Potent antioxidative DNA damage of selected Saudi medicinal plants in cultured human lymphocytes

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Abstract: Oxidative stress is a condition that might predispose the individuals to diseases including cancer. The 8-hydroxydeoxyguanosine (8-OHdG) is a marker that reflects oxidative DNA damage in the body. In this study, seven Saudi medicinal plants were investigated for their potential against oxidative DNA damage using the 8-OHdG assay in cultured human lymphocytes. Extracts at 10-100µg/mL from *Nigella sativa* black seeds, *Olea chrysophylla* (aerial parts) and *Pulicaria crispa* (aerial parts) significantly decreased levels of 8-OHdG ($P<0.01$), suggesting their usefulness as protective agents against oxidative DNA damage. The order of the antioxidative DNA damage effect of the extracts at 100µg/mL was *Pulicaria crispa* (36%) >*Olea chrysophylla* (24%) >*Nigella sativa* (18%). On the other hand, *extracts of Bupleurum falcatum* L at 100ug/mL induced significant increases in the 8-OHdG biomarker ($P<0.01$). Finally, *Ficus palmate*, *Zygophyllum Simplex*, *Citrullus colocynthis* did not modulate levels of 8-OHdG in cultured human lymphocytes at examined concentrations (10 and 100µg/mL, $P>0.05$). In conclusion, extracts from *Nigella sativa*, *Olea chrysophylla* and *Pulicaria cripa* medicinal plants can be used as useful agents to counteract oxidative DNA damage in cultured cells.

Keywords: DNA damage, 8-OHdG, plant extract, cultured lymphocytes, antioxidants.

INTRODUCTION

Oxidative stress is the condition where an imbalance between the generation of reactive oxygen species (ROS) in the body and antioxidant exist (Farias *et al.*, 2017). Oxidative stress can arise as a result of infection and as part of the inflammatory response (Butcher *et al.*, 2017, Guzik and Touyz, 2017). In addition, oxidative stress is associated with chronic diseases such metabolic disorders, renal dysfunction, cardiovascular diseases, auto-immune diseases, and others (Miranda-Diaz *et al.*, 2016, Zinellu *et al.*, 2016). Several therapeutic drugs have been also shown to increase reactive oxygen species inside the body (Chirino and Pedraza-Chaverri, 2009).

Oxidative stress can destroy macromolecules such as proteins and nucleic acids. This can lead to tissue and organ damage and the subsequent development of diseases and the addition of more complications to existing conditions (Zhao *et al.*, 2007). For example, oxidative stress associated with diabetes plays a major role in the development of complications related to the disease (Pitocco *et al.*, 2013).

The use of antioxidant supplements has been shown to be useful in the managements of oxidative stress associated with chronic diseases and therapeutic drugs. For example, the use of antioxidant vitamin C and E emulate oxidative stress associated with sickle cell disease (Amer *et al.*, 2006). In addition, antioxidant use prevents oxidative damage induced by therapeutic drugs such as diazepam (El-Sokkary, 2008).

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Saudi Arabia, the largest country in the Middle East, is rich in medicinal plants that that have been used in the treatmentof human diseases (Rahman *et al.*, 2004). In addition, several of such plants have been shown to possess antioxidant and anticancer activity in both in vitro and in vivo systems (Al-Howiriny *et al.*, 2004, Albadawi *et al.*, 2017).

In this study, several Saudi medicinal plants were screened for their antioxidative DNA damage properties using 8-OHdG. The plants were selected based on their antioxidant properties and include:*Nigella sativa*, *Olea chrysophylla*, *Citrullus colocynthis*, *Bupleurum falcatum*, *Zygophyllum simplex*, *Ficus palmate* and *Pulicaria crispa*. The 8-OHdG assay has been shown to be useful for screening medicinal plants for their antioxidative properties and to prevent DNA damage induced by different drugs (Alkofahi *et al.*, 2016, Valavanidis *et al.*, 2009).

