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Full Length Research Paper

Antioxidant activities of Uyghur medicinal tea in human HL-60 cell line and rat hepatic microsomes

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Uyghur medicinal tea (UMT) is preferably used as a common nutritional additive by Uyghur population, especially by the aged people including centenarians from southern part of Xinjiang, along the Silk Road of China. The study showed an evidence for the antioxidant properties of UMT extract by using two different oxidative systems *in vitro*: (1) the myeloperoxidase/H_2O_2/HOCl enzymatic system in human HL-60 cell line and (2) the cytochrome P450/NADPH reductase system in rat liver microsomes. The EGB 761 G*inkgo biloba* extract was used as the reference. Reactive oxygen species (ROS) production was measured by fluorescence of 2',7'-dichlorofluorescein (DCF) originated from 2',7'-dichlorofluorescein diacetate (DCFHDA) probe oxidation. UMT extract inhibited ROS formation by 81, 86 and 73% in the HL 60 cell line system (at the concentrations of 0.25, 0.50 and 0.75 mg/ml) and at similar rates in the microsomal system. In the same conditions, *G. biloba* extract inhibited ROS activity by 39, 78 and 81% in HL 60 cells, and by 78, 87 and 89% in the microsomal system. The present study shows that UMT possesses relatively high antioxidant activity, comparable to the free radical scavenger properties of EGB 761 extract. This effect could explain its benefic preventive action against age-related pathologies. Habitual consumption of UMT may provide beneficial effects in prevention of atherosclerosis and oxidative stress in aging process.

Key words: Uyghur medicinal tea, antioxidant activity, HL 60 cells, hepatic microsomes.

INTRODUCTION

Cell damage by free radicals is believed to play a central role in the aging process and disease progression. Antioxidants are the first line of defense against free radical damage. The need for antioxidants becomes critical with increased exposure to reactive oxygen species (ROS) originating from environmental pollution, cigarette smoking, drugs, illness and stress (Ranjindar et al., 2002). Because many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. The antioxidant supplementation could serve as an important factor in improving free radical protection, disease prevention and treatment of some pathologies related to oxidative stress that is atherosclerosis, cardiovascular diseases, neurodegenerative diseases and cancer (Klapcinska et al., 2000).

In the recent years, substantial progress has been made in the knowledge of bioactive components with antioxidant properties in plant foods and their links to health (Orzechowski et al., 2000). Increasing interest in the health benefits of some special nutritional plants, that is medicinal tea, has been made also for use as dietary supplements and functional foods due to their potential antioxidant activity (Higdon et al., 2003).

The Uygur medicinal tea (UMT), one kind of tea, containing approximately 12 kinds of medicinal plants and growing on the Asia regions, is daily consumed as

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common nutrition additive for its aromatic, digestive and energetic properties by Uyghur population, especially by the old men and centenarians along the ancient silk road region from Xinjiang, North-West of China (Mouhemmed et al., 2001). Regular consumption of UMT should provide health benefit and protection against oxidative process in aging. We investigated comparatively the antioxidant properties of UMT extract by using two different *in vitro* models: myeloperoxidase (MPO) /H_2O_2/HOCl enzymatic system in human HL-60 cell line and the cytochrome P450/NADPH reductase system in rat liver microsomes. As a control, we used EGB 761 *Ginkgo biloba* extract, because it is widely known for its free radical scavenging properties (Christen et al., 2002; Rimbach et al., 2001).

MATERIALS AND METHODS

Chemicals

Phorbolmyristate acetate (PMA) and 2', 7'-dichloro-dihydrofluorescein diacetate (DCFH-DA) were obtained from Sigma (France). UMT was kindly provided by Pharmaceutical Laboratory of Kashgar Uygur Medicine (UMT contain approximately 12 kinds of medicinal plants such as *Rhizoma Alpiniae galanga, Cinnamomum cassia presl, Flos caryophylli, Fructus apii, Fructus piperis* Longi, *Fructus tsaoko, Fructus foeniculi, Flos cinnamomi, Fructus cardamomy, Rhizoma valerianae, Fructus anisi, Fructus gardeniae).* The UMT extract was prepared by simple decoction method (decoction with pure water at 75°C for 20 min and filtration) and drayed by vacuum evaporation. The EGB 761, a standardized extract from dried leaves of *G. biloba*, was kindly provided by Beaufour-Ipsen (Paris, France). The UMT and EGB 761 were prepared as stock solutions at a concentration of 10 mg/ml in ultrapure water, and then they were diluted at three different concentrations for experimental use. DCFH-DA was dissolved in ethanol at a concentration of 30 mM whereas PMA was dissolved in dimethylsulfoxide at a concentration of 1.6 mM and stored at –20°C.

