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# Inhibitory effect of chamomile essential oil on the sister chromatid exchanges induced by daunorubicin and methyl methanesulfonate in mouse bone marrow

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**Abstract**

Different preparations of chamomile (*Matricaria chamomilla*) are used to treat various diseases, including inflammation and cancer; however, no studies on the plant's antigenotoxic capacity have been made. The aim of the present work was to determine the inhibitory effect of the chamomile essential oil (CO), on the sister chromatid exchanges (SCEs) produced by daunorubicin and methyl methanesulfonate (MMS) in mouse bone marrow cells. CO was analyzed and was found to contain 13 compounds, mainly bisabolol and its oxides, chamazulene, farnesene, germacrene and other sesquiterpenes. Initially, a toxic and a genotoxic analysis of CO were made; both showed negative results. To determine whether CO can inhibit the mutagenic effects induced by daunorubicin, one group of mice was administered corn oil, another group was treated with the mutagen (10 mg/kg), a third group was treated with 500 mg/kg of CO; three other groups were treated first with CO (5, 50 and 500 mg/kg) and then with 10 mg/kg of daunorubicin. In the case of MMS, the experimental groups consisted of the following: the negative control group which was administered corn oil, a group treated with 25 mg/kg of MMS, a group treated with 1000 mg/kg of CO, and three groups treated first with CO (250, 500 and 1000 mg/kg) and then with MMS (25 mg/kg). The results indicated a dose-dependent inhibitory effect on the SCEs formed by both mutagens. In the case of daunorubicin, a statistically significant result was observed in the three tested doses: from the lowest to the highest dose, the inhibitory values corresponded to 25.7, 63.1 and 75.5%. No alterations were found with respect to the cellular proliferation kinetics, but a reduction in the mitotic index was detected. As regards MMS, the inhibitory values were 24.8, 45.8 and 60.6%; no alterations were found in either the cellular proliferation kinetics or in the mitotic indices. Our results suggest that CO may be an effective antimutagen that could be considered for further study. 

*Keywords:* Chamomile; Genotoxicity; Antigenotoxicity; Daunorubicin; Methyl methanesulfonate

## 1. Introduction

Plants have been used effectively throughout history to treat a variety of illnesses, a practice that has furthered the synthesis of plant-specific ther-

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apeutic compounds. Interest in such a practice has recently been reinforced due to the knowledge that chemicals such as proteases and antioxidants may prevent or reduce the development of cancer by blocking genetic damage (Berhow et al., 2000).

The chamomile plant (*Matricaria chamomilla*), which belongs to the Asteraceae family, is commonly used as a medicinal tea to treat inflammatory disorders, fever, diarrhea, menstrual pain, and intestinal and hepatic tumors (Mann and Staba, 1986); it is also the active principle in creams used for atopic eczema (Patzelt-Wenczler and Ponce-Poschl, 2000). More than a hundred metabolites have been mentioned as components of the plant, some of which are known to be concentrated in its essential oil (CO); this oil makes up from 0.24 to 1.9% of the plant (Trease, 1986). Pharmacological studies on several components of CO have revealed positive results. For example, the terpene, bisabolol, reduces inflammation and arthritis, prevents the development of gastric ulcer, and acts against bacteria and fungi; it is also a wound-healing principle, and a constituent of cosmetic ointments made to treat dermatitis (Jakovlev et al., 1979; Torrado et al., 1995; Szelenyi et al., 1979; Villegas et al., 2001; Grassi et al., 2000). The sesquiterpenes matricine and chamazulene have shown anti-inflammatory effects (Jakovlev et al., 1983). In the case of chamazulene, this effect might be explained by its inhibitory action on the formation of leukotriene in neutrophilic granulocytes as well as by its antioxidative properties (Safayhi et al., 1994).

Free radicals such as superoxide, OH and $H_2O_2$, are noxious to cells and attack polyunsaturated fats, proteins and DNA, where they produce molecular alterations related to aging, arteriosclerosis and cancer (Gardner, 1997). Thus, the prevention of damage by free radicals is a feasible procedure for improving human health. One of the main actions of the antineoplastic daunorubicin on DNA occurs through the induction of free radicals, which in turn may produce several types of genotoxic damage. Among these, an increase in the rate of sister chromatid exchanges (SCEs) is known (Szabova, 1996; Noviello et al., 1994).