MATERIALS AND METHODS

Collection of medicinal plant materials

The used plant part to prepare extracts (see table 1) were collected from Almedina area, Saudi Arabia between January and April, 2016. Plants were identified by a specialist in medicinal plants (Professor Sami Zalat) from the Botany Division of Biology Department at Taibah University, Saudi Arabia. A voucher specimen from each plant used in the study was deposited at the Plant Laboratory of above division.

Crude extracts preparation

Plant materials (200 gram from each used part, table 1) were air dried in the shade and then grounded in a Wiley grinder with a 2mm diameter mesh. The grounded material was then refluxed in (2 L) 70% ethanol per each 500g at 50°C for 36 hours in continuous extraction (soxhlet) apparatus. Ethanol extract was filtered and concentrated under reduce pressure at 40°C using a rotary evaporator to produce dried residues (active principles). The net yield was 28.5 g/kg.

Blood donors

Two healthy male volunteers (ages: 26 and 24 years) donated their blood to be used for lymphocyte cultures. Both volunteers were never smokers and non-alcoholic were not using any supplements or medications at least 6 months prior to participation in the study. Fifty mL of blood was obtained from volunteers each time they donated in sterile heparinized tubes. Volunteers gave written informed consent as required by the institutional ethics committee at Taibah University.

Blood cultures

The p –Euro clone Chromosome medium (Italy) that contains: RPMI 1640, fetal bovine serum, glutamine, penicillin-streptomycin and phytohaemagglutinin was used to culture human lymphocytes. The percentage of whole blood to culture media was 1:9 (Azab *et al.*, 2017)About one mL of withdrawn blood was added to tissue-culture flasks containing media under laminar hood (Thermo Fisher Scientific, Waltham, MA USA) and sterile conditions. Cultures were then incubated at 37°C in a dark CO_2 incubator for 72 hours (Esmadi *et al.*, 2016). Cultures were then used in the 8-OHdG assay as described below.

The 8-OHdG assay

After 72 hours of blood culture incubation, the cultures were centrifuged at 1500 xg for 5 minutes to precipitate cells. , Cellular pellet was then washed five times in bovine serum-free chromosome medium. Cellular pellet were then re-suspended in 1ml of wash medium and then exposed to different concentrations (10 µg/mL and 100 µg/Ml) of the plant extract for six hours(Alzoubi *et al.*, 2014). Cisplatin (0.8 µg/mL, Sigma-Aldrich, St. Louis, MO, USA) and Vitamin E (alpha tocopherol; 1µg/mL; Acros Organics, Turkey) were used as additional controls to validate the assay. At the end of the incubation period, tubes were centrifuged at 1500 xg and 100µL of the supernatant was used for 8-OHdG measurements. Untreated cultures were used as controls. The 8-OHdG marker was measured using commercially available kit that was obtained from Sigma-Aldrich (City, USA) as described by the manufacturer instructions. Changes in the absorbance were analyzed at 405 nm using an ELISA Epoch Biotekreader (BioTek, Winooski, VT, USA). From each experiment, mitotic indices were evaluated as previously described (Khabour *et al.*, 2016) to examine the cytotoxicity of plant extracts. None of the included extracts presented in the study were found cytotoxic at examined concentrations when cultures were treated for six hours.

