

*Full Length Research Paper*

## ***In vitro* testing of a laxative herbal food supplement for genotoxic and antigenotoxic properties**

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**Chronic constipation (CC) is one of the most common functional gastrointestinal disorders and despite it is a rarely life-threatening condition, its effects can lead to discomfort and diminish the quality of life. Laxatives are commonly prescribed in CC patients including herbal products in standardized forms. The aim of the present study was to evaluate a commercially available herbal formulation (*Sollievo Bio*, Aboca, Sansepolcro, Italy), for potential cytotoxicity, genotoxicity and antigenotoxicity on a human colorectal adenocarcinoma cell line (HT-29). The cytokinesis-block micronucleus test was used for genotoxicity and antigenotoxicity testing, three concentrations of *Sollievo Bio* (0.2, 0.4 and 0.8 mg/ml) were assayed and mitomycin C (MMC: 3 µg/ml) was used as an inductor of micronuclei. *Sollievo Bio* did not induce any significant variation in spontaneous micronucleus (MN) frequency and, interestingly, reduced the MN frequency induced by MMC in a dose-dependent manner. In conclusion, our findings confirmed the safety of *Sollievo Bio* and suggested that *Sollievo Bio* may possess strong antigenotoxic and protective effects on MMC *in vitro*.**

**Key words:** Herbal laxative, HT-29 cells, genotoxicity, antigenotoxicity, cytokinesis-block micronucleus (CBMN) test.

### **INTRODUCTION**

Chronic constipation (CC) is one of the most common functional gastrointestinal disorders (FGID) in the general population, which can be attributed to dietary, psychologic, as well as anatomical factors. Although constipation is a rarely life-threatening condition, its effects can lead to discomfort and diminish the quality of life. Estimated prevalence rates range from 0.7 to 30% in children and from 2 to 28% in the general population with an average of about 15% (Cook et al., 2009). In CC patients laxatives are the most commonly prescribed pharmacological agents, but current drug therapies are not without risk. Based on low levels of satisfaction both with treatment and side effects, a large number of CC patients turn to complementary and alternative medicine practices including herbal products. Herbal therapy in

standardized forms (for quality and quantities of ingredients) represents an emerging therapeutic opportunity for FGID.

*Sollievo Bio* tablets, commercialized by Aboca Società Agricola (Sansepolcro, Italy), are a fixed combination of herbs that are recognized as playing an important role in regulating intestinal function. *Sollievo Bio* contains powdered Senna (*Cassia angustifolia*) leaves, powdered Dandelion (*Taraxacum officinale*) roots and Chicory (*Cichorium intybus*), leaf juice of Aloe (*Aloe ferox*), powdered fruits of Caraway (*Carum carvi*) and Cumin (*Cuminum cyminum*), and an essential oil from sweet Fennel (*Foeniculum vulgare*); these plants have been reported to have multiple pharmacological properties relevant in gastrointestinal pathophysiology. The formulation reported in Table 1 has been suggested as a means of restoring normal intestinal function and relieving occasional constipation.

Fruits and leaves of *C. angustifolia* (Family name, Fabaceae) are among the most important natural drugs

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**Table 1.** Composition of *Sollievo* Bio<sup>(a)</sup> tablets.

<i>Sollievo</i> Bio ingredients	%	mg/dose die min (1 tablet) (mg)	mg/dose die max (3 tablet) (mg)
Senna ( <i>Cassia angustifolia</i> ) leaves bio <sup>(b)</sup>	30.50	122	366
Dandelion ( <i>Taraxacum officinale</i> ) radix bio	24.27	97.08	291.24
Aloe ( <i>Aloe ferox</i> ) juice bio <sup>(c)</sup>	22.86	91.44	274.32
Chicory ( <i>Cichorium intybus</i> ) radix bio	8.06	32.24	96.72
Sweet Fennel ( <i>Foeniculi fructus</i> ) fruit bio	6.92	27.68	83.04
Essential oil	0.08	0.32	0.96
Caraway ( <i>Carum carvi</i> ) fruit bio	5.76	23.04	69.12
Cumin ( <i>Cuminum cyminum</i> ) fruit bio	1.55	6.2	18.6

<sup>(a)</sup> Bio = from organic agriculture, <sup>(b)</sup> 2.5% in hydroxyanthracen derivatives calculated as Sennoside B - equivalent to 2.40 mg of Sennoside B/dosage form, <sup>(c)</sup> 8.8% of Aloin A+B - equivalent to 16.6 mg of Aloin A+B/ dosage form

worldwide for treating constipation through enhanced peristalsis. Leaves and fruits of *C. angustifolia* contain several laxative constituents such as anthranoids, mostly sennosides A and B, aloe-emodin, emodin, and chrysophanol, fatty oils, flavonoids, polysaccharides and tannins (Siddique et al., 2010). Senna has been widely used as an analgesic, febrifuge, diuretic, hepatoprotective, vermifuge and cholagogue remedy as well as for treating amoebic dysentery, tuberculosis, gonorrhoea, dysmenorrhoea, anemia, flu, liver and urinary tract diseases (Picon et al., 2010).

