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Antigenotoxic Properties of Different Plant Oils and the Influence of Olfactory Bias

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ABSTRACT

In this study, antigenotoxic effects and developmental toxicity of frankincense and blue anemone oils were aimed to be analysed and the olfactory bias was aimed to be checked to see the possibility of an interaction between the olfactory perception and antigenotoxicity of the plant oils. The somatic mutation and recombination test was used to analyze genotoxicity, developmental process of *Drosophila melanogaster* was screened and the feeding assay was used to perform an olfactory bias test. Genotoxicity test results showed that none of the oils affected the spot frequencies compared to negative control and they caused 73.3 - 100 % inhibitions after the cotreatment with H_2O_2. None of them caused any significant difference in puparation and eclosion. The frankincense and blue anemone oils were also found antigenotoxic in this study and these effects were independent from the olfactory perception because the rates of feeding were similar to the one observed with negative control.

Keywords: Plant oils, Somatic mutation and recombination test, Drosophila melanogaster, Olfactory perception.

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Introduction

Plant oils which are mainly isolated from different part of the plants like fruits, seeds and pulps are used in cooking, cosmetic products and health supplements [1]. They are considered as the important part of the human nutrition because they account for 75% of daily dietary lipids worldwide [2]. The nutritional value is very critical considering the fact that the essential fatty acids found in plant oils are crucial for the healthy function of the human body and the developmental pathways [2]. Biomaterial industry also takes the advantage of plant oils because they are considered as renewable resources and their unique structures improve the biomaterial characteristics [3]. Plant oils are composed of triglycerides which are the esters of three fatty acids with a glycerol and the fatty acid chain contains unique functional groups affected by the type and growth conditions of the plant [4]. They are rich in important phytochemicals like tocopherols, carotenoids, phenolic compounds, sterols, minerals and vitamins [2]. The cold pressed plant oils are especially known to have antioxidant phenolic compounds and they were shown to prevent inflammations, hyperlipidaemia, allergic reactions and oedema [5]. According to the previous findings, plant oils like canola, peanut, sesame and olive oils were found protective against type two diabetes and inflammations in addition to their antioxidant and anticarcinogenic properties [6-10]. The plant oils can be categorized as fixed and essential oils. The plant seeds are known to contain fixed oils in addition to proteins, sugars, mucilage, organic acids, alkaloids, tannins, minerals, vitamins etc. [11]. Fixed oils are considered as the provider of energy, essential fatty acids and fat soluble vitamins for body so they are the important components of the immune system [12]. On the other hand, essential oils are the complex mixtures of low molecular weight compounds extracted from plants by solvent extraction or distillation methods. They contain terpenoids and phenylpropanoids responsible for the biological properties of the plants.

Several essential oils isolated from the plants are known to have antimicrobial/viral, anticancer/mutagenic, antidiabetic and antiinflammatory properties [13]. Therefore, the evaluation of the nutritional and health impacts of these oils is very important considering the fact that the ease of the access to the public. In this study, the toxicologic effects of two different plant essential oils (frankincense (*Boswellia sacra* Flück.) (Burseraceae) and blue anemone (*Anemone apennina* Auct. Orient. ex Boiss.) (Ranunculaceae) essential oils) and the influence of olfactory bias were aimed to be investigated. Frankincense (*Boswellia sacra* Flück.) oil was chosen because of the beneficial properties of the *Boswellia* species reported before. It is known that frankincense is a medicinal plant with anti tumorigenic, anti inflammatory properties and have been used traditionally against asthma and wounds. The *Boswellia* species are known to have terpenes and boswellic acids that are responsible for the medical properties for the traetment of age-related disorders, neurorecovery, skin disorder, cancer and depression [14, 15]. In addition, the clinical studies have shown the antiinflammatory properties of these plants