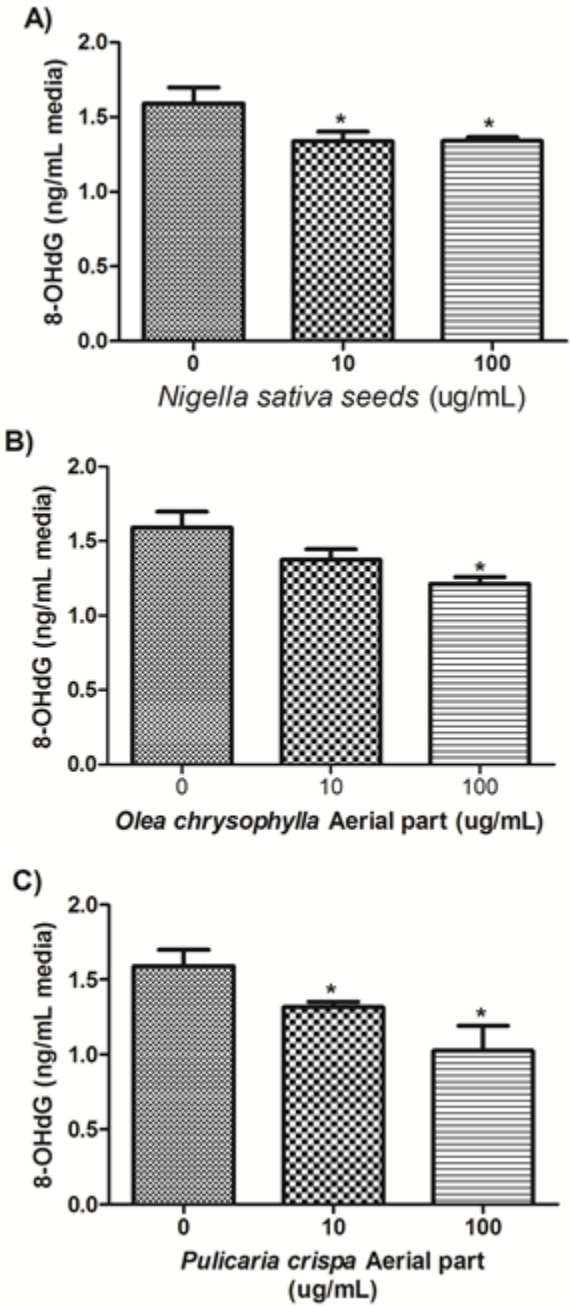

Fig. 1: Plant extracts that decreased 8-OHdG biomarker in cultured human lymphocytes.

Cultures were treated with different concentrations of plant extracts for 6 hours. 8-OHdG biomarker was measured in the supernatant. Significant decrease in the 8-OHdG was induced by treatment of cultures with A) *Nigella sativa*, B) *Olea chrysophylla* and C) *Pulicaria crispa* plant extracts. * indicates significant difference using ANOVA, $P < 0.05$.

STATISTICAL ANALYSIS

Statistical comparisons were performed using GraphPad Prism statistical software (version 4). ANOVA followed by Tukey posthoc test was used for the three group analysis. A $P<0.05$ was used as a threshold for statistical significant.

RESULTS

In this study, extracts from 7 Saudi medicinal plants were screened for their anti-mutagenic activity using the 8-OHdG assay and cultured human lymphocytes. This assay has been shown to be useful for such purpose (Alkofahi, Alzoubi *et al*., 2016). To validate the assay, cultures were treated with Cisplatin (0.8µg/mL) that is known to induce oxidative stress or Vitamin E (1µg/mL) that has potent antioxidant activity(Khabour *et al.*, 2015, Khabour *et al.*, 2014). Treatment of cultures with Cisplatin significantly increased 8-OHdG by approximately 78% ($P<0.01$). Vitamin E, on the other hand, significantly decreased 8-OHdG by approximately 33% ($P<0.01$).

Fig. 1 shows plant extracts that were found to possess anti-mutagenic activity. These plants are *Nigella sativa* seeds (fig. 1A), *Olea chrysophyll a*aerial part (fig. 1 B) and *Pulicaria crispa* aerial part. Extract of *Nigella sativa* black seeds showed significant anti-mutagenic activity at all examined concentrations (10ug/mL and 100 ug/mL, $P<0.001$). The magnitude of the decrease in 8-OHdG is about 18% for both concentrations. In the *Olea chrysophylla* case, significant decrease in the 8-OHdG marker was found in the group treated with 100ug/mL of the extract with a magnitude of decrease equal 24% ($P< 0.05$, fig. 1B). With respect to *Pulicaria crispa*, dose dependent effect was observed for 10ug/mL and 100 ug/mL concentrations with magnitude of decreasesequal to 16% and 36% respectively ($P<0.01$, fig. 1C).

