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Screening of selected medicinal plants from Jordan for their protective properties against oxidative DNA damage

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ABSTRACT

Herbal medicinal products represent a major focus for drug development and industry and it holds a significant share in drug-market all over the globe. In here, selected medicinal plant extracts from Jordan with high antioxidative capacity were tested for their protective effect against oxidative DNA damage using *in vitro* 8-hydroxydeoxyguanisine assay in cultured human lymphocytes. The following plant extracts were tested *Cupressus sempervirens* L., *Psidium guajava* (L.) Gaerth., *Silybum marianum* L., *Malva sylvestris* L., *Varthemia iphionoides* Boiss., *Eminium spiculatum* L. Blume, *Pistachia palaestina* Boiss., *Artemisia herba-alba* Asso, *Ficus carica* L., *Morus alba* Linn, *Cucumis sativus* L., *Eucalyptus camaldulensis* Dehnh., *Salvia triloba* L., *Zizyphus spina-christi* L. Desf., and *Laurus nobilis* L. A fractionation scheme for the active plant extracts of the above was followed. Plants extract fractions with best protective properties against DNA damage included hexane fraction of *S. marianum* L. (aerial parts), chloroform fractions of *P. palaestina* Boiss. (Fruits), ethanolic fractions of *E. camaldulensis* Dehnh (leaves), *S. triloba* L. (leaves), and ethanolic fractions of *Z. spina-christi* L. Desf. (Fruits/leaves). On the other hand, the ethanolic extracts of *V. iphionoides* Boiss was found to increase oxidative DNA damage. In conclusion, plant extracts with antioxidative DNA damage properties might have clinical applications in cancer prevention.

1. Introduction

The geographic location of Jordan lies at the bridge of Asia, Africa and Europe (Al-Eisawi, 1998; Feinbrun-Dothan, 1986). This gives Jordan a unique natural flora, which is very diverse, yet, largely unstudied (Abu-Irmaileh and Afifi, 2000; Al-Khalil, 1995). Thus, studying local flora of Jordan is expected to provide information and plant products that are unique. Jordanian plants were reported for their general antioxidant properties (Alali et al., 2007), but not for their oxidative DNA damage prevention effects (Table 1). Because of their antioxidative effect, these plants have strong potential to reduce spontaneous DNA damage rates.

The molecular mechanism of cancer development involves induction of three to seven mutations in the tumor suppressor gene and oncogenes (Ueda and Komaru, 2011). The main mutagenic source for such mutations is free radicals that are produced during normal body metabolism and due to chronic oxidative stress and inflammation (Lawless et al., 2010). It has been estimated that more than 60% of cancers can be prevented by appropriate diet that includes consumption of fruits and vegetables with significant antioxidant activity (Donaldson, 2004; Norppa et al., 2006). In this study, the antioxidative DNA damage profiles of selected plant extracts were assessed using the 8-hydroxy-2-deoxyguanosine (8-OH-dG) assay. The 8-OH-dG assay reflects the level of the oxidative DNA damage inside cells (Valavanidis et al., 2009). These DNA damages occur spontaneously at certain frequencies in all cells, and various endogenous and exogenous mutagenic agents can enhance these frequencies (Ray et al., 2001; Wilson and Thompson, 2007). Thus, consumption of certain diets, and the use of certain natural plants supplements that lower oxidative stress and the subsequent mutation rate might have powerful effects against cancer development (Donaldson, 2004).

In the current study, we tested the hypothesized that lowing oxidative stress will decrease oxidative DNA damage. Therefore, the objective of the current study was to test the effect of administration of extracts of medicinal plants with high antioxidative capacity on oxidative DNA damage using the standard 8-OHdG assay.

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Table 1
Candidate Jordanian plants with anti-oxidative activity, and their other pharmacological effects.

