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Taxifolin mitigates oxidative DNA damage *in vitro* and protects zebrafish (*Danio rerio*) embryos against cadmium toxicity

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ABSTRACT

Taxifolin (TAX) is a natural source of bioflavonoid found in various conifers. In this study, initially we investigated the antioxidant potential of TAX under *in vitro* assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), ferric-ion reducing power (FRAP) and hydroxyl radical (OH). The activities of DPPH, ABTS, FRAP and OH radical levels were significantly inhibited by TAX with an IC_{50} values of 16.48, 66.34, 18.17 and 11.42 μg/ml, respectively. Secondly, TAX exhibited a strong protection against OH mediated DNA damage on pUC19 plasmid DNA at 1.0 μg/ml. Finally, we evaluated the protective mechanism of TAX against cadmium intoxicated zebrafish embryos (*Danio rerio*). We found that embryos exposed to 100 μM Cd exhibited significantly reduced survival, delayed hatching and phenotypic abnormalities at 24, 48, 72 and 96 hours post fertilization (hpf). Similarly, Cd intoxicated embryos showed significantly increased cardiac function (131 beats/min) at 60 hpf. Conversely, treatment with TAX (0.1, 1.0 and 10 μM) significantly enhanced the antioxidant enzyme levels (SOD, CAT, GPx and GR) by reducing the lipid peroxidation (MDA) in zebrafish embryos. Collectively, our results concluded that TAX could act as a potent redox scavenger against oxidative DNA damage and also functions as a crucial suppressor of Cd toxicity in zebrafish embryos.

1. Introduction

Oxidative stress induced by metabolites of free radicals such as hydrogen peroxide, superoxide anion and hydroxyl radicals is generated ubiquitously in biological systems through respiratory chain mechanism and various exogenous factors (Chatti et al., 2011). Some early studies reported that under pathological conditions, reactive oxygen species (ROS) triggered overproduction of biologically active radical metabolites, which impacts protective cellular antioxidant levels. These processes are linked with the pathogenesis of several diseases in biological systems such as cellular damage, cancer, neurodegenerative diseases and also the aging process (Wang and Yang, 2012; Sastre et al., 2003). Epidemiological studies have indicated that administration of exogenous antioxidants

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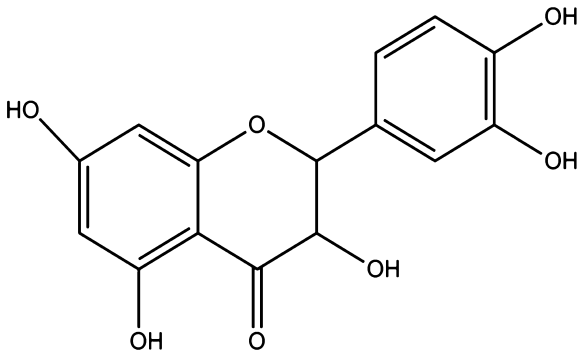

Fig. 1 – Structure of taxifolin.

like carotenoids, polyphenols, anthocyanins, vitamin C and vitamin E can protect cellular components thereby reducing the mortality of ROS-driven oxidative damage. Such exogenous antioxidants have low or minimal toxicity and are commonly derived from a diet rich in vegetables and fruits (Joshipura et al., 2004). Therefore, in recent years, plant derived antioxidants have gained much demand in biopharmaceuticals and nutraceuticals.

The zebrafish (*Danio rerio*) is a freshwater small tropical vertebrate has emerged as an ideal organism for investigating drug toxicity because of its physiological and genomic similarities to humans (Hill et al., 2005). Drug absorption is rapid in the Zebrafish embryo *via* the skin and gills and it provides a simple platform for observation in the assessment of developmental deformities (Berry et al., 2009). Taxifolin (Fig. 1), a derivative of flavonoids has been naturally found in citrus fruit, onions (Slimestad et al., 2007) and milk thistle (Wallace et al., 2005). Several studies have shown that TAX exerts chemopreventive effect against various diseases in which oxidants and/or free radicals are implicated [such as anti-inflammatory (Gupta et al., 1971), cerebral ischemia perfusion (Wang et al., 2006), anticancer (Brusselmans et al., 2005) and antimicrobial activities (Young et al., 2007)]. Accumulating reports outline the antioxidant potential of TAX but to date, more detailed studies on the radical scavenging effect of TAX are lacking. Therefore, in this study, we investigated the protective effect of TAX against oxidative plasmid DNA damage *in vitro* and also evaluating its inhibitory potential against cadmium (Cd) induced developmental toxicity in zebrafish embryos.