Three plant extracts did not show anti-mutagenic effects at examined concentrations (fig. 2). These plants are Ficus palmate aerial parts (fig. 2A, $P = 0.30$), *Zygophylum Simplex* aerial part (fig. 2B, $P = 0.49$), and *Citrullus colocynthis* fruit (fig. 2C, $P = 0.45$).

Finally, extract from two *Bupleumum falactum* aerial part at 100ug/uL (fig. 3, $P<0.01$) was found to increase 8-OHdG biomarker (fig. 3). This result indicates induction of oxidative DNA damage by this plant in cultured human lymphocytes.

DISCUSSION

In this study, seven Saudi medicinal plants were screened for their anti-mutagenic activity in cultured human lymphocytes using 8-OHdG biomarker that reflects oxidative DNA damage. The extracts of *Nigella sativa* (black seeds), *Olea chrysophylla* (aerial parts) and *Pulicaria crispa* (aerial parts) significantly decreased levels of 8-OHdG, suggesting their usefulness as protective agents against oxidative DNA damage.

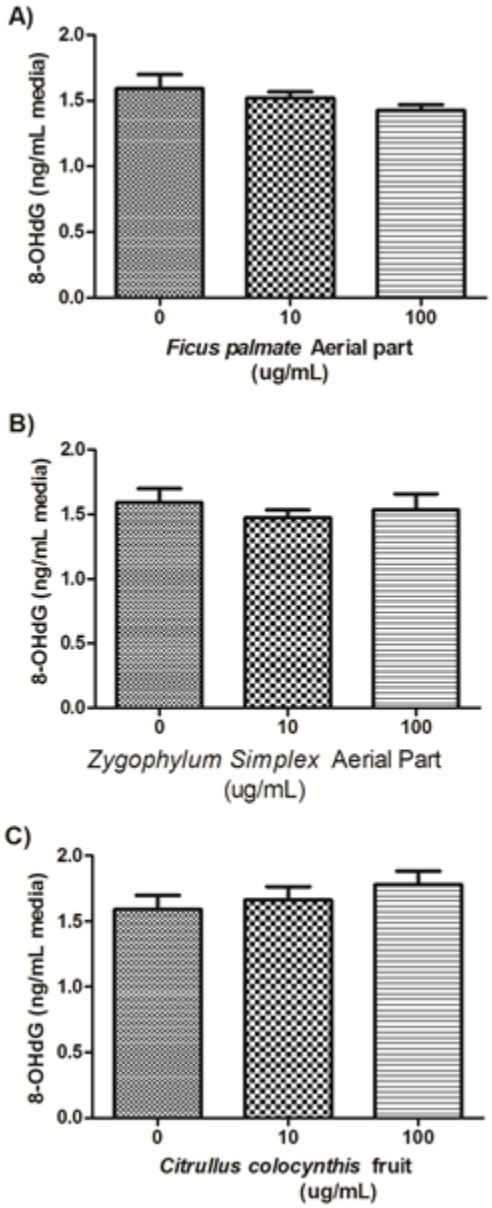

Fig. 2: Plant extracts that did not affect 8-OHdG biomarker in cultured human lymphocytes.
Cultures were treated with different concentrations of plant extracts for 6 hours. 8-OHdG biomarker was measured in the supernatant. No significant change in the 8-OHdG was observed by treatment of cultures with A) Ficus palmate, B) *Zygophylum Simplex* and C) *Citrullus colocynthis*.