HL-60 cell culture and cell treatment

The human promyelocytic leukemia HL-60 cell line was grown in RPMI-1640 medium (Sigma) supplemented with 15% fetal bovine serum, 2 mM glutamine (Sigma) and antibiotics /antimycotics (Sigma). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Cellular differentiation was induced by suspending cells at initial density of 2.5 x 10^5 cells/ml in a 75 cm^2 culture flask containing RPMI-1640 medium and 1.3% dimethylsulfoxide (DMSO) for 4 days. Cell counting was performed using Trypan blue exclusion. HL-60 cells were plated in 12-well plates at a density of 2 x 10^6 cells/well in RPMI 1640 medium, with 980 µl of PBS, 20 µl PMA (10 µM) and treated with different final concentrations of UMT or EGB 761 (250, 500 and 750 µg/ml) for 4 h. After this treatment, the cells were collected by centrifugation, washed and suspended in PBS. The cell pellet was conserved in ice bath until preparing the mixture for the immediate quantification of ROS production and for the measurement of protein concentration.

Animals and microsome preparation and treatment

Male Sprague-Dawley rats (150 to 180 g, Iffa-Credo, France) were housed in a controlled environment room with a 12 h light/dark photoperiod. The rats were fasted overnight prior to sacrifice and liver microsomes were prepared by the method described (Ekstrom et al., 1989).

Measurement of reactive oxygen species and protein concentration

ROS production was measured by fluorescence of 2',7'-dichlorofluorescein (DCF) originated from 2',7'-dichlorofluorescein diacetate (DCFHDA) probe oxidation. 2.5 x 10^5 cells were incubated with 20 µl DCFH-DA (40 µM) at 37°C for 30 min in a 96-well plate. Fluorescent DCF formation was monitored using a FL 600 Microplate Fluorescence Reader (Bio-Tek, USA) at an excitation and emission wavelength 485 and 530 nm, respectively. Microsomes (0.5 mg/ml) were pre-incubated for 15 min with 4 µM of DCFH-DA for de-esterification by endogenous esterase in 50 mM phosphate buffer, pH 7.4 at 37°C, and then for an additional 30 min incubation in the absence or presence of 0.5 mM NADPH and UMT or EGB 761 extracts at three different concentration (100, 250 and 500 µg/ml) according to Choi et al. (2002). The NADPH-initiated microsomal ROS production was quantitated with the fluorescent DCF formation originated from DCFH-DA oxidation. This is a probe that has been utilized extensively for the measurement of microsomal ROS production (Puntarulo et al., 1998; Serron et al., 2000).

Protein concentrations were determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. All results were expressed by fluorescent units / µg of protein.

RESULTS

Effect of treatments with both extracts on ROS production in HL-60 cells

Variation of ROS production after treatment of cells with UMT and EGB 761 extracts are shown on Figure 1. With both extracts, we observed a significant diminution of fluorescence, as compared to the control which suggests an antioxidant effect, whatever the concentration used. At the lower concentration, EGB 761 and UMT extracts inhibited the ROS formation by 39 and 81%, respectively. When cells are incubated with 500 and 750 µg/ml of extracts, ROS production is decreased by 86 and 73% for UMT and by 78 and 81% for EGB 761. Variation of fluorescence observed with UMT extract show no significative dose-dependent effect (Figure 1). Cells were incubated with 250, 500 and 750 µg/ml of EGB 761 (2,3,4) and UMT (5,6,7) extracts, respectively. Control (1): untreated cells. Values are the mean±SD of three individual determinations. Number signs indicate a significant difference from the control value (one-way ANOVA followed by Newman-Keuls multiple range test). (1) *p < 0.05, **p < 0.01 signified the difference vs. the Control (2): °p < 0.05, °°p < 0.01 signified the difference vs. the first concentration (3): #p <0.05, ##p < 0.01 signified the difference vs. EGB 761.