[15]. In another study, cardiac, genetic, hepatic and neuromuscular toxicity of *Frankincense* essential oil from *Boswellia sacra* using the zebrafish embryo were analysed and it didn't show any toxicity on zebrafish [16]. The frankincense plant also showed antigenotoxic properties when it was used in a gum form [17]. However, it is the first time that antigenotoxic effects and developmental toxicity of the frankincense oil were analysed in this study using *Drosophila melanogaster* model organism and the olfactory bias was checked to see the possibility of an interaction between the olfactory perception and antigenotoxicity of the plant oil. The blue anemone (*Anemone apennina* Auct. Orient. ex Boiss.) (Ranunculaceae) essential oil was also chosen in this study because of its characteristics reported before. *Anemone* genus members have been used against cancer, microbial infections and inflammations in addition to their sedative, analgesic, anti-convulsant and anti-histamine properties [18-20]. It was also shown that the triterpenoids and saponins found in those plants could be responsible for their anti-cancer activities [21]. In fact, researchers provided the evidence that triterpenoid saponins isolated from *Anemone flaccida* induced apoptosis by COX-2/PGE2 pathway in HeLa cancer cells [21]. In another study, *Anemone nemorosa* L. extracts, a close relative of *Anemone apennina* Auct. Orient. ex Boiss., showed high antioxidant activities (0.1 - 5 µg [GAE] per 1 mL sample) and cytotoxic activities against Caco-2 cancer cells [22]. However, there isn't any study about the genotoxicity of blue anemone oil in literature and it is the first time that antigenotoxic effects and developmental toxicity of the blue anemone oil were analysed in this study using *D. melanogaster* model organism and the olfactory bias was checked to see the possibility of an interaction between the olfactory perception and antigenotoxicity of the plant oil.

A compound that can cause genetic damage is regarded as a genotoxic agent and there are many bioactive substances counteracting the effects of these genotoxic compounds in nature [23]. These bioactive compounds are present in many plants abundantly and effective against genotoxicity [24]. Many plant oils have antigenotoxic properties and this was generally attributed to the antioxidant activities of their phytochemical compositions [23]. Therefore, the antigenotoxicity studies should be performed for all of the plant oils available on the market to ensure that existing medicinal potentials are not overlooked.

One of the important antigenotoxicity tests is somatic mutation and recombination test (SMART). It is based on the loss of heterozygosity caused by genotoxic damage resulting in mutated wing hair patterns on *Drosophila melanogaster* [25]. *D. melanogaster* is a frequently used model organism in biomedical sciences because approximately 75% of the genes responsible for human diseases have homologs in this organism and the short observation time is needed for screening the entire developmental process [26, 27]. Thus, antigenotoxic effects of frankincense and blue anemone oils were analyzed in this study by using SMART. In addition, developmental toxicity was analyzed to evaluate the effects of plant oils on normal developmental process of *D. melanogaster*.

The influence of the olfactory bias was also analyzed in this study to show the influence of the odor evoked preference on the antigenotoxicity of plant oils. Olfactory bias means a prejudice in favor of or against a specific olfactory perception which makes an organism aware of an odor through nose, sensory neurons and cerebral centers [28]. In traditional medicine, the sense of odor is very important for humans and the aromatic essential oils have been used for a long time as painkillers, anxiety relievers and energy boosters [29]. According to the previous findings, a strong relationship between the odor and oxidative stress was also observed [30]. The researchers found that the antioxidant activity was increased after inhalation of some plant odors [29, 30]. Not only humans but also most of the terrestrial animals use olfactory perception for the detection of dangers, nutritional sources or available mating partners and *D. melanogaster* is one of those animals which is accepted as an excellent model organism having a simple version of the olfactory system [31]. Olfactory bias can allure organisms to specific compounds and this may increase or decrease the appetite, so the protective effects of the compounds can be seen better or worse. Therefore, the independency of the antigenotoxic effects of the plant oils from the behavioural effects should be examined. In this study, feeding assay was used to perform an olfactory bias test to be able to check the existence of an interaction between the olfactory perception and antigenotoxicity of the plant oils.

Material and Methods

Materials

H_2O_2 and Brilliant Blue For Coloring Food (FCF) dye were purchased from Merck (Sigma Aldrich). The plant oils were purchased from local bazaar. The frankincense (*Boswellia sacra* Flück.) (Burseraceae) essential oil was obtained by cold pressing method and it complies with Turkish food codex (TR-34-K-000495/ 700 14 060). The blue anemone (*Anemone apennina* Auct. Orient. ex Boiss.) (Ranunculaceae) essential oil was obtained by water vapor distillation method and it complies with Turkish food codex (TR-34-K-000495/ 700 14 094).

Model Organisms and Their Growth Conditions

Wild type (*Oregon R*), flr^3 (flr^3/ *In (3LR) TM3* Bd^s) and *mwh* (*y; mwh j*) strains of *D. melanogaster* used in this study. The flies were kept at 22 °C. The growth media was prepared according to the classical method [32, 33]. In order to prepare the medium, 8.6 % sugar (w/v), 1.8 % agar (w/v), 18 % semolina (w/v), 5 % yeast (w/v), 0.001 % antifungal drug (v/v, MikostatinDeva Holding, 228/97) and 1 % propionic acid (v/v) were dissolved in dH_2O.

