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Research Article

EVALUATION OF THE GENOTOXICITY OF ESSENTIAL OIL FROM *ORIGANUM COMPACTUM* BENTH. IN HUMAN LYMPHOCYTES

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ABSTRACT

Objective: Essential oils with antimicrobial and antioxidant activities have gained importance. This study was conducted to investigate the genotoxic potential of the essential oil of *Origanum compactum*.

Methods: Micronucleus test and proliferation index (PI) were used to evaluate the genotoxic effect of the essential oil of *O. compactum* on human lymphocytes.

Results: The results demonstrated that the PI values were influenced by the tested essential oil. An increase in concentration of essential oil also caused the appearance of micronuclei.

Conclusion: The results of the present work suggest that *O. compactum* essential oil exhibited genotoxic activity at higher concentration. Further studies are necessary to confirm these findings.

Keywords: *Origanum compactum,* Human lymphocytes, Proliferation index, Micronucleus test.

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INTRODUCTION

Natural products have historically been an extremely productive source of therapeutic agents. According to the World Health Organization, due to poverty and lack of access to modern medicine, approximately 65-80% of the population in developing countries depends mainly on medicinal plants for their primary health care [1]. In addition, despite the remarkable progress in synthetic organic chemistry, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants [2].

Essential oils are complex mixtures of odorous and volatile compounds extracted from plants by mechanical pressing or hydro- and steam-distillation. They are mainly composed of monoterpenes, sesquiterpenes, alcohols, ethers, aldehydes, esters, and ketones [3]. Essential oils are widely used as flavor enhancers in many food products and as odorants in fragrances [4]. Their lipophilic compounds can cross cell membranes and be absorbed through the skin, and for this reason, they have a long history of use for various medical applications [5]. Essential oils are known to possess several biological properties including antibacterial, antioxidant, antifungal, insecticidal, and anticancer activities [6-9]. However, it is also important to determine the potential genetic hazards of compounds present in medicinal plants, and few studies have investigated the genotoxic and mutagenic properties of essential oils.

The genus *Origanum* (Lamiaceae family) is among the most important aromatic plants worldwide. 49 taxa, divided into 10 sections, belong to this genus, most of them having a very local distribution around the Mediterranean [10]. *Origanum compactum* possesses very compact pinkish flowers and is the endemic oregano in Morocco. Known locally as “zaatar,” it is traditionally considered as a panacea for health. In aqueous infusion, zaatar is traditionally used to treat dysentery, colitis, bronco-pulmonary, gastric acidity, and gastrointestinal diseases [11]. In addition, it has been used as a tonic, aphrodisiac, and appetite stimulant as well as a flavoring and preservative for melted butter [11].

O. compactum essential oil has been shown to possess antioxidant, antibacterial, and antifungal, activities [12,13]. Nevertheless, few studies have evaluated its genotoxic properties. In this context, the aim of this study was to evaluate the genotoxic effect of the essential oil of *O. compactum* using the micronucleus (MN) assay and proliferation index (PI). To our knowledge, this is the first study exploring the genotoxicity of essential oil of *O. compactum* on human peripheral blood lymphocytes.

METHODS

Essential oil

The essential oil used in the current study was produced by Santis Company. It was extracted by steam distillation from the flowers, leaves, and stems of *O. compactum*. Samples were collected during the months of April, May, June, and July 2009 in Taounat, Morocco. The chemical composition of the essential oil was determined in our previous work [14]. The major components were carvacrol (43.97%), p-cymene (17.78%), and thymol (11.56%).

MN assay

Human peripheral blood cells were used as a test system. Peripheral venous blood was collected from healthy, non-smoking persons aged 25 years. Donors provided informed consent for the participation in this study. Cells were cultured according to a slightly modified standard protocol for the MN test [15]. 0.5 mL of whole blood was cultured under sterile conditions in a culture tube containing 5 mL of RPMI 1640 medium supplemented with fetal bovine serum, 1% phytohaemagglutinine, and 1% antibiotics (penicillin/streptomycin). Essential oil was dissolved in dimethyl sulfoxide (DMSO). Because longer exposure times caused considerable inhibition of cell division, the essential oil of *O. compactum* was added 24 hrs after culture initiation. 100 µL of each dilution was added to the culture medium to produce the following final concentrations of essential oil: 32×10^{-4}, 16×10^{-4}, 8×10^{-4}, 4×10^{-4}, 2×10^{-4}, 1×10^{-4} or 0.5×10^{-4} µL/mL. A solvent control (DMSO) was also included. Cultures were incubated at 37°C for 72 hrs. 28 hrs before harvest, 0.1 mL of cytochalasin-B was added

Table 1: Proliferation index and distribution of micronuclei in binucleate lymphocytes after treatment with *O. compactum* essential oil

Concentration of EO (μL/mL)	Number of binucleated cells analyzed	Binucleated with 1 MN	Binucleated with 2 MN	Binucleated with 3 MN	Total number of MN	PI
0	1000	0	0	0	0	1.53
0.5×10^{-4}	1000	0	0	0	0	1.52
1×10^{-4}	1000	0	0	0	0	1.52
2×10^{-4}	945	1	0	0	1	1.50
4×10^{-4}	1000	2	0	0	2	1.52
8×10^{-4}	800	2	0	0	2	1.45
16×10^{-4}	788	2	0	0	2	1.44
32×10^{-4}	655	1	0	0	1	1.40