Effect of treatments with both extracts on microsomal ROS production

Addition of NADPH to the rat liver microsomes initiated

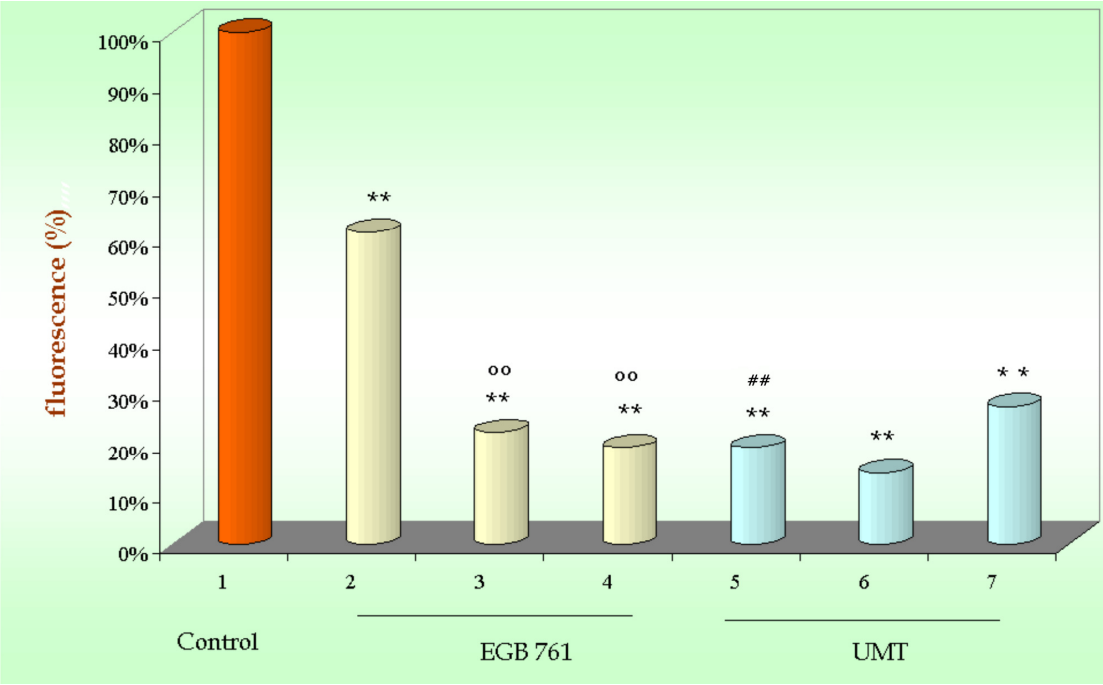

Figure 1. Variation percentage of fluorescence in Uyghur Medicinal Tea (UMT) and EGB 761 *G. biloba* extract treated HL-60 cells.

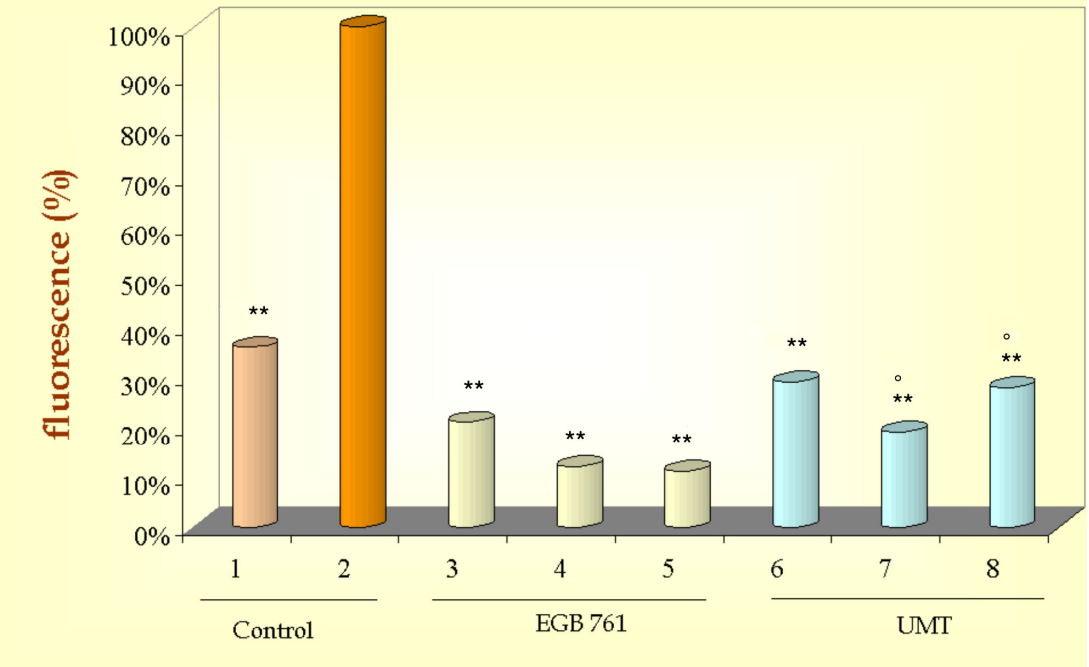

Figure 2. Modification of fluorescence in microsomes in the presence of EGB 761 and UMT extract.