Nigella sativa is an herb known as a black cumin that has been used to treat diseases such as hypertension, diabetes and neurodegeneration (Kooti *et al.*, 2016). The seeds are rich in thymoquinone and monoterpenes compounds that have been shown to reduce cisplatin induced

Table 1: List of the investigated plants, used parts, and their medicinal use

Plant Scientific Name	Vernacular name	Part used	Medicinal use	References
Nigella sativa L.	black seed	Seeds	Antimicrobial, anticancer, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective.	Kooti *et al.*, 2016, Cascella *et al.*, 2017, Bourgou *et al.*, 2008, Badary *et al.*, 2007, Badary, Abd-Ellah *et al*., 2007, Khader *et al*., 2010.
Olea chrysophylla Lam subsp. europaea	Wild olive	Leaf	Antiarrhythmic, antiatherosclerotic, antimicrobial, antiviral, anticancer and anti-inflammatory activity	Katsiotis *et al*., 1998, Rigacci and Stefani, 2016, (Li, Liu *et al*., 2016) Topalovic *et al*., 2015, Cabarkapa *et al*., 2014, Fabiani *et al*., 2008
Pulicaria crispa	Jithjath	Leaf	Antiepileptics, antimicrobial, anti-inflammatory, antioxidant activities	Abou-Zeid *et al*., 2007, Kuete *et al*., 2013, Stavri *et al*., 2008, Marwah *et al*., 2007, al-Yahya *et al*., 1988.
Bupleurum falcatum L.	abu za'aryr, sickle hare's ear	Leaf	Immunomodulatory, antiulcerative, anti-atherosclerosis, hepatoprotective and nephroprotective activities, antioxidant effects.	Lee *et al*., 2010, Park *et al*., 2015, Niikawa *et al*., 1990, Zhang *et al*., 2014, Liu *et al*., 2014.
Zygophyllum simplex	Jarmal, garmal	Arial parts	Used against aches, calm thirst. treatment of dental caries, antibacterial, antihelmintic and anti-inflammatory.	Abdallah and Esmat, 2017
Ficus palmata	Wild Fig	Leaf	Antitumor, anti-inflammatory, treatment of epilepsy, jaundice, bronchitis, influenza whooping cough, and tonsillitis, antioxidant activities	Lansky *et al*., 2008; Noumi and Fozi, 2003; Çalişkan and Polat, 2011.

nephrotoxicity and tetrachloride hepatotoxicity (Cascella *et al.*, 2017).The antimutagenic potential of *Nigella sativa* seeds has been demonstrated using the Ames test (Bourgou *et al.*, 2008). In addition, it deduced DNA damage induced by benzo(a)pyrene in mice (Badary *et al.*, 2007) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in cultured rat hepatocytes (Khader *et al.*, 2010).

*Olea chrysophylla*is a synonym of *Olea europaea* subsp. *Cuspidate*, which is known as "wild olive"(Katsiotis *et al.*, 1998). This plant grows in various regions of Saudi Arabia and the region. Little research has been done on this variety of olive plant. In general, olive leaf polyphenols has been shown to be useful as anti-hypertensive, anti-diabetic, anti-carcinogenic, anti-atherosclerotic and anti-inflammatory (Rigacci and Stefani, 2016). Olive leaves extract is rich in Oleuropein that has been shown to be powerful against many foodborne pathogens(Li *et al.*, 2016). In accordance with our findings, the dry olive leaf extract has been shown to counteract L-thyroxine-induced genotoxicity in human peripheral blood leukocytes via lowering oxidative stress (Topalovic *et al.*, 2015) and to protect human leukocytes from adrenaline induced DNA damage as evaluated using in vitro comet assay (Cabarkapa *et al.*, 2014). In addition, oxidative DNA damage is significantly reduced by treatment with olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells (Fabiani *et al.*, 2008)

The third plant that showed an antioxidative DNA damage effect is *Pulicaria crispa*, which is known in Saudi Arabia as "geethgath". This plant is a member of the Compositae family and is used by people of the region to treat inflammation and infection (Abou-Zeid *et al.*, 2007, Kuete *et al.*, 2013). Phytochemical studies of this herb revealed that the plant is rich in sesquiterpene lactones of the guaianolide (Stavri *et al.*, 2008). Extracts from this plant has been shown to possess strong antioxidant activity (Marwah *et al.*, 2007) and cancer chemopreventive properties (al-Yahya *et al.*, 1988).

