Cytogenetic, cytotoxic and GC–MS studies on concrete and absolute oils from Taif rose, Saudi Arabia

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Abstract Taif rose (*Rosa damascena trigintipetala* Dieck) is a sort of damask rose, which is considered as one of the most important economic products of Taif. In this study, the authors investigated the possible cytotoxic, genotoxic, antimutagenic and anticancer effect of concrete and absolute rose oils. The results showed that both concrete and absolute rose oils were cytotoxically and genotoxically safe at a dose of 10 µg/ml when tested on cultures of normal human blood lymphocytes. Also, the results showed significant antimutagenic activity at $p < 0.001$ for absolute rose oil at the same dose level when tested on cultures of normal human blood lymphocytes supplemented with 300 ng/ml mitomycin C (MMC). On the other hand, concrete and absolute oils exerted a cytotoxic activity against two kinds of human cancer cell lines: HepG2 and MCF7. Concrete oil showed cytotoxic activity against HepG2 and MCF7 with a half maximal inhibitory concentration (IC₅₀) of 16.28 and 18.09 µg/ml, respectively, whereas absolute rose oil showed its cytotoxic

activity against HepG2 and MCF7 with an IC₅₀ of 24.94 and 19.69, respectively. From this study, it is concluded that concrete and absolute rose oils are cytotoxically and genotoxically safe at a dose of 10 µg/ml when tested on cultures of normal human blood lymphocytes. In addition, absolute oil has an antimutagenic activity at the same dose. Further investigations are needed to study the activity of higher doses of both oils in vitro and in vivo in experimental animals in order to evaluate the capability of using these oils as therapeutic for treatment of some kinds of cancers.

Keywords Mutagenic · Antimutagenic · HepG2 · MCF7

Introduction

A carcinogen is a substance that is capable of causing cancer in human and animal. Carcinogenic substances can be classified into physical, biological and chemical ones. Chemical carcinogens are toxic substances, which are organ specific, target epithelial cells, and cause genetic damage (genotoxic) (Kumar et al. 2010). Today, several industrial chemicals were identified as risk factors for cancer e.g. arsenic, nickel, chromium, benzene, and polycyclic aromatic hydrocarbons, etc. In addition, the role of life style factors such as tobacco smoking and alcohol consumption in the development of cancer is evident from numerous epidemiological and experimental studies (Weinstein 1991).

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To overcome the effect of these chemical carcinogens, it is recommended that our work should focus on two approaches. The first approach is to decrease the amount of these carcinogens. The second approach is the use of antimutagens and anticarcinogens in our daily life because it is the most effective procedure to prevent human cancer and genetic diseases. Dietary antioxidants are antimutagens and anticarcinogens which decrease the highly genotoxic and mutagenic effect of reactive oxygen species (ROS) and reactive nitrogen species in normal function in human (Mantena et al. 2008; Cornelli 2009). Hence, there is increasing demand for natural antioxidant molecules to replace the synthetic additives used in the food industry (Vatan et al. 2011). Therefore, a new approach is the detection of naturally occurring and biologically active substances with genoprotective and anticancer properties that can be used in pharmaceutical and food industries. In fact, plants and herbs are considered as the main sources of these genoprotective and anticancer substances. Antimutagenic activity was detected in a number of plant oils such as cape gooseberry, corn, sunflower, sea-buckthorn, rice, beech, olive, rose, wheat embryo, pumpkin seeds, etc. (Agabeyli and Mirzazadeh 2011).

Roses are important ornamental plants and have been referred to as the queen of flowers. Over 150 rose species and more than 2,000 cultivars have been registered and are spread in Europe, Asia, Middle East, and North America (Cai et al. 2005). Members of the Rosaceae family have long been used in perfumes, cosmetics, foods, and for medicinal purposes. The Taif rose (*Rosa damascena trigintipetala* Dieck) is a sort of damask rose which is considered as one of the most important economic products of Taif. The essential oil of Taif rose has an excellent reputation as perfume. In this study, the authors investigated the possible cytotoxic, genotoxic, antimutagenic and anticancer effect of concrete and absolute oils of Taif rose. In addition, major components of Taif concrete and absolute rose oils have been analyzed using GC–MS spectroscopy technique.

