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Protective role of naringin against cisplatin induced oxidative stress, inflammatory response and apoptosis in rat striatum *via* suppressing ROS-mediated NF-κB and P53 signaling pathways

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ABSTRACT

Cisplatin (Cis) is an effective chemotherapeutic agent successfully used in the treatment of a wide range of malignancies while its usage is limited due to its dose-dependent toxicity. The present study was conducted to investigate the efficacy of naringin, an ubiquitous flavonoid, against Cis-induced striatum injury in Wistar aged rats. Briefly, the experimental procedures were divided in two sets of experiments. In the first, the animals were divided into 4 groups: control, Nar 25 mg/kg, Nar 50 mg/kg and Nar 100 mg/kg. In the second, the animals were divided into 4 groups: Cis (5 mg/kg/week for 5 consecutive weeks), Cis + Nar (25 mg/kg), Cis + Nar (50 mg/kg) and Cis + Nar (100 mg/kg). The administration of Cis (5 mg/kg/week for 5 consecutive weeks) resulted in a decline in the concentrations of reduced glutathione and ascorbic acid. The activity of membrane bound ATPases and glutathione peroxidase (GPx) were decreased while the activity of catalase (CAT) and superoxide dismutase (SOD) were increased. Further, in striatum tissue, Cis significantly enhance the mRNA gene expression of P53, nuclear factor κB pathway (NFκB) and tumor necrosis factor (TNF-α). Oxidative/nitrosative stress was evident in Cis group by increased malondialdehyde (MDA), protein carbonyls (PCO), reactive oxygen species (ROS) and nitrite concentration (NO). Naringin (25, 50 and 100 mg/kg) administration was able to protect against deterioration in striatum tissue, abrogate the change in antioxidant enzyme activities and suppressed the increase in MDA, PCO, NO and TNF-α concentrations. Moreover, Nar inhibited P53, NFkB and TNF-α pathways mediated inflammation and apoptosis, and improved the histological changes induced by Cis. Thus, these findings demonstrated the neuroprotective nature of Nar by attenuating the pro-inflammatory and apoptotic mediators and improving antioxidant competence in striatum tissue. These results imply that Nar has perfect effect against Cis-induced striatum injury in aged rats, which should be developed as an effective food and healthcare product for the treatment of brain injury in the future.

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1. Introduction

Chemotherapy is one of the most valuable cancer treatments and many anticancer agents have been developed to achieve this over the years. Nevertheless, their serious side effects and consequent systemic toxicity has contributed to the limitations for cancer treatment [1,2]. Several investigators have observed that anticancer agents have been utilized for the chemotherapy of various cancers such as sarcoma small cell lung cancer, ovarian cancer, lymphomas germ cell tumors and CNS disability [3–5], but they have also been implicated in causing various kinds of toxicity after treatment in cancer patients measured as adverse effects [6]. The anti-cancer mechanisms of cisplatin are complex and involve multiple events that include inflammation by activating proinflammatory cytokines [7], production of reactive oxygen, [8] and nitrogen species [9], and activate several signal transduction pathways, such as those involving the p53 protein, that culminate in the activation of cell apoptosis [6,10–12]. Although the production of reactive

Abbreviations: CAT, catalase; DNPH, dinithrophenylhydrazine; DCHF-DA, 2′,7′-dichlorofluorescein diacetate; GPx, glutathione peroxidase; MDA, malondialdehyde; NFκB, nuclear factor κB; PCO, protein carbonyls; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species; SOD, superoxide dismutase; TNFα, tumor necrosis factor-α; TBARS, thiobarbituric acid reactive substances; TBA, thiobarbituric acid.

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oxygen species (ROS) by the cisplatin has been implicated in the pathogenesis of cisplatin-induced neurotoxicity [13], the precise mechanisms underlying the neuropathy remain to be unknown and still a matter of debate; however, evidence suggests that cisplatin interacts with both nuclear and mitochondrial DNA, forming adducts that interfere with gene transcription and the synthesis of proteins involved in the maintenance of neurons, thus contributing to its cytotoxicity and neurotoxicity [14].

In recent years, a growing interest in the use of phytochemicals as free radical scavengers and inhibitors of oxidative stress has been considerably exploited. For instance, anti-oxidant rich foods have been suggested to play an essential role in the prevention of cardiovascular diseases (CVDs) [15], metabolic disorders (diabetes, metabolic syndrome) [16] and cancer [17,18]. The widespread distribution of flavonoids, coupled with relatively low toxicity compared to other active plant compounds (for instance alkaloids) makes them potential candidates to be developed as therapeutic entities. Naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside) is a well-known flavanone glycoside of grape fruits, e.g., *Citrus paradise, Citrus sinensis, Citrus unshiu*, and *Artemisia selengensis* [19]. Many biological activities of naringin have been recognized such as anti-inflammatory activity *in vitro* [20–22], *in vivo* [23,24] and possess cardioprotective, neuroprotective and anti-apoptotic properties by inhibiting tumor development and suppressing tumor cell proliferation [22,25–27]. It is also reported that naringin up-regulates the gene expression of superoxide dismutase, catalase and glutathione peroxidase in high cholesterol-fed rabbits [28]. However, the protective role of naringin against cisplatin-induced striatum injury has not been investigated. Hence we proposed to evaluate whether administration of naringin, a natural flavonoid offers protection against cisplatin-induced neurotoxicity and also to study the mechanism of its neuroprotection.