Bupleurum falcatum L is an herb that has been used globally as anti-inflammatory agent (Lee *et al.*, 2010, Park *et al.*, 2015).*The results showed that extracts of Bupleurum falcatum* L at 100 ug/mL induced significant increase in oxidative DNA damage. In agreement with this finding, extracts from *Bupleurum falcatum* L has been shown to enhance the mutagenicity of Trp-P-1, Trp-P-2 and benzo[a]pyrene (Niikawa *et al.*, 1990). However, Saikosaponin-D that derived from *Bupleurum falcatum* L. has been shown to attenuate heat stress-induced oxidative damage in LLC-PK1 cells by increasing the expression of

anti-oxidant enzymes and HSP72 (Zhang *et al.*, 2014). In addition, this compound has been shown to protect the body against acetaminophen-induced hepatotoxicity (Liu *et al.*, 2014). Thus, extracts of *Bupleurum falcatum* L. might have different effects depending on treated cell type/preparation used.

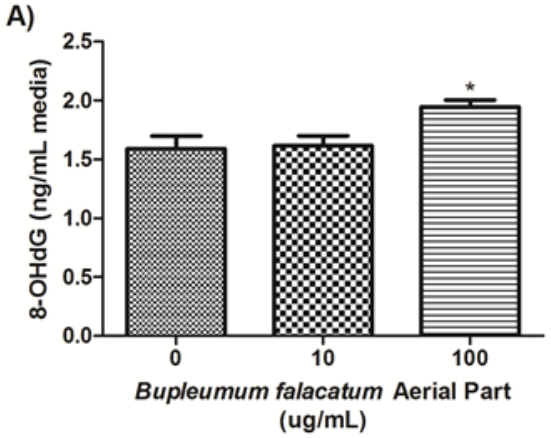

Fig. 3: *Bupleumum falactum* extract increased 8-OHdG biomarker in cultured human lymphocytes.

Cultures were treated with different concentrations of *Bupleumum falactum* extract for 6 hours. 8-OHdG biomarker was measured in the supernatant. Significant increase in the 8-OHdG was induced by the treatment. * indicates significant difference using ANOVA, $P < 0.05$.

Three plant extracts, *Ficus palmate*, *Zygophyllum Simplex*, *Citrullus colocynthis* did not modulate levels of 8-OHdG in cultured human lymphocytes at examined concentrations. These plants have been shown to possess antioxidative activities and to be useful for the treatment of several diseases(Abdallah and Esmat, 2017, Hajjar *et al.*, 2017, Hussain *et al.*, 2014, Ostovan *et al.*, 2017). In addition, extracts obtained from *Citrullus colocynthis* fruit have been shown to protect against genotoxicity induced by cyclophosphamide in mouse bone marrow cells (Shokrzadeh *et al.*, 2013). Thus, the present findings do not rule out their usefulness as medicinal plants against tissue damage and treatment of diseases.

Oxidative stress is a serious condition that predisposes the body to tissue damage via the destruction of macromolecules including DNA. This leads to the development of chronic diseases, cancer and aging. The consumption of certain diets rich in antioxidants might protect tissues from oxidative damage and subsequent prevention or delaying such diseases. The present findings indicate the usefulness of some plants in decreasing oxidative DNA damage and expected protection against disease such as cancer.

In this study, we examined anti-oxidative DNA damage activity of the Saudi medicinal plants using the 8-OHdG assay. To better characterize this property, it is recommended in future studies to expand present findings to include other genotoxic compounds. In addition, further fractionation and identification of antimutagenic active compounds from the plants that gave positive results is strongly recommended.

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