Materials and methods

Collection of roses and preparation of concrete and absolute rose oil

The fresh roses were collected from a rose farm (April 2011) in the Taif governorate. The green parts of roses

were removed and the remaining was cut into small pieces. The fresh cut petals of roses (250 g) were defatted by soaking in 1,500 ml *n*-hexane (Sigma-Aldrich, St. Louis, MO, USA) for 1 week at room temperature with shaking from time to time followed by filtration. The *n*-hexane was removed under vacuum yielding rose concrete (soft as a wax material with rose-like odour). This extraction process was repeated four times. The absolute rose oil was prepared by stirring the concrete oil with 20-fold weight of ethyl alcohol (Sigma-Aldrich). The mixture was cooled at -20°C for 6 h followed by filtration. The alcoholic solution was evaporated under vacuum resulting in absolute rose oil (semiliquid material also with rose-like odour). The process was repeated four times. The two oils were stored at -20°C in brown glass vials.

Viability assay

Preheparinized syringe was used to collect 10 ml of venous blood from healthy, non-smoking and non-alcoholic persons who were tested to be free of viruses and common diseases. Lymphocytes were separated according to McFee et al. (1997) and counted. Cells were cultured at a concentration of one million/ml complete medium. Complete medium was set up by adding 4 ml of RPMI-1640 with L-glutamine (Biochrom AG, Berlin, Germany), 1 ml fetal calf serum (Biochrom AG), 100 μl penicillin/streptomycin (100 $\times$) (Biochrom AG), 100 μl antimycotic (100 $\times$) (Biochrom AG) and 25 μl preservative heparin (5,000 U/ml) (Biochrom AG) per culture tube. Culture tubes were divided into three groups. The first and second groups of culture tubes were treated with different doses of concrete and absolute oils, respectively (5, 10, 50, 75 and 100 $\mu\text{g/ml}$). The third group of culture tubes was used as untreated control. Lymphocytes were incubated at 37°C for 72 h. Culture tubes were centrifuged at $115\times g$ for 10 min (Mikro 22R, Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany). The concentration of lymphocytes in the sample was adjusted to be less than 10^4 cells/ μl ($10^{10}/\text{l}$) using phosphate buffer saline (Biochrom AG). Twenty microliter of 7-amino-actinomycin D (7-AAD) dye solution (Immunotech S.A., Beckman Coulter Company, Marseille, France) and 100 μl of the test sample were added to each test tube and mixed well. Test tubes were incubated for 15–20 min at room temperature ($18\text{--}25^{\circ}\text{C}$), protected from light. Preparations were

analyzed within 1 h on Beckman Coulter EPICS XL flow cytometer (Beckman Coulter Company, Miami, FL, USA) using 620 or 675 nm “band pass” filter (Schmid et al. 1992; Fetterhoff et al. 1993). The relative viability percent was calculated according to the following equation:

$$\text{Relative viability (\%)} = [\text{viability of each tested dose} / \text{viability of control}] \times 100$$

Genotoxicity/antimutagenicity assays

Heparinized peripheral blood obtained from ten healthy non-smoking and non-alcoholic donors (4 females and 6 males) were used in this experiment. Lymphocytes were prepared as mentioned in viability assay and cultured in the same complete medium supplemented with 96 µg/ml phytohemagglutinin (PHA-L) (Biochrom AG). Lymphocytes of each donor were divided into six culture tubes, the first was left as control, the second was treated with 10 µg/ml rose concrete oil, the third was treated with 10 µg/ml rose absolute oil, the fourth was treated with 300 ng/ml mitomycin C (MMC) (Sigma-Aldrich) as a mutagenic agent for induction of oxidative and chromosomal damage, the fifth was treated with 10 µg/ml rose concrete oil and 300 ng/ml MMC and finally the last tube was treated with 10 µg/ml rose absolute oil and 300 ng/ml MMC. All culture tubes were incubated at 37 °C for 72 h. Metaphases were obtained by adding colcemid (0.4 µg/ml) (Biochrom AG) 2 h prior to harvesting. Cells were centrifuged at 115×*g* for 10 min, then cells were suspended in 5 ml of pre-warmed (37 °C) 0.075 M KCl (hypotonic solution) (Merck Chemicals, Darmstadt, Germany) and incubated at 37 °C for 30 min. Cells were centrifuged at 115×*g* for 10 min, then suspended in 5 ml of fixative (methanol:glacial acetic acid; 3:1) (Merck) and were incubated at room temperature for 10 min. The supernatant was removed after centrifugation for 10 min at 115×*g*, the cells were suspended in another fixative, and the step was repeated three times. To prepare slides, 2–3 drops of fixed cell suspension were dropped on clean slides, stored in cold ethanol and then they were flame dried. Slides were stained in 4 % Giemsa solution (Sigma-Aldrich) in phosphate buffer (pH 6.8) for 7–10 min and microscopically screened (Savage 1975; Pereira et al. 1988).