O. compactum: Origanum compactum, MN: Micronucleus, , EO: Ethylene oxide

to each culture. Cells were harvested by centrifugation (800 rpm, 8 minutes), and pellets were resuspended in a hypotonic solution of 0.075 M KCl at 4°C. The cells were again centrifuged and fixed in a methanol: Acetic acid (3:1) mixture. The fixation procedure was applied three times. Formaldehyde was added to the first fixative to preserve the cytoplasm. Slides were produced by dropping concentrated cell suspensions onto slides and then air drying. Slides were stained with 5% Giemsa (pH 6.8), washed in distilled water, and dried at room temperature. MN analysis was performed on coded slides by scoring 1000 binucleate lymphocytes for each concentration of essential oil. The identification of MN was carried out according to Fenech [16]. The PI was calculated according to the following formula [17]:

$$PI=\frac{(1\times N1)+(2\times N2)+(3\times N3)+(4\times N4)}{1000\,Analyzed\,cells}$$

Where *N*1-*N*4 are the number of cells with 1-4 nuclei, respectively.

Statistical analysis

Statistical analysis was performed by linear regression, Pearson correlation and one-way ANOVA using SPSS 20.0 software.

RESULTS AND DISCUSSION

In this study, we investigated the genotoxic potential of essential oil from *O. compactum* using the MN assay. The MN test is one of the preferred methods for assessing DNA damage at the chromosome level because it enables both chromosome loss and chromosome breakage to be measured reliably [18]. The MN test has become one of the most commonly used methods for genotoxicity testing and biomonitoring populations at risk [19-21]. This test has been recommended for monitoring in product development and regulatory testing of new drugs [22].

The treatment of lymphocytes with increasing concentrations of *O. compactum* essential oil-induced the appearance of micronuclei (Table 1). In fact, micronuclei first appeared at an essential oil concentration of 2×10^{-4} μL/mL. The number of micronuclei was stable for the concentrations of 8×10^{-4} and 16×10^{-4} μL/mL but decreased at an essential oil concentration of 32×10^{-4} μL/mL. This decrease can be explained by the decrease in cell viability. PI values were also influenced by the concentration of essential oil. The lowest PI value was observed in cultures treated with 32×10^{-4} μL/mL essential oil indicating high genotoxicity.

The PI values were negatively correlated with the concentrations of essential oil (Table 2), while a positive correlation was observed between the number of MN and essential oil concentrations. There was no significant correlation between MN frequency and PI ($p>0.05$).

Our results did not accord with the results previously shown by other authors using different assays; which demonstrated the absence of the genotoxicity of essential oils from different *Origanum* species. In fact, mutagenic and antimutagenic activities of *O. compactum* were investigated by the somatic mutation and recombination test (SMART)

Table 2: Correlation analysis of the PI and number of MN in lymphocytes after treatment with *O. compactum* essential oil

Factors tested	Pearson correlation	p
Concentration/PI	−0.931	p<0.001
Concentration/number of MN	0.499	NS
PI/Number of MN	−0.510	NS

NS: Not significant. *O. compactum: Origanum compactum*, MN: Micronucleus, PI: Proliferation index

test in *Drosophila melanogaster*. The essential oil did not show any significant increase in the number of somatic mutations, as measured by the standard and high bio-activation cross. *O. compactum* essential oil also showed a strong inhibitory effect against urethane-induced mutagenicity. However, a weak inhibitory effect on the mutagenicity induced by methyl methanesulfonate was observed [23].

Furthermore, it was reported that *Origanum onites* L. oil has strong antimutagenic activity in TA98 and TA100 strains of *Salmonella typhimurium*. Inhibition of mutagenicity was around 40-50% for TA98 and 60% for TA100 in the presence or absence of the metabolic activity. This essential oil strongly reduced the mutagenicity induced by 4-nitro-o-phenylenediamine and 2-aminofluorene in the absence and presence of metabolic activation [24]. In other studies, *O. onites* L. and *O. minutiflorum* have not shown any significant genotoxic effect using the SMART in *D. melanogaster*. Moreover, these essential oils were able to abolish the genotoxic effects induced by potassium dichromate ($K_2Cr_2O_7$) and cobalt chloride ($CoCl_2$) [25]. Similar results were obtained in other studies. In fact, the antimutagenic effect of *O. compactum* might be related to the presence of some major components such as carvacrol and thymol. It was reported that thymol and carvacrol did not induce DNA stand breaks at any concentration using the standard comet assay [26]. In human lymphocytes, carvacrol induced DNA damage at concentrations >0.05 mM, and thymol induced DNA damage at higher concentrations (0.2 mM). Below the DNA damaging concentrations, carvacrol, and thymol may protect against DNA-damage-induced by mitomycin C and 2-amino-3-methylimidazo [4,5-f]-quinoline [27,28]. Similarly, carvacrol and thymol have been reported to reduce the number of DNA lesions induced by H_2O_2 in human hepatoma HepG2 and colonic Caco-2 cells [29]. However, in other studies, the genotoxic potential of carvacrol and thymol was reported to be very weak in the Ames assay and negative in the SOS chromotest [30].

The genotoxicity of essential oils can vary depending on the organism and the genotoxic assay used. Moreover, possible antagonistic effects of compounds in the oil should also be taken into consideration.

CONCLUSION

In this study, we investigated the genotoxic effect of *O. compactum* essential oil by the MN test. The tested essential oil increases the number of micronuclei in a dose dependent manner. Further studies are necessary to confirm these finding.

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