2. Materials and methods

2.1. Chemicals and reagents

Nitro blue tetrazolium (NBT), TAX, DPPH, ascorbic acid, ABTS, thiobarbutric acid (TBA), pUC19 plasmid DNA, trichloroacetic acid (TCA), Cadmium chloride (CdCl2), H_2O_2, and ethylene diamine tetra acetic acid (EDTA) were purchased from Sigma Chemical Co., St. Louis, MO, USA. The purified plasmid DNA of pUC19 was procured from Bangalore Genei Pvt. Ltd., India. Other chemicals and solvents not listed above were from Himedia, Mumbai, India.

2.2. In vitro antioxidant studies

2.2.1. DPPH radical scavenging assay

The DPPH radical was assayed as described by Liyana-Pathiranan and Shahidi (2005). The reaction tubes containing 0.135 mM DPPH in methanol followed by 1.0 ml of TAX (20–100 μg/ml) in methanol. The mixture was maintained in the dark for 30 min at 37 °C and the absorbance was read at 517 nm in UV-spectrophotometer (Systronics-2203, India) using ascorbic acid as standard. The percentage inhibition was calculated by the following formula: DPPH (%) inhibition = $[(ABS_{control} - ABS_{sample})/(ABS_{control})] \times 100$.

2.2.2. ABTS radical scavenging assay

The assay was performed by the method of Re et al. (1999). Briefly, 7.0 mM ABTS and 2.4 mM potassium persulfate of stock solution was prepared and the working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at 37 °C in the dark. The solution was then diluted by mixing 1 ml ABTS with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm. Varying concentrations of TAX (20–100 μg/ml) were allowed to react with ABTS and the absorbance was taken at 734 nm and the scavenging capacity of the compound was compared with ascorbic acid. The percentage inhibition calculated as ABTS radical scavenging activity (%) = $[(ABS_{control} - ABS_{sample})/(ABS_{control})] \times 100$.

2.2.3. Ferric reducing antioxidant property assay (FRAP)

The FRAP process employed was that of Oyaizu (1986). The reaction tube contains 1.0% potassium ferrocyanide and different concentration of TAX (20–100 μg/ml) in 0.2 M phosphate buffer (pH-6.6). The mixture was kept at 50 °C for 20 min, and then 2.5 ml of 10% TCA was added after centrifugation at 3000 × *g* for 10 min. To the supernatant, an equal volume of distilled water and 1.0% ferric chloride was added. The absorbance was measured at 700 nm using ascorbic acid as standard. The absorbance intensity determines the antioxidant activity of TAX.

2.2.4. Hydroxyl radical scavenging capacity

To 0.2 ml of different concentrations of TAX and/or standard, 0.2 ml of each solution (3 mM deoxyribose, 0.1 mM ferric chloride, 0.1 mM EDTA, 0.1 mM ascorbic acid and 2 mM H_2O_2) was added and incubated at 37 °C for 30 min. Then 0.2 ml of ice cold TCA (15%, w/v) and TBA (1%, w/v) was added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm (Halliwell et al., 1987).

2.3. DNA damage protection activity

The DNA damage, protecting activity of TAX was measured as described by Lee et al. (2002) on pUC19 plasmid DNA with some modification. Briefly, 2.0 μl of pUC19 plasmid DNA (100 ng/μl) and 4.0 μl of TAX with variable concentrations (0.2–1.0 μg/ml) were incubated for 10 min at 37 °C followed by the addition of 4.0 μl of Fenton's reagent (50 μM ascorbic acid, 30 mM H_2O_2 and 80 μM $FeCl_3$). After 30 min incubation (37 °C), the reaction mixture (10 μl) along with 3.0 μl of loading dye was placed on