For each culture, 25 metaphases were examined microscopically for chromosomal aberrations and the ratio of metaphase to interphase nuclei in 1,000 cells for determining the mitotic index. Statistical analysis was performed using Student *t* test.

Anticancer activity

The effect of concrete and absolute oils of the Taif rose were investigated in vitro on two kinds of human cancer cell lines: liver carcinoma cell line (HepG2) and breast carcinoma cell line (MCF7) [Obtained frozen in liquid nitrogen (−180 °C) from the American Type Culture Collection (Manassas, VA, USA) and were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing] using Sulphorhodamine-B (SRB) assay as described by Skehan et al. (1990). The SRB assay is a colorimetric method and cell numbers are indirectly estimated by staining total cellular protein with the SRB dye (Sigma-Aldrich). This dye is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under moderate acidic conditions to provide a sensitive index of cellular protein content. Cells were seeded in 96-well microtiter plates at a concentration of 5×10^4 – 10^5 cell/well in a fresh medium and left to attach to the plates for 24 h. Wells were prepared for each individual sample and incubated for 48 h at 37 °C in 5 % CO₂. After 24 h, cells were incubated with the appropriate different concentrations of the fraction under test (0, 5, 12.5, 25 and 50 µg/ml), completed to total of 200 µl volume/well using fresh medium and incubation was continued for 24, 48 and 72 h. Following 24, 48 and 72 h treatment, the cells were fixed with 50 µl cold 50 % trichloroacetic acid (Sigma-Aldrich) for 1 h at 4 °C. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid (Sigma-Aldrich). The wells were then washed 4 times with 1 % acetic acid. The plates were air-dried and the dye was solubilized with 100 µl/well of 10 mM Tris base (pH 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Hamburg, Germany) at 57×*g*. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Metertech Σ 960, Metertech Inc., Taipei, Taiwan). The mean background

absorbances were automatically subtracted and mean values of each sample concentration were calculated.

The experiment was repeated 3 times for each cell line. The percentage of cell survival was calculated according to the following equation:

$$\text{Survival fraction (\%)} = [\text{OD of treated cell} / \text{OD of control cells}] \times 100$$

According to the National Cancer Institute guideline an extract and/or a compound with IC_{50} values $<20 \mu\text{g/ml}$ is considered active (Boik 2001).

GC–MS analysis of concrete and absolute rose oil

The analysis of the samples was performed using gas chromatograph (GC, Model CP 3800, Varian, Walnut Creek, CA, USA) coupled with a mass spectrometer (MS, Model Saturn 2200, Varian) and auto sampler (Model Combi Pal, Varian) system. The separation was done using a VF-5 fused silica capillary column (30 m $\times$ 0.25 i.d. mm film thickness 0.25 μm , Varian). For MS detector, electron impact (EI) ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant low rate of 1 ml/min. Injector and mass transfer line temperature were set at 250 and 300 $^{\circ}\text{C}$, respectively. The oven temperature was programmed for 5 min at 60 $^{\circ}\text{C}$, 60–290 $^{\circ}\text{C}$ at 6 $^{\circ}\text{C}/\text{min}$ and held for 5 min at 290 $^{\circ}\text{C}$, solvent delay time 3 min. The injection of the samples was carried out with the auto-sampler for 1 μl with a split ratio 1/20. The conditions of analysis and specification of the instrument were optimized for a better separation and resolution. Identification of components of concrete and absolute oils was based on matching with Wiley and NIST electronic library.