#	Plant with antioxidant activity	Vernacular name/s	Plant part	Some other pharmacological effects
1	*Cupressus sempervirens* L. (Mothana et al., 2009)	Serou	Fruits, leaves	Antibacterial activity (Mothana et al., 2009)
2	*Psidium guajava* (L.) Gaerth. (Gull et al., 2012)	Guava	Leaves	Nephroprotective, anti-hyperglycemic and anti-hyperlipidemic effects (Deguchi and Miyazaki, 2010; Huang et al., 2011; Kuang et al., 2012)
3	*Silybum marianum* L. (Anestopoulos et al., 2012)	Khurfaish	Aerial parts	Hepatoprotective (Ahmad et al., 2012), and antidiabetes properties (Huseini et al., 2006)
4	*Malva sylvestris* L. (Gasparetto et al., 2012)	Khubaizeh	Leaves	Anti-inflammatory, anti-complementary, skin tissue integrity and anti-ulcerogenic activity (Gasparetto et al., 2012)
5	*Varthemia iphionoides* Boiss. (Al-Dabbas et al., 2006; Al-Mustafa and Al-Thunibat, 2008)	shaileh	Aerial parts	Antihyperglycemic (Kasabri et al., 2011), antibacterial (Al-Dabbas et al., 2006), and antiplatelet activity (Afifi and Aburjai, 2004)
6	*Eminium spiculatum* L. Blume (Janakat and Al-thnaibat, 2008)	Je'ddeh, erquita, arqat	Leaves	Antiproliferative (Alkofahi and Al-Khalil, 1995), and antibacterial (Obeidat, 2011) activity
7	*Pistachia palaestina* Boiss. (Alali et al., 2007)	Butum	Fruits	Antianthropod pests (Cetin et al., 2011)
8	*Artemisia herba-alba* Asso (Abid et al., 2007)	Sheeh	Aerial parts	Antihyperglycaemic and antihyperlipidemic effect (al-Shamaony et al., 1994; Hamza et al., 2011)
9	*Ficus carica* Linn L. (Ali et al., 2012)	Teen	Leaves	Antiinflammatory (Ali et al., 2012), and hepatoprotective (Singab et al., 2010)
10	*Morus alba* Linn (Nade et al., 2010; Singab et al., 2010)	Toot	Leaves	Hepatoprotective (Singab et al., 2010), and Immunomodulatory (Bharani et al., 2010)
11	*Cucumis sativus* L. (Ibrahim et al., 2010; Kumar et al., 2010)	Khiar	Aerial parts	Analgesic (Kumar et al., 2010)
12	*Eucalyptus camaldulensis* Dehnh. (Barra et al., 2010; El-Ghorab et al., 2003)	Keena, Abu caleptus	Leaves	Antifungal (Barra et al., 2010), anti *Mycobacterium tuberculosis* (Lawal et al., 2012) and *Helicobacter pylori* (Adeniyi et al., 2009)
13	*Salvia triloba* L. (Ciesla et al., 2011; Mahdy et al., 2012; Yildirim et al., 2000)	Miarameieh	Leaves	Antibacterial (Al-Bakri et al., 2010), antiinflammatory (El-Sayed et al., 2006), and antiproliferative (Al-Kalaldeh et al., 2010)
14	*Zizyphus spina-christi* L. Desf. (Al-Busafi et al., 2007)	Nabaq, Sedr	Fruits, leaves	Antihyperglycemic (Abdel-Zaher et al., 2005; Michel et al., 2011; Nesseem et al., 2009), and protects against aflatoxin B1-induced heptotoxicity (Abdel-Wahhab et al., 2007).
15	*Laurus nobilis* L. (Berrington and Lall, 2012; Emam et al., 2010; Saab et al., 2012)	Ghaar	Leaves	Cytotoxicity in cancer cell lines (Berrington and Lall, 2012), anti-inflammatory (Berrington and Lall, 2012), neuroprotective (Koo et al., 2011)

2. Methods

2.1. Collection and identification of plant material

Materials of 15 plants in Table 1 are collected from different locations of Jordan. Taxonomic identity of plants was confirmed by Professor Jameel Lahaam, Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid, Jordan. A voucher specimen from each plant was deposited at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and technology, Irbid, Jordan.

2.2. Preparation of crude plant extracts

The plant material was shade-dried, then, it was grounded in a Wiley grinder with a 2 mm diameter mesh. The ground material was percolated in ethanol (95%), then, it was evaporated under vacuum using rotary evaporator at 40 °C. The combined ethanolic extracts were concentrated in vaccum to give a gummy material, which were subjected to further fractionation (Fig. 1).

2.3. Screening for oxidative DNA damage using 8-OH-dG assay in cultured human lymphocytes

2.3.1. Subjects

Blood samples were obtained from healthy, young, adult, male, and non-smoking volunteers recruited from the blood bank unit at King Abdullah University Hospital (KAUH). Informed consents were obtained from each volunteer according to the institutional review board of Jordan University of Science and Technology. Volunteers were not taking any medication at the time of the experiment. Lymphocyte cultures were established by adding 1 ml of fresh heparinized whole blood to 9 ml of PB max complete karyotyping media (Gibco-Invitrogen, Paisley, UK). Cultures were incubated in the dark at 37 °C in a CO_2 incubator with appropriate humidity. Blood from each donor was used in the controls as well as treated cultures of all tested plant extracts.

2.4. The 8-OH-dG assay

After 66 h of incubation of human blood lymphocytes, cultures were centrifuged, the pallete were resuspended in serum-free media, and cultures were treated with different concentrations of plant extracts (10 μg/ml, 100 μg/ml, 500 μg/ml and 1000 μg/ml, n = 8/dose) for 6 h (Alzoubi et al., 2012). After that, cultures were centrifuged and 8-OH-dG was measured in the supernatant using ELISA kit according to the manufactures protocol (Abcam Inc., Cambridge, MA, USA). Plates were read at 405 nm using Epoch Biotek microplate reader (BioTek, Winooski, VT, USA). Dose response curves were developed for each plant extract and the optimum concentration that showed high antioxidative DNA damage activity (based on ANOVA and post hoc analysis) was used in succeeding assays. Plant extracts that give negative results (i.e. do not affect or increase 8-OH-dG levels) were excluded from further examination. Part of the treated cell cultures were examined for cytotoxicity of the plant extracts. None of the plant extracts were found to be cytotoxic when cultures were treated for 6 h (data not shown).

2.5. Statistical analysis

Statistical analysis was performed using Graphpad Prism Statistical Software (version 5, USA). Data were expressed as mean ± standard deviation (SD). The comparisons of parameters were performed using one-way ANOVA followed by Tukey's multiple comparison.