Somatic Mutation and Recombination Test (SMART)

SMART was performed using previous protocols in literature [34, 35]. For this test, *flr3* female and *mwh* male flies were crossed and 72 ± 4 hours later third instar larvae with *mwh+/+flr3* genetic background were removed from the vial and washed with dH_2O. 50 larvae were added into the experimental bottles prepared with growth medium containing plant oils (5 % v/v) or H_2O_2 (6.5 mg/L) and the negative control group was prepared without adding anything into the medium. The cotreatment group was prepared by adding plant oils (5 % v/v) with H_2O_2 (6.5 mg/L) into the same medium. When they became adults, the wings were collected and hair patterns were investigated using light microscope (400x). Genotoxicities were determined by the the frequencies of spots per wing (*Fr.)*. The spots were named as single spots (*mwh* or *flr* phenotype) or twin spots (both mutated clones adjacent). *Fr.* values were calculated by dividing the number of spots (n) by the number of wings (N). [34-36]. Inhibition % values were also calculated for the cotreatment group using equation 1 [37, 38].

$$\text{Inhibition \%} = \frac{(\text{Fr.of H2O2 Group} - \text{Fr.of Cotreatment Group})}{\text{Fr. of H2O2 Group}} \text{x}100 \quad (1)$$

Developmental Toxicity Test

Developmental toxicity test was performed to screen the developmental process from the larval to adult stages of wild type *D. melanogaster*, *in vivo* [31, 39-42]. For this test, first instar larvae were added into the growth medium composed of plant oils (5 % v/v) or H_2O_2 (6.5 mg/L). The negative control group was prepared without adding anything into the medium. The cotreatment group was prepared by adding plant oils (5 % v/v) with H_2O_2 (6.5 mg/L) into the same medium. The puparation, eclosion and survival % were calculated by the equations 2, 3 and 4 [31, 39-42].

$$\text{Puparation \%} = \frac{\text{Pupae \#}}{\text{Larvae \#}} \text{ x } 100 \quad (2)$$

$$\text{Eclosion \%} = \frac{\text{Adult \#}}{\text{Pupae \#}} \text{ x } 100 \quad (3)$$

$$\text{Survival \%} = \frac{\text{Adult \#}}{\text{Larvae \#}} \text{ x } 100 \quad (4)$$

Olfactory Bias Test

Olfactory bias test (feeding assay) was performed according to the previous studies [31]. In order to perform this test, blue-growth medium containing 0.5 % (w/v) Brilliant blue FCF dye was prepared for this study. 50 larvae were added into the blue-growth medium composed of plant oils (5 % v/v) or H_2O_2 (6.5 mg/L). The negative control group was prepared without adding anything into the blue-growth medium. The cotreatment group was prepared by adding plant oils (5 % v/v) with H_2O_2 (6.5 mg/L) into the same blue-growth medium. The larvae were fed on those media for 30 minutes and washed in sterile serum physiologic solution. Then, the larvae were dried by filter paper and frozen overnight at −80 °C. The absorbances showing a direct relationship with the amount of blue-growth media ingested were measured at 625 nm.

Results

SMART Results

In Table 1 and Table 2 SMART results are given. According to the results, it was observed that an oxidative agent, H_2O_2, caused important small single, large single and twin spot frequency increases ($p<0.05$). On the other hand, frankincense and blue anemone oils didn't affect the spot frequencies compared to negative control ($p>0.05$). Cotreatment groups also showed similar results. None of the cotreatment group significantly affect the spot frequencies. In addition, inhibition % values calculated with the cotreatment group SMART results given in Table 2 showed that frankincense and blue anemone oils caused 73.3 - 100 % inhibitions.

Table 1. SMART results

Treatments	Small single spots		Large single spots		Twin spots	
	Fr.	SE	*Fr.*	SE	*Fr.*	SE
Negative Control	1.33[b]	0.33	0[b]	0	0[b]	0
H_2O_2 (6.5 mg/L)	5[a]	0.58	4.33[a]	0.67	10[a]	0.58
Frankincense oil	0.67[b]	0.33	0.67[b]	0.33	0[b]	0
Blue anemone oil	0.67[b]	0.33	0.33[b]	0.33	0[b]	0
Frankincense oil + H_2O_2	1[b]	0	0[b]	0	0.33[b]	0.33
Blue Anemone Oil + H_2O_2	1.33[b]	0.33	0[b]	0	0[b]	0