Results

Viability assay

The results showed a decrease of the percent of relative viability with increasing the dose for both concrete and absolute oils (Table 1). Also, the results showed that concrete oil was less toxic than absolute oil. The doses of 5 and 10 $\mu\text{g/ml}$ of both oils seemed to be non-toxic (depending closely on the same results between treated groups and normal control groups), so we chose 10 $\mu\text{g/ml}$ as a highly safe dose for using in the antimutagenic test.

Table 1 Relative viability percent of human peripheral blood lymphocytes in groups treated with different doses of concrete and absolute Taif rose oils

Agent	Dose ($\mu\text{g/ml}$)				
	5	10	50	75	100
Concrete rose oil	99.3	96.6	82.1	76.2	61.9
Absolute rose oil	100	98.8	77.2	59	42.4

Table 2 The effect of concrete and absolute rose oils on mitotic indices of peripheral blood lymphocytes of both normal control and MMC treated cultures

Group	No. of individuals	MI ($X \pm SE$)
Normal control	10	22.2 ± 4.5
Concrete rose oil	10	18.0 ± 3.7
Absolute rose oil	10	25.8 ± 4.19
MMC	10	$2.9 \pm 1.05^{***}$
Concrete rose oil + MMC	10	$2.6 \pm 1.02^{***}$
Absolute rose oil + MMC	10	$6.1 \pm 1.97^{**}$

MI no. of metaphase/1,000 cell

MMC mitomycin C

$^{**} p < 0.01$, $^{***} p < 0.001$ in relation to normal negative control group

Genotoxicity/antimutagenicity assays

The mitotic index indicated proliferating activity of cultured cells. The mitotic index (mitotic index = number of metaphases per 1,000 observed cells) was determined in all groups. The exposure to 300 ng/ml MMC for 72 h showed high inhibition of mitotic activity at $p < 0.001$. Concrete and absolute rose oils (10 $\mu\text{g/ml}$) did not show differences in the mitotic activity in relation to the non-treated group. Adding 10 $\mu\text{g/ml}$ of concrete and absolute oils to MMC culture showed no improvement in the mitotic activity in case of concrete and a small improvement in the mitotic activity in case of absolute oil (Table 2). Complete inhibition of mitotic activity appeared in culture tubes from three individuals treated with MMC, concrete rose oil + MMC and absolute rose oil + MMC, so the samples of the remaining seven individuals were only used for examining chromosomal aberrations as shown in Table 3.

The number of chromosomal aberrations induced in all groups is shown in Table 3. The frequencies of

Table 3 The effect of concrete and absolute oils on total chromosomal aberrations in human peripheral blood lymphocytes of both normal control and MMC treated cultures

Group	No. of individuals	No. of total chromosomal aberrations/cell ($X \pm SE$) with gaps	No. of total chromosomal aberrations/cell ($X \pm SE$) without gaps
Normal control	10	0.11 ± 0.02	0.05 ± 0.01
Concrete rose oil	10	0.12 ± 0.03	0.04 ± 0.02
Absolute rose oil	10	0.05 ± 0.01	0.03 ± 0.01
MMC	7	$3.49 \pm 0.26^{***}$	$2.98 \pm 0.18^{***}$
Concrete rose oil + MMC	7	$3.04 \pm 0.12^{***}$	$2.55 \pm 0.11^{***}$
Absolute rose oil + MMC	7	$1.67 \pm 0.29^{***} \quad \bullet\bullet\bullet$	$1.54 \pm 0.25^{***} \quad \bullet\bullet\bullet$