The genotoxic potential of CO has not been determined; therefore, the initial aim of this investigation was to evaluate the capacity of this mixture to form SCEs in mouse bone marrow. Considering the therapeutic uses of chamomile, as well as the antioxidant species contained in it, the second aim defined for the research was to determine the antigenotoxic capacity of CO by evaluating its effect on the SCEs induced by daunorubicin in the same model. On the other hand, methyl methanesulfonate (MMS) is a direct alkylating agent that forms covalent bonds with DNA and metilates guanine residue causing genotoxic damage; this effect has been detected as SCEs in in vivo and in vitro studies (Madrigal-Bujaidar et al., 1998). Based on this information, it was decided that the inhibitory effect of the extract on the SCEs induced by MMS was to be evaluated.

## 2. Material and methods

### 2.1. Chemicals

The extract of the essential oil was obtained from Gritman Co. (Friendswood, TX). It is a blue colored mixture obtained through a vapor distillation process, from the flowers of *M. chamomilla*. The components of the extract were identified and quantified with gas chromatography. Thirteen compounds were determined with this assay, including bisabolol and its oxides, chamazulene, farnecene, germacrene, and sesquiterpenes (Table 1). The extract was maintained at 4 °C in the dark until it was used.

Activated charcoal (200–400 mesh), corn oil, 5-bromodeoxyuridine (99% pure, BrdU), chlorhydric acid, Hoechst 33258, and MMS (99% pure) were obtained from Sigma Chemicals (St Louis, MO). Potassium chloride, sodium phosphate, potassium phosphate, sodium citrate, distilled water, and ammonium hydroxide were purchased from Baker S.A. (Mexico City), and deionized water was obtained from Hycel (Mexico City). Daunorubicin (97% pure) was obtained from Lemery Laboratories (Mexico City).

Table 1
Components of the tested chamomile essential oil

| Compound | RT[a] | CAS no. | Area (%) |
|---|---|---|---|
| (*E*)-β-Farnesene | 38.46 | 28973-97-9 | 28.17 |
| Germacrene-D | 39.23 | 23986-74-5 | 2.19 |
| Unidentified sesquiterpene | 40.07 | | 1.40 |
| Unidentified sesquiterpene | 41.17 | | 0.78 |
| (*Z*,*E*)-α-Farnesene | 41.35 | 26560-14-5 | 1.59 |
| Unidentified sesquiterpene | 48.52 | | 0.71 |
| α-Bisabolol oxide B | 49.28 | 26184-88-3 | 4.31 |
| α-Bisabolol oxide | 50.65 | 22567-38-0 | 5.30 |
| α-Bisabolol | 51.18 | 515-69-5 | 2.31 |
| Chamazulene | 52.80 | 529-05-5 | 2.39 |
| α-Bisabolol oxide A | 54.46 | 22567-36-8 | 41.77 |
| 1,6-Dioxaspiro[4.4]non-3-ene,2-(2,4hexadyn-1-ylidene) | 60.73 | | 2.19 |
| Hexatriacontane | 67.49 | 630-06-8 | 0.50 |

[a] RT, Retention time obtained with gas chromatography.

### 2.2. Animals

Male mice (NIH) weighing 25 g were used for the experiment; they were obtained from the National Institute of Hygiene. The animals were maintained at 24 °C in a 12-h dark–light period, and were allowed to consume food (Purina) and tap water freely.

### 2.3. Lethal dose50

A procedure that required few animals was followed for this determination (Lorke, 1983). The assay was made in two steps: doses of 10, 100 and 1000 mg/kg were tested first, and showed no lethality among the animals; then the effect of 1600, 2900, 4300 and 5600 mg/kg was tested, and no mortality appeared among the treated mice either.

### 2.4. Genotoxicity of the essential oil

Five groups with five mice each were used for this assay and organized as follows: a negative control group that was administered corn oil orally, three groups of animals that were administered CO (10, 100 and 1000 mg/kg, respectively) orally, and a positive control group that was ip administered an aqueous solution of MMS (25 mg/kg). Immediately after inoculation with the compounds, the animals were injected with an aqueous suspension of BrdU adsorbed to activated charcoal (1.7 mg/kg) (Madrigal-Bujaidar et al., 1999); 21 h later an ip inoculation of colchicine (10 μg/g) was administered to each mouse in order to deter the mitotic process in metaphase. Three hours after this inoculation, the mice were sacrificed by cervical dislocation and the bone marrow of both femurs was dispersed in KCl (0.075 M) at 37 °C for 45 min, centrifuged at 2000 rpm for 5 min and fixed three times with a solution of methanol–acetic acid (3:1). A few drops of the cell suspension were put on slides with methanol; the slides were then dried and stained according to the method of Hoechst–Giemsa to differentiate the sister chromatids (Madrigal-Bujaidar et al., 1999).