[a] $p< 0.05$, in contrary to negative control
[b] $p< 0.05$, in contrary to H_2O_2
SE: Standard error, *Fr.*: Frequency
Plant oil concentration: 5 % v/v, H_2O_2 concentration: 6.5 mg/L

Table 2. Spot inhibition % values of the cotreatments

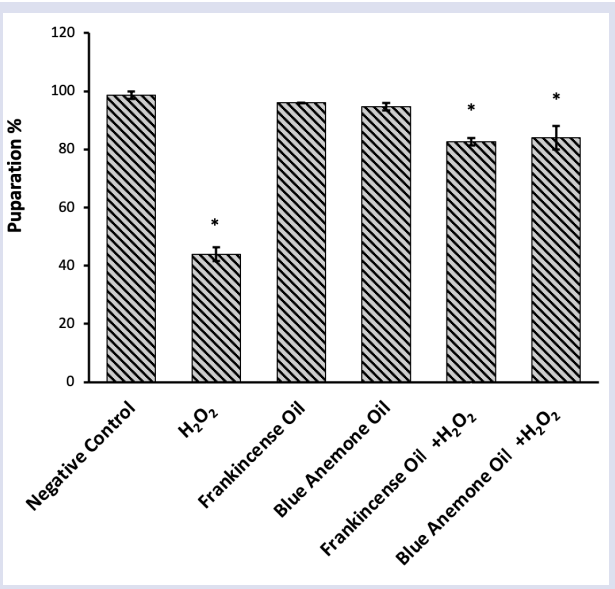
Treatments	Small single spots	Large single spots	Twin spots
	Inhibition %	Inhibition %	Inhibition %
Frankincense oil + H_2O_2	80	100	96.7
Blue Anemone Oil + H_2O_2	73.3	100	100

Plant oil concentration: 5 % v/v, H2O2 concentration: 6.5 mg/L

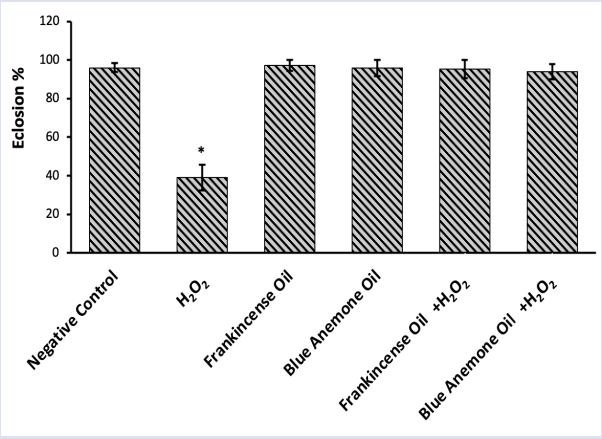
Developmental Toxicity Test Results

The developmental toxicity test results are given in Figure 1. The results showed that H_2O_2 caused important decreases in puparation, eclosion and survival %. The frankincense and blue anemone oils didn't cause any significant difference in puparation, eclosion and survival %. When the cotreatment groups are examined, it was observed that the frankincense and blue anemone oils

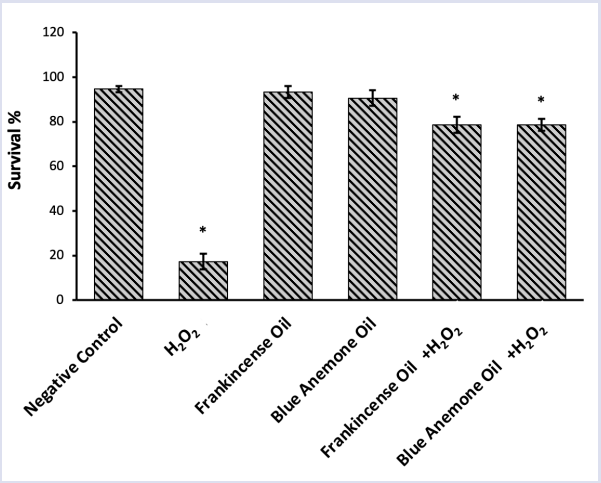
caused significant decreases in puparation and survival % ($p< 0.05$, Figure 1 (a)(c)). On the other hand, none of the oils cotreated with H_2O_2 caused any significant decrease in eclosion % ($p>0.05$, Figure 1 (b)).