MMC mitomycin C

*** $p < 0.001$ in relation to the normal negative control group

••• $p < 0.001$ in relation to the MMC positive control group

different types of chromosomal aberrations were counted in 25 cells per individual for each group. In MMC treated groups, few individuals did not complete the 25 metaphases, and then we calculated the mean of aberrations per the number of counted cells. The results showed no increase in chromosomal aberrations in groups treated with concrete and absolute oils. All groups treated with MMC showed a significant increase in the frequencies of most types of chromosomal aberration and in the frequencies of total chromosomal aberrations with or without gaps. Adding absolute oil to MMC caused a significant decrease in the frequencies of most types of chromosomal aberrations and in the frequencies of total chromosomal aberrations with or without gaps. Each of the treated and control groups showed only one polyploid cell and one pulverized cell, so these two parameters were not suitable for statistical analysis and are not presented in Table 4. Gaps, breaks, deletions and radial forms were the most frequent types of chromosomal aberrations found in MMC treated groups. Several representative metaphase figures revealing different types of aberrations are shown in Fig. 1.

Anticancer agents

Concrete and absolute oils were tested for cytotoxic activity against two kinds of human cancer cell lines; liver carcinoma cell line (HepG2) and breast carcinoma cell line (MCF7). Results showed that both oils were active toward both cell lines (Fig. 2).

GC–MS analysis

According to the extraction process of concrete rose oil, the compounds responsible for the distinguishable odor constitute 53.90 % of the oil and the remaining (46.10 %) is a waxy material as per the GC–MS chromatogram calculation. The GC–MS showed five major aroma compounds in the concrete oil: phenyl ethanol (37.83 %), β -citronellol (8.2 %), geraniol (4.04 %), eugenol (1.48 %) and methyl eugenol (0.72 %). The analysis of GC–MS chromatogram of absolute oil showed that aroma compounds constitute 56.89 % and the waxy material 43.11 % of the oil. The major aroma compounds in absolute oil are: phenyl ethanol (33.31 %), β -citronellol (12.45 %), geraniol (6.28 %), eugenol (2.03 %) and methyl eugenol (1.05 %).

Discussion

7-Amino-actinomycin D (7-AAD) is a cytochemical probe for DNA depending on the ability of 7-AAD to insert itself between the tops of successive cytosine/guanine bases of the DNA double strand, when the interior of the cell and the nuclear chromatin are accessible. Non-viable cells can be characterized by loss of membrane integrity, so they are permeable to 7-AAD and are stained whilst viable cells retaining their membranous integrity are impermeable to 7-AAD and are unstained (Gill et al. 1975). Our results showed a dose dependent decrease in the percentage of viable cells for both oils. The results

Table 4 The effect of concrete and absolute oils on different types of structural chromosomal aberrations in peripheral blood lymphocytes of both normal control and MMC treated cultures