DCFH-DA oxidation in the presence of 0.5 mM NADPH. The addition of EGb 761 decreased the DCFH-DA oxidation by 78, 87 and 89%, respectively (Figure 2). We observed that UMT possessed a significant antioxidant activity by inhibiting the ROS formation by 70, 81 and 72% in the microsomal system. For both products, there is no significant difference among two lower concentrations used, however, at higher concentration, EGB 761 has

higher activity than UMT.

The reaction mixture was composed by the microsome suspension containing 100, 250 and 500 µg/ml, of EGB 761 (3,4,5) and UMT (6,7,8), respectively. Control 1: microsomes alone; control 2: microsomes + NADPH; EGB 761: extract of *G. biloba*; UMT: Uygur medicinal tea. The results indicate a significant difference from the control value. Values are the mean±SD of three individual determinations, one-way ANOVA followed by Newman-Keuls multiple rage test. (1) *p < 0.05, **p < 0.01 signified the difference vs. the Control 2; (2) °p < 0.05, °°p < 0.01 signified the difference vs. EGB 761.

In both *in vitro* models which we used, we globally measured an antioxidant effect of UMT and *G. biloba* extracts (EGB 761). The HL-60 cell myeloperoxidasic enzymatic system is currently used for the evaluation of the antioxidant effect of natural products (Rajbhandari et al., 2001). In HL-60 cells, at the lowest concentration, treatment with UMT lead to a significant decrease of ROS production, as comparable with values measured in the presence of EGB 761. For higher rates, there was no difference according to the nature of extract as well as to the concentration used. Following assays at lower concentrations of extracts could be investigated.

DISCUSSION

The main findings of this study are that UMT possesses relatively high antioxidant activity, comparable to the free radical scavenger properties of EGB 761 extract. Nowadays, the strongest evidence supports the role of antioxidant defenses, rates of ROS production, level of oxidative damage and cellular repair mechanisms and its implication in oxidative aging and development of various age-related pathologies (Gustavo et al., 2002). ROS and oxidative damage both increase with age and vary inversely with MLS (maximum life span) of different species (Dudas et al., 1995). Long-term dietary antioxidants supplementation may be an effective way for keeping or enforcing the antioxidant defenses capacity, and may contribute to slow the oxidative aging process and to longevity.

The UMT contain approximately 12 kinds of medicinal plants, among them, one of the main constituents is Rhizoma *Alpiniae galanga* (rhizomes of *Alpinia galanga* L). The hot water soluble polysaccharide extract of *A. galanga* showed relatively strong immunostimulating activity both *in vitro* and *in vivo* study (Bendjeddou et al., 2003). The second main component of UMT, the *Cinnamomum cassia* Presl was demonstrated its high antioxidant activity *in vitro* (Lin et al., 2003), furthermore, this plant is also used for cancer treatment in traditional Korean medicine (Seo et al., 2005).

UMT is preferably consumed for its digestive, aromatic, vital energy improvement characteristics, by local Uygur people of the southern area of Xinjiang, especially by the aged people including centenarians (Mohammed et al., 2001). We consider that regular consumption of antioxidants present in teas, as well as in UMT, could provide beneficial effects for health and may be valuable in prevention of atherosclerosis, oxidative process at aging and in longevity.

Furthermore, our study is the first report to date investigating the effect of UMT on HL-60 cells which ROS production is particularly linked to the MPO enzymatic system. Recent findings indicate that MPO is involved in cardiovascular diseases through its strong oxidative activity (Zhang et al., 2003; Hoy et al., 2002), and inversely, MPO deficiency seems to be protective against cardiovascular disease (Kutter et al., 2000). Our study shows that UMT leads to decreased HL-60 cell mediated ROS production, and this could provide an additional mechanism of contribution of UMT to a local protective effect in atherosclerosis.

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