The following scoring was made on each mouse treated with all the tested doses: (a) the rate of SCEs in 30 second-division mitosis; (b) the mitotic index (MI) in 1000 cells; and (c) the cellular proliferation kinetics in 100 cells. The proportion of cells in the 1st, 2nd and 3rd cellular divisions (M1, M2 and M3, respectively) was identified so as to calculate the average generation time (AGT), which was equal to $24/(M1+2M2+3M3)100$. The statistical analysis of the data obtained was made with the ANOVA and the Student's *t*-test.

### 2.5. Antigenotoxicity of the essential oil on daunorubicin

Six groups with five animals each were organized for this assay: a negative control group that was administered corn oil orally, a group treated orally with CO (500 mg/kg), three groups that were administered CO (5, 50 and 500 mg/kg) orally and then treated with an ip inoculation of daunorubicin (10 mg/kg), and the sixth group which was ip treated with 10 mg/kg of daunorubicin. The experimental procedure as well as the staining, scoring, and statistical analysis were carried out as described in the previous assay. The percentage of the genotoxic inhibition produced by CO on the SCEs induced by daunorubicin (Dau) was calculated with the following equation:

$$1-(\text{SCE increase of CO}+\text{SCE of Dau}/\text{SCE increase of Dau})100.$$

where the SCE increase of Dau = mean SCEs of Dau − mean SCEs of corn oil.

### 2.6. Antigenotoxicity of the essential oil on methyl methanesulfonate

For this assay, the animals were organized in groups with five individuals each: a negative control group that was administered corn oil orally, another group that was administered CO (1000) mg/kg) orally, three groups treated orally with the essential oil extract (250, 500 and 1000 mg/kg) first, and then with an i.p. inoculation of 25 mg/kg of MMS; one more group was i.p. inoculated with 25 mg/kg of MMS alone. The experimental procedure as well as the staining, scoring, statistical analysis, and the calculation of the genotoxic inhibition were carried out as indicated for the antigenotoxic assay on daunorubicin.

## 3. Results

The capacity of CO to induce SCEs is shown in Table 2. It was determined that the mixture is not genotoxic under the experimental conditions tested. The three tested doses showed a level similar to that obtained in the control treated animals, i.e. an induction of not more than 1.1 SCEs; the results contrasted sharply with the high degree of damage observed in the MMS treated animals. Table 2 also shows a non-significant cytotoxic effect produced by CO with respect to the control value.

Table 3 shows the inhibitory effect of chamomile oil on the frequency of SCEs produced by daunorubicin in bone marrow cells. No SCE formation was determined above the control level with the oil, and a clearly high frequency was detected with daunorubicin alone; however, the administration of CO to daunorubicin treated animals gave rise to a statistically significant dose-dependent reduction in the genotoxic damage. The antigenotoxic response corresponded to 25.7, 63.1 and 75.5% with 5, 50 and 500 mg/kg, respectively, of the tested CO. The cellular proliferation kinetics revealed no alteration by administering CO and daunorubicin together, with respect to the control treated mice; however, a MI decrease was detected with the two high doses of the plant oil plus the mutagen (Table 3). This result parallels the one obtained with daunorubicin alone, and suggests a lack of cytotoxic protection against the mutagen with the chamomile oil extract.

The effect of the tested antimutagen on the SCEs induced by MMS was similar to the result described earlier. A statistically significant dose-dependent genotoxic decrease was observed in the damage produced by the mutagen; nevertheless, the protection in this case was lower than that obtained with daunorubicin. The three tested doses of CO (250, 500, and 1000 mg/kg) showed an inhibition of 24.8, 45.8 and 60.6%. With respect to the AGT and the MI values, no alterations were detected when the chemicals were administered together or separately (Table 4).

## 4. Discussion

Cancer and other chronic diseases may be related with mutations produced by environmental agents; therefore, minimizing the exposure to harmful agents has been recommended as a way to prevent these diseases. Unfortunately, it is not

Table 2
Sister chromatid exchanges (SCEs), cellular proliferation kinetics (CPK), and the mitotic index (MI) induced by chamomile essential oil (CO) in mouse bone marrow cells

| Agent | Dose (mg/kg) | Mice no. | SCE | C | P | K | AGT (h) | MI (%) |
|---|---|---|---|---|---|---|---|---|
| | | | $\bar{X}\pm$E.D. | M1 | M2 | M3 | $\bar{X}\pm$E.D. | $\bar{X}\pm$E.D. |
| Corn oil | 0 | 5 | 1.06±0.35 | 14.4 | 77.6 | 8.4 | 12.56±0.45 | 3.98±0.91 |
| CO | 10 | 5 | 1.05±0.35 | 18.4 | 74.8 | 6.2 | 13.17±0.11 | 3.02±1.12 |
| CO | 100 | 5 | 0.92±0.28 | 17.2 | 76.8 | 6.4 | 13.19±0.44 | 2.96±0.89 |
| CO | 1000 | 5 | 0.86±0.11 | 12 | 79.2 | 8.6 | 12.40±0.42 | 3.02±0.65 |
| MMS | 25 | 5 | 18.6±3.17* | 19.2 | 76.6 | 4.4 | 13.16±0.13 | 4.82±0.67 |