Group	No. of individuals	Number of types of aberration/cell ($\bar{X} \pm \text{DS}$)					
		Gap	Isochromatid gap	Break	Isochromatid break	Deletion	Fragment
Normal control	10	0.056 $\pm$ 0.018	0.004 $\pm$ 0.004	0.04 $\pm$ 0.008	0.004 $\pm$ 0.004	0 $\pm$ 0	0 $\pm$ 0
Concrete rose oil	10	0.064 $\pm$ 0.023	0.008 $\pm$ 0.005	0.008 $\pm$ 0.005**	0 $\pm$ 0	0.016 $\pm$ 0.012	0 $\pm$ 0
Absolute rose oil	10	0.012 $\pm$ 0.009*	0.004 $\pm$ 0.004	0.008 $\pm$ 0.005**	0.008 $\pm$ 0.005	0.004 $\pm$ 0.004	0 $\pm$ 0
MMC	7	0.469 $\pm$ 0.096***	0.034 $\pm$ 0.02	1.999 $\pm$ 0.108***	0.06 $\pm$ 0.036	0.29 $\pm$ 0.06***	0.12 $\pm$ 0.05*
Concrete rose oil + MMC	7	0.441 $\pm$ 0.08***	0.044 $\pm$ 0.016*	1.737 $\pm$ 0.08***	0.024 $\pm$ 0.012	0.041 $\pm$ 0.018**	0.047 $\pm$ 0.016**
Absolute rose oil + MMC	7	0.109 $\pm$ 0.04**	0.039 $\pm$ 0.015*	1.106 $\pm$ 0.199***	0.033 $\pm$ 0.021	0.05 $\pm$ 0.022**	0.017 $\pm$ 0.012
Number of types of aberration/cell ($\bar{X} \pm \text{DS}$)							
Group	Minute	Number of types of aberration/cell ($\bar{X} \pm \text{DS}$)					
		Acentric fragment	Double minute	Radial form	Complex rearrangement	Centromeric attenuation	
Normal control	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.008 $\pm$ 0.008	
Concrete rose oil	0 $\pm$ 0	0.004 $\pm$ 0.004	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.016 $\pm$ 0.009	
Absolute rose oil	0 $\pm$ 0	0.008 $\pm$ 0.005	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.004 $\pm$ 0.004	
MMC	0.08 $\pm$ 0.03**	0.117 $\pm$ 0.046**	0.009 $\pm$ 0.009	0.266 $\pm$ 0.057***	0.04 $\pm$ 0.021*	0 $\pm$ 0	
Concrete rose oil + MMC	0.047 $\pm$ 0.021*	0.289 $\pm$ 0.021***	0.01 $\pm$ 0.01	0.273 $\pm$ 0.027***	0.06 $\pm$ 0.019**	0.02 $\pm$ 0.02	
Absolute rose oil + MMC	0.016 $\pm$ 0.011	0.16 $\pm$ 0.06**	0 $\pm$ 0	0.126 $\pm$ 0.038**	0.031 $\pm$ 0.019	0 $\pm$ 0	

MMC mitomycin C

** $p < 0.01$ in relation to the MMC positive control group* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in relation to the normal negative control group

Fig. 1 **a** Normal metaphase plate from human peripheral blood lymphocytes without any treatment. **b** and **c** are metaphase plates from treated human peripheral blood lymphocytes showing breaks (*b*), fragment (*f*), radial form (*r*), acentric fragment (*af*), minute (*m*), deletion (*d*) and complex rearrangement (*cr*). Human peripheral blood lymphocytes were separated and cultured in complete medium supplemented with phytohemagglutinin incubated at 37 °C for 72 h. Lymphocytes of each donor were divided into six culture tubes, the first one was

left as control and while the other tubes were treated with 10 µg/ml rose concrete oil, 10 µg/ml rose absolute oil, 300 ng/ml mitomycin C (MMC), 10 µg/ml rose concrete oil + 300 ng/ml MMC and 10 µg/ml rose absolute oil + 300 ng/ml MMC, respectively. The cultures were treated with hypotonic solution at 37 °C for 30 min, and then they were centrifuged and the pellets were fixed with methanol glacial trice. The fixed cells were dropped on clean slides, dried, stained with 4 % Giemsa solution, and microscopically screened

revealed that concrete oil is less toxic for normal human peripheral blood lymphocytes than absolute oil. Also, the doses 5 and 10 µg/ml for both oils were considered safe due to the close reading values between normal control and treated groups (percent of relative viability ≥ 95 %), so the dose of 10 µg/ml was chosen for the antimutagenic assay.

Chromosomal aberration assays are considered to be very sensitive end points for recognizing the genotoxic effects induced by chemicals (Sweify et al. 2005). The present data showed no change in the frequency of chromosomal aberrations in groups treated with concrete and absolute oils alone when compared with non-treated group. These results proved that concrete and absolute oils were not genotoxic at the tested dose (10 µg/ml).

Mitomycin C (MMC) is an antitumor antibiotic. The primary action of MMC has been considered to inhibit the deoxyribonucleic acid (DNA) synthesis by cross-linking of the complementary strand of DNA (Iyer and Szybalski 1964). This would inhibit DNA replication and probably might be the antitumor action of this drug. Despite the fact that MMC showed antitumor action, many reports proved that it may cause high frequency of chromosomal aberrations, sister chromatid exchanges and micronuclei formation (Latt 1974; Fauth et al. 2000; Yogesh and Fulzele 2009). In this study, we used this antitumor-antibiotic

