blood samples were collected from all rats via *retro*-orbital bleeding. Blood samples were left at room temperature for 20 min to clot. The clotted blood samples were centrifuged at 1000 × *g* for 15 min for serum separation. The obtained sera were kept at −20 °C till use.

2.3.2. Specimen collection

Kidneys were excised after dissection of the animals and then washed by physiological saline. The kidney samples were divided into two parts. The first part of the kidney (0.5 g) was suspended in 5 ml ice cold phosphate buffered saline (pH: 6.8) for homogenization (*Teflon Homogenizer, India*). The kidney tissue homogenate was then centrifuged at 20,000 × *g* for 10 min at 4 °C using high speed cooling centrifuge. The supernatants were kept on −20 °C till the time of determination of oxidative/ antioxidant parameters (Lin et al., 2010). The second part of the kidney was preserved at −80 °C for molecular investigation.

2.3.3. Detection of BAX and BCL-2 gene expression by real time-polymerase chain reaction (RT-PCR)

Total RNA was isolated from kidneys using Ribozol™ RNA Extraction Reagents with the code N580 (AMRESCO, LLC Corporate Headquarters, 28,600 Fountain Parkway, Solon, OH 44,139, USA) according to manufacturer's instruction. The concentration of RNA was measured using a UV spectrophotometer “Hitachi spectrophotometer, Model U-2000, Hitachi Ltd. Tokyo, Japan”.

2.3.3.1. c DNA synthesis. Five µg RNA was reverse transcribed using oligonucleotide (dT)18 primer (final concentration, 0.2 mM) and was denatured at 70 °C for 2 min. Denatured RNA was placed on ice and reverse transcription mixture containing 50 mM KCl, 50 mM Tris HCl (pH 8.3), 0.5 mM of deoxyribonucleotide triphosphate (dNTP), 3 mM MgCl2, 1 U/mL RNase inhibitor, and 200 units of moloney murine leukemia virus reverse transcriptase. The reaction tube was located at 42 °C for 1 h, followed by heating to 92 °C to stop the reaction.

2.3.3.2. Real-time quantitative polymerase chain reaction (RT-PCR). For real-time quantitative PCR, 5 µL of first-strand cDNA was used in a total volume of 25 µL, containing 12.5 µL 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer as shown in Table 1. PCR reactions consisting of 95 °C for 10 min (1 cycle), 94 °C for 15 s, and 60 °C for 1 min (40 cycles), were done on step one plus real-time PCR system (Applied Biosystems). The data were analyzed with the ABI Prism 7500 sequence detection system software and quantified using the v1.7 Sequence Detection Software from PE Biosystems (*Foster City, CA*). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the beta-actin genes, all these steps were performed according the method described by Kenneth and Thomas (2001).

Table 1
Sequences of the primers used for amplification of mRNAs encoding BCL-2 and BAX by quantitative real-time PCR.

mRNA	Sequences (5′ → 3′)	Gene Accession No
BCL-2	Forward primer: 5′-CATGTGTGTGGAGAGCGTCAA-3′ Reverse primer: 5′-GCCGGTTCAGGTACTCAGTCA-3′	NM_016993
BAX	Forward primer: 5′-GGGACGAACTGGACAGTAACAT-3′ Reverse primer: 5′-GGAGTCTCACCCAACCACCCT-3′	NM_017059
β-actin	Forward primer: 5′-ATGAGCCCCAGCCTTCTCCAT-3′ Reverse primer: 5′-CCAGCCGAGCCACATCGCTC-3′	NM_007393