MMS, methyl methanesulfonate. Each SCE value is the mean of 30 second-division cells per mouse, the CPK value is the mean of 100 cells per mouse, and the MI is the mean of 1000 cells per mouse. M1, M2, and M3 = frequency of cells in first, second and third cellular division. Average generation time (AGT) = 24/(M1+2M2+3M3)100.

* Statistically significant difference with respect to the corn oil value. ANOVA and Student's $t$-test, $P < 0.05$.

easy to eliminate the source of genotoxicity completely in modern society. In view of this situation, the identification and application of well-known antimutagens is a valid complementary strategy for improving human health (De Flora, 1998). A number of plants have been tested to determine their antimutagenic potential; among these, green, black and white teas are known to be strong mutagenic and carcinogenic inhibitors (Sarkar and Bhaduri, 2001; Anderson et al., 2001; Santana-Rios et al., 2001). Catechins, theaflavins, and thearubigins have been detected as some of the main active components in such inhibitors (Sarkar and Bhaduri, 2001). Studies on these agents have also suggested that the whole mixture may be more effective than its specific constituents (Sarkar and Bhaduri, 2001; Santana-Rios et al., 2001); this observation is congruent with the use of the CO extract in our study. In fact, favorable effects have been suggested even with the consumption of a mixture of green tea and the antineoplastic sulindac and tamoxifen (Fujiki et al., 2001).

Our data are congruent with this line of research, indicating that other plant teas and their constituents may be effective antimutagens. The effect was shown with CO in a dose-dependent manner, and with positive activity in a broad dose range. The low dose tested against daunorubicin (5 mg/kg) corresponds to the CO found in 20 g of

Table 3
Effect of chamomile essential oil (CO) on the sister chromatid exchanges (SCEs), the cellular proliferation kinetics (CPK), and the mitotic index (MI) induced by daunorubicin (Dau) in mouse bone marrow cells

| Agent | Dose (mg/kg) | Mice no. | SCE | Inhibition (%) | C | P | K | AGT (h) | MI (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | $\bar{X}\pm$E.D. | | M1 | M2 | M3 | $\bar{X}\pm$E.D. | $\bar{X}\pm$E.D. |
| Corn oil | 0 | 5 | 1.57±0.31* | – | 21.4 | 68.6 | 9.6 | 13.04±0.32 | 5.86±1.74 |
| CO | 500 | 5 | 1.53±0.30* | – | 20.4 | 69.8 | 10.2 | 13.31±0.30 | 4.70±0.94 |
| Dau | 10 | 5 | 11.0±1.49 | – | 24.4 | 66.8 | 8.8 | 14.00±0.92 | 2.58±0.50** |
| CO+Dau | 5+10 | 5 | 8.59±1.20* | 25.79 | 25.0 | 65.4 | 10.0 | 13.00±0.48 | 4.12±1.00 |
| CO+Dau | 50+10 | 5 | 5.06±0.59* | 63.10 | 25.0 | 68.8 | 6.2 | 13.42±0.64 | 2.64±1.17** |
| CO+Dau | 500+10 | 5 | 3.88±0.65* | 75.58 | 23.2 | 68.4 | 8.4 | 13.36±0.37 | 2.34±0.93** |

Each SCE value is the mean of 30 second-division cells per mouse, the CPK value is the mean of 100 cells per mouse, and the MI is the mean of 1000 cells per mouse. MI, M2, and M3 = frequency of cells in first, second and third cellular division. Average generation time (AGT) = 24 (M1+2M2+3M3) 100.

* Statistically significant difference with respect to the value of Dau.