drug to test the possible antimutagenic activity of concrete and absolute oils. The study results of MMC in this experiment were coincided with previous reports (Fauth et al. 2000; Yogesh and Fulzele 2009; Unal et al. 2013). The group treated with MMC alone showed a significant elevation of chromosomal damage. In addition, chromatid gaps, chromatid breaks, deletions and radial forms were the most common aberrations found. Adding absolute oil to the culture caused a significant decrease in the frequency of chromosomal aberrations especially with respect to frequency of chromatid gap, chromatid break, deletions and the number of total aberrations. This indicates antimutagenic activity for rose absolute oil. In case of concrete oil, a slight improvement (decrease) in the frequency of chromosomal aberrations had been observed but did not reach significance, so we cannot consider it to have an antimutagenic activity.

Many reports proved that many essential oils have antimutagenic activity e.g. lavender, sage, *Teucrium ramosissimum*, *origanum compactum* and rose essential oils (Evandri et al. 2005; Knezevic et al. 2005; Mezzoug et al. 2007; Sghaier et al. 2010; Agabeyli and Mirzazadeh 2011). As mentioned before the addition of rose concrete oils showed some improvement in the frequency of chromosomal aberrations which is less than for rose absolute oil. This may be due to the

Fig. 2 Cytotoxicity of concrete (a) and absolute (b) rose oils against liver carcinoma cell line (HepG2) and breast carcinoma cell line (MCF7) at different concentrations using Sulphorhodamine-B (SRB) assay. The horizontal axis corresponds to different concentrations of oil and the vertical axis corresponds to percent of survival fraction. SRB is a colorimetric assay estimating cell numbers indirectly by staining total cellular proteins with the dye. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader. The percentage of survival fraction was calculated according to the following equation: Survival fraction (%) = [OD of treated cell/OD of control cells] × 100

higher percent of the compounds responsible for aroma in absolute than concrete rose oil.

Eugenol as one of rose concrete and absolute oils constituents was reported as antimutagenic agent against the mutagenicity caused by tobacco and benzo(a)pyrene (Bhattacharjee and Sengupta 2009; Yokota et al. 1986).

The results demonstrated that concrete and absolute oils were toxic against HepG2 and MCF7 cells. Concrete rose oil showed a more toxic effect in the HepG2 cell line ($IC_{50} = 16.28 \mu\text{g/ml}$) than in MCF7 ($IC_{50} = 18.09 \mu\text{g/ml}$). On the contrary absolute rose oil showed more toxic effects against the MCF7 cell line ($IC_{50} = 19.69 \mu\text{g/ml}$) than HepG2 ($IC_{50} = 24.94 \mu\text{g/ml}$). There are three mechanisms for the action of anticancer drugs; the first is to damage the DNA of the

affected cancer cells; the second is to inhibit the synthesis of new DNA strands and preventing the cell from replicating which allows the tumor to grow; and the third is to stop mitosis or the actual splitting of the original cell into two cells that ultimately halt the progression of the cancer (Ophardt 2003). Rose oil is an essential oil and due to a number of chemical constituents, essential oils apparently have no specific cellular targets (Hussain 2009). The cytoplasmic membrane is passed through easily by essential oils that make it permeabilized and may cause its damage. Also, essential oils have the ability to coagulate the cytoplasm, hence damaging lipids and proteins (Ultee et al. 2000; Burt 2004; Hussain 2009). Injury to the cell wall and cell membrane can lead to lysis and the escape of macromolecules (Lambert et al. 2001;