(a)



(b)



(c)

Figure 1. Developmental toxicity test results

Olfactory Bias Test Results

The olfactory bias test results are given in Figure 2. The absorbances showing the rate of feeding observed with H_2O_2, frankincense oil and blue anemone oil were similar to the one observed with the negative control ($p>0.05$).

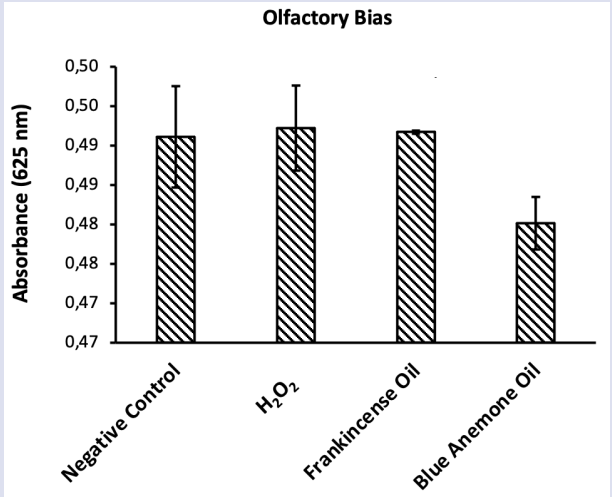


Figure 2. Olfactory bias test results

Discussion

The potential antigenotoxic effects of the frankincense oil and blue anemone oil were analysed in this study by using SMART assay. The results given in Table 1 and 2 showed that all of the plant oils used in this study was non-genotoxic. It was also clearly seen that the frankincense and blue anemone oils were able to inhibit the genotoxic effects of H_2O_2 (inhibition % of the cotreatment groups were between 73.3 - 100 %). Therefore, the frankincense and blue anemone oils can be considered as antigenotoxic oils.

As an additional toxicity assessment, the developmental toxicities were examined. The results given in Figure 1 showed that none of the plant oils studied here showed any detrimental effect on puparation and eclosion processes of *D. melanogaster*. However, according to the results of the cotreatment groups it was clearly observed that plant oils couldn't protect the organism's puparation process and survival % from the harmful effects of H_2O_2 (Figure 1(a) (c)).

An olfactory bias test was also analysed in this study to be able to check the existence of an interaction between the olfactory perception and antigenotoxicity of the plant oils. According to the results given in Figure 2 it was observed that H_2O_2, frankincense oil and blue anemone oil didn't affect the feeding rate positively or negatively ($p>0.05$). Therefore, it can be said that there isn't any olfactory bias against those plant oils.

When the assay results obtained in this study were combined, it was clearly observed that the frankincense oil and blue anemone oil were antigenotoxic and, most importantly, these effects were independent from the olfactory perception. According to the developmental toxicity test all of the plant oils were safe. In previous findings, it was also observed that frankincense oil had

anti-cancer (melanoma) activities *in vitro* and *in vivo*, while protecting the normal human epithelial melanocyte cell growth [14]. The researchers also showed that frankincense plant in a gum form was able to decrease the nuclear abnormalities and cytotoxicity observed as a result of tobacco smoking [17]. There isn't any study about the genotoxicity of blue anemone oil in literature but there are some studies about the other members of the *Anemone* genus. For example, *Anemone nemorosa* L. extracts showed antioxidant and antiproliferative activities on cancer cells (Caco-2) [22].

Conclusions

In this study, antigenotoxic effects of the frankincense and blue anemone oils were analysed by SMART, developmental toxicity was analysed to evaluate the effects of plant oils on normal developmental process of *D. melanogaster* and feeding assay was used to perform an olfactory bias test to check the existence of an interaction between the olfactory perception and antigenotoxicity of the plant oils. According to the results, all of the plant oils used in this study was non-genotoxic and none of the plant oils studied here showed any detrimental effect on puparation and eclosion processes of *D. melanogaster* while they couldn't protect the organism's puparation process and survival % from the harmful effects of H_2O_2. In addition, there wasn't any olfactory bias against frankincense oil and blue anemone oil. To conclude, the frankincense oil and blue anemone oil were found antigenotoxic in this study and, most importantly, these effects were independent from the olfactory perception.

A lot of plant oils have been introduced to the market recently while the data on the comprehensive toxicologic potential of most of them have not been reported. The evaluation of the nutritional and health impact of these oils is very important considering the fact that the ease of the access to the public. In this study, antigenotoxic effects of some plant oils (the frankincense and blue anemone oils) were analysed. In future, the medical potentials of these plant oils should be evaluated more.

Conflicts of interests

The author reports no conflicts of interest.

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