The aim of our study is to investigate the potency of naringin to modulate cisplatin associated neuronal functional and structural changes through modulation of nitro-oxidative stress, the expression of proinflammatory mediators (NFκB and TNF-α) and effector proapoptotic P53 implicated in the neurotoxicity insult caused by cisplatin.

2. Materials and methods

2.1. Chemicals and reagents

Unless otherwise specified, naringin and all other chemicals required for biochemical assays were obtained from Sigma Chemicals Co. (St. Louis, France).

2.2. Animals

Middle-aged (18 month-old, 57–61% lifespan) Wistar rats were purchased from the Central Pharmacy (SIPHAT, Tunisia). Animals were kept in an air conditioned room (22 ± 2 °C) with free access to water and commercial diet supplied by the Company of Animal Nutrition, Sfax, Tunisia [29]. All manipulations were carried out between 08:00 A.M. and 04:00 P.M. All animal procedures were conducted in strict conformation with the local Institute Ethical Committee Guidelines for the Care and Use of laboratory animals of our Faculty.

2.3. Cisplatin exposure and treatment with naringin

Experimental procedures were divided in two sets of experiments. In the first set, animals were randomly divided into four groups (10 rats in each group): control (CT); naringin (25 mg/kg body weight) (Nar 25); naringin (50 mg/kg body weight) (Nar 50) or naringin (100 mg/kg body weight) (Nar 100).

In the second experimental set, animals were divided into 4 groups (10 rats in each group): cisplatin (Cis) group (5 mg/kg/once a week *via* the intraperitoneal (i.p.) route for 5 consecutive weeks); cisplatin with naringin (25 mg/kg body weight) (Cis + Nar 25); cisplatin with naringin (50 mg/kg body weight) (Cis + Nar 50); and cisplatin with naringin (100 mg/kg body weight) (Cis + Nar 100). Naringin was diluted with buffered saline and propylene glycol 25/75 (v/v), administered daily by oral gavage, and did not exceed 1.0 ml/kg body weight.

2.4. Biochemical analysis of the striatum

At the end of the treatment, animals were euthanized by cervical decapitation to avoid stress conditions. Brain structures were quickly removed from skull, rinsed in ice-cold Tris–HCl buffer (10 mM, pH 7.4), placed on filter paper moistened with the same buffer on top of a Petri dish filled with ice and the following striatum regions were dissected using consistent anatomical landmarks as criteria for dissection and was homogenized in the appropriate buffer as indicated in the procedures of each parameter measurements.

The homogenates were then centrifuged at 12,000×*g* for 15 min at 4 °C to remove nuclei and debris. The supernatants were separated, aliquoted, and stored at −80 °C until analysis.

2.4.1. Determination of oxidative stress markers

2.4.1.1. Determination of lipid peroxidation. Lipid peroxidation in the striatum tissue was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) which were expressed in terms of malondialdehyde content according to Draper and Hadley [30] method. Briefly, 100 µl of trichloroacetic acid (5%) was added to 100 µl of striatum supernatants and centrifuged at 4000×*g* for 10 min. 100 µl of the supernatants were transferred to a Pyrex tube and incubated with 200 µl of thiobarbituric acid reagent (TBA, 0.67%) on a boiling water bath for 15 min. The TBARS were determined in microplate reader at 532 nm. The MDA values were calculated using 1,1,3,3-tetraethoxypropane as the standard and expressed as nmoles of MDA/mg protein.

2.4.1.2. Determination of protein carbonyls. Protein carbonyl (PCO) contents were detected by the reaction with 2,4-dinitrophenylhydrazine (DNPH) method as reported by Levine et al. [31]. Briefly, the DNPH reaction proteins were precipitated with an equal volume of 20% (w/v) trichloroacetic acid and washed three times with 2 ml of an ethanol/ethyl acetate mixture (1:1). Finally, the precipitates were dissolved in 6 M guanidine HCl solution. The absorbance was measured at 370 nm, using the molar extinction coefficient of DNPH, $e = 22{,}000\ M^{-1}\ cm^{-1}$ and the results were expressed as nmol/mg protein.

2.4.1.3. Determination of hydroperoxide levels. Hydroperoxide assay (H_2O_2) was determined by the method of Gay et al. [32]. Briefly, 50 µl of the sample were added to 950 µl of FOX 1 reagent (25 mM H_2SO_4, 250 µM ferrous ammonium sulfate, 100 µM xylenol orange and 0.1 M sorbitol) and incubated for 30 min at room temperature. This assay is based on the ability of H_2O_2 to oxidize the ferrous Fe^{2+}-ions to the ferric Fe^{3+} ions which react with xylenol orange to a colored complex. The absorbance of the samples was read at 570 nm and the concentration of H_2O_2 was determined using standard peroxide solutions in the same microtiter plate.

2.4.1.4. Determination of oxygen reactive species (ROS) levels in the striatum. In order to determine the presence of oxidative imbalance caused by Cisplatin, ROS levels in striatum from aged rats