Oussalah et al. 2006). Essential oils are liable to stimulate depolarization in mitochondrial membranes of eukaryotic cells by decreasing the membrane potential, affect Ca^{2+} cycling and other ionic channels and reduce the pH gradient (Vercesi et al. 1997; Bakkali et al. 2005, 2008; Hussain 2009). The fluidity of membranes is disturbed by them, hence becoming noticeably permeable which results in leakage of radicals, cytochrome C, calcium ions and proteins as in case of oxidative stress. Permeabilization of the outer and inner mitochondrial membranes causes cell death by necrosis and apoptosis (Armstrong 2006; Hussain 2009). Although reduced forms of phyto-phenolics are powerful antioxidants, the phenoxyl radical produced through antioxidation reactions is a potential prooxidant which may exhibit cytotoxic activity when the life time of a radical is prolonged (Sakihama et al. 2002; Burt 2004; Barbehenn et al. 2005). In our study phenyl ethanol, one of the phenolic constituents, comprised of about 33 and 37 % of total volume of the absolute and concrete oil, respectively. Phenyl ethanol, which has a rose-like aroma, is an important fragrance in the cosmetic industry and possesses organoleptic characteristics that contribute to the quality of beverage and foods. It has been estimated that approximately 700,000 kg of phenyl ethanol is consumed annually as a food component (Mo and Sung 2007). The antibacterial properties of some essential oils were attributed to their high phenyl ethanol content (Boskabady et al. 2011; Singh et al. 2012). To the best of our knowledge, there is no published report about the anticancer properties of phenyl ethanol. Then according to the high level of phenyl ethanol in the oil, it may be one of the components responsible for the anticancer properties of this oil.

Geraniol—one of rose absolute and concrete oil constituents, a monoterpene alcohol—elicited a dramatic reduction in the amounts of thymidylate synthase (TS) and thymidine kinase (TK) expression in colon cancer cells (Carnesecchi et al. 2004). These two enzymes are involved in 5-fluorouracil (5-Fu) toxicity, in that a decrease in these enzymes is related to enhanced 5-Fu cytotoxicity (Mans et al. 1999). Geraniol lowered the resistance of cancer cells to 5-Fu, thus potentiate the inhibition of tumor growth by the drug, and increased the survival time of nude mice grafts with the human colorectal tumor cell TC118. Geraniol acts on at least two different targets involved

in the resistance of cancer cells to 5-Fu: it increases cell membrane permeability leading to enhanced uptake of 5-Fu by colon cancer cells and causes a significant change in the resulting potential and cell membrane polarization, which may trigger modifications of membrane bound protein activity and alteration in intracellular signaling pathways (Carnesecchi et al. 2002a, b). Also, the growth inhibition effect of eugenol (another constituent of concrete and absolute rose oils) has been observed in the hepatoma cell line HepG2 and human promyelocytic leukemia cell HL-60. In leukemia cells, eugenol treatment induced cytotoxicity and internucleosomal DNA fragmentation (Okada et al. 2005). Eugenol transduced the apoptotic signal via reactive oxygen species generation, thereby inducing mitochondrial permeability transition, reducing the level of the anti-apoptotic protein bcl-2, and inducing cytochrome C release to the cytosol and subsequent apoptotic cell death (Yoo et al. 2005). Eugenol inhibited the proliferation of HT-29 cells and the mRNA expression of CoX-2 (Vidhya and Devaraj 2011). Also, the methylated form of eugenol (methyl eugenol) was found to inhibit melanoma cells growth by about 20–40 % (Pisano et al. 2007). Review of literature showed that essential oils including the previous compounds in other investigated plants showed antimutagenic and anticancer activities (Edris 2007; Hussain 2009; Adorjan and Buchbauer 2010).

Conclusion

In conclusion, the data presented here showed that the concrete and absolute oils have a cytotoxic effect toward normal human blood lymphocytes which is dose dependent. In addition, at a dose of 10 $\mu\text{g/ml}$ both oils are cytotoxically safe and have no genotoxic effect. This study implies that pretreatment of rose absolute oil (10 $\mu\text{g/ml}$) has a strong inhibitory role against mutagenic action of MMC (300 ng/ml) which is an anticancer/antibiotic drug causing secondary genetic damage. On the other hand, rose concrete oil shows antimutagenic activity at the same dose level but without statistical significance. Both rose concrete and absolute oils showed anticancer activity against HepG2 and MCF7 cell with an IC_{50} nearly falling within the NCI criteria ($\text{IC}_{50} < 20 \mu\text{g/ml}$), thus these agents can be considered as having a promising

anticancer potential. Further investigations are needed to study the activity of higher doses of both oils in vitro and in vivo in experimental animals to evaluating the capability to use these oils as therapeutic for the treatment of different kinds of cancers.

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