** Statistically significant difference with respect to the control value. ANOVA and Student's $t$-test, $P < 0.05$.

Table 4
Effect of chamomile essential oil (CO) on the sister chromatid exchanges (SCEs), the cellular proliferation kinetics (CPK), and the mitotic index (MI) produced by methyl methanesulfonate (MMS) in mouse bone marrow cells

| Agent | Dose (mg/kg) | Mice no. | SCE | Inhibition (%) | C | P | K | AGT (h) | MI (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | $\bar{X}\pm$E.D. | | M1 | M2 | M3 | $\bar{X}\pm$E.D. | $\bar{X}\pm$E.D. |
| Corn oil | 0 | 5 | $1.05\pm0.38^{*}$ | – | 12.6 | 81.6 | 5.8 | $13.04\pm0.40$ | $5.52\pm0.67$ |
| CO | 1000 | 5 | $0.95\pm0.22^{*}$ | – | 17.8 | 77.4 | 4.8 | $13.23\pm0.16$ | $5.92\pm0.56$ |
| MMS | 25 | 5 | $14.88\pm2.48$ | – | 19.8 | 71.4 | 8.8 | $13.33\pm0.44$ | $4.62\pm0.07$ |
| CO+MMS | 250+25 | 5 | $11.46\pm2.17^{*}$ | 24.83 | 18.8 | 75.6 | 5.6 | $13.29\pm0.70$ | $5.04\pm0.49$ |
| CO+MMS | 500+25 | 5 | $8.55\pm0.52^{*}$ | 45.85 | 13.0 | 84.2 | 3.2 | $13.12\pm0.38$ | $5.82\pm0.94$ |
| CO+MMS | 1000+25 | 5 | $6.24\pm1.39^{*}$ | 62.47 | 14.8 | 81.4 | 3.8 | $13.33\pm0.43$ | $5.48\pm1.51$ |

Each SCE value is the mean of 30 second-division cells per mouse, the CPK value is the mean of 100 cells per mouse, and the MI is the mean of 1000 cells per mouse. MI, M2, and M3 = frequency of cells in first, second and third cellular division. Average generation time (AGT) = 24/(M1+2M2+3M3)100.

* Statistically significant difference with respect to the value of MMS. ANOVA and Student's $t$-test, $P \leq 0.05$.

chamomile, which is the amount needed to prepare about ten cups of tea. Also, the efficacy of CO was made evident in the case of damage produced by a direct and an indirect mutagen. These characteristics of the extract, as well as its lack of toxicity, suggest that it is pertinent to extend the research to other models, using CO and the whole tea in order to verify the reported antigenotoxicity and determine the mechanism of action involved. As regards the genotoxic potential of CO, it is interesting to point out that no SCE increase or alteration in the MI or the CPK was detected with an amount of the mixture as high as 1000 mg/kg. These data are encouraging signs in the search for antimutagens, because it is known that good inhibitory agents at low doses may sometimes become ineffective or comutagenic when the doses are increased (Salvadori et al., 1994; Madrigal-Bujaidar et al., 1998).

Several constituents of the tested mixture are terpens. Various chemical structures of this type of compounds have been reported as chemopreventive agents, showing a reduction in mammary carcinogenesis, when the 7,12-dimethylbenz[a]anthracene-induced rat mammary carcinogenesis model was used (Russin et al., 1989). Terpens were also found to have a potent protective effect on D-galactosamine/lipopolysaccharide-induced acute liver injury in mice (Matsuda et al., 2001). Other specific constituents of CO (germacrene and farnecene) have hardly been studied with respect to protective or pharmacological effects; however, their antimicrobial and molluscicidal activities are known (Biavatti et al., 2001; Khallouki et al., 2000).

*M. chamomilla* is therapeutically used for conditions that may have to do with oxidative stress. Since compounds from the CO tested in our study, like bisabolol and chamazulene, are antioxidants (Khallouki et al., 2000; Rekka et al., 1996), a possible explanation for the inhibitory effect of this oil on the SCEs produced by daunorubicin is that such compounds trap the free radicals formed during the mutagen biotransformation. The formation of free radicals by daunorubicin occurs through a reduction of its quinone and hydroquinone structures to a semiquinone, which in turn may form superoxide and hydrogen peroxide (Bachur et al., 1977). Under comparable circumstances, a reduction in human oxidative stress has been reported when green tea is consumed (Klaunig et al., 1999); therefore, it should be interesting to test whether the consumption of chamomile tea produces a similar effect.

With respect to the protective action on MMS, antioxidants in CO may provide strong nucleophilic centers which would enable them to react with electrophilic carcinogens such as MMS; this process would intercept the mutagen, and consequently reduce the methylation of MMS on DNA (Madrigal-Bujaidar et al., 1998; Horváthová et al., 1998; Diaz Barriga et al., 1999; Rivas Olmedo et al., 1992). Our results on the two tested mutagens,

suggest that the CO inhibitory mechanism of action may be specifically related with the chemical involved, as has been reported for other mixtures (Marnewick et al., 2000).

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