*T. officinale* is a member of the Asteraceae/Compositae family. Traditionally, it has been used as a herbal medicine for the treatment of dyspepsia, flatulence, gallstones. It is also used for stimulating appetite and bile stimulation, and as a laxative, diuretic, circulatory tonic, skin toner, blood tonic, digestive tonic and for the treatment of viral and bacterial infections (Rodriguez-Fragoso et al., 2008). Recent studies have provided evidence that it may reduce the risk of other diseases, including inflammation, hepatitis (Jeon et al., 2008), alcohol-induced toxicity in the liver, and tumors (Sigstedt et al., 2008). The most important biologically active components of Dandelion are quercetin, luteolin, luteolin-7-O-glucoside, *p*-hydroxyphenylacetic acid, germacranolide acids, chlorogenic acid, chicoric acid, monocaffeoyltartaric acid, scopoletin, aesculetin, aesculin, cichoriin, arnidiol, faradiol, caffeic acid, taraxacoside, taraxasterol. *T. officinale* also contains large amounts of the polysaccharide inulin, as well as a high potassium concentration (Kisiel and Michalska, 2005).

Chicory (Asteraceae/Compositae family) is a biannual crop plant. During the first year, the roots accumulate inulin-type fructans and at the end of the growing season inulin is the most abundant storage carbohydrate molecule in chicory roots (up to 20% of the root fresh weight) (Degand et al., 2009). Chicory inulin is a natural linear of fructan with  $\beta$ -(2-1) glycosidic linkages. Because of the  $\beta$ -configuration of the bonds between fructose monomers, inulin-type fructans are resistant to hydrolysis

by human digestive enzymes ( $\alpha$ -glucosidase, maltase-isomaltase, sucrase) specific for  $\alpha$ -glycosidic linkages and are subsequently fermented in the colon (Kelly, 2008). As a result, inulin-type fructans (that can be then considered as prebiotics) cause significant changes in the composition of the gut microflora and have shown beneficial physiologic and nutritional effects. These effects are probably related to an improved calcium bioavailability and are associated with hypotriacylglycerolemia and hypoinsulinemia in experimental models and with a reduced risk of developing precancerous lesions in the colon (Roberfroid, 2000).

Genus *Aloe* plants (Aloeaceae family) appear in a considerable number of published ethnopharmacological studies. Most of the research on this plant has been done on the species *Aloe vera* and *Aloe arborescens*, however at least two additional *Aloe* species are reported to have therapeutic effects, namely *Aloe ferox* and *Aloe perryi baker* (Jia et al., 2008). Traditionally, *Aloe* plants are used for their laxative, anti-inflammatory, immunostimulant, antiseptic, antiulcer, antitumor and antidiabetic properties. Recently, Loots Du (2007) have identified the phytochemical content of an *A. ferox* lyophilized leaf gel, including phenolic acids/polyphenols, phytosterols, fatty acids, indoles, alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic acids, ketones, and alcohols.

*Carum carvi* (Apiaceae, Umbelliferae family) fruits are rich in cyclic monoterpenes (carvone and limonene), which constitute over 95% of the total essential oil component (Seidler-Lozykowska et al., 2010). They are used in the pharmaceutical industry as carminative and are found to be effective against spasmodic gastrointestinal complaints, flatulence, irritable stomach, indigestion, lack of appetite and dyspepsia (Adam et al., 2006; Holtmann et al., 2003). Fruits and essential oil are also used as antibacterial (Iacobellis et al., 2005), diuretic (Lahlou et al., 2007) and antiulcerogenic agents (Khayyal et al., 2001). Furthermore, experimental studies have

revealed antihyperglycemic (Eddouks et al., 2004) and cancer chemopreventive (Naderi-Kalali et al., 2005) activities.

Fruits of *C. cyminum* (in the Apiaceae family) contain green seeds which are one of the commonly used spices in food preparations. The fruits have medicinal application in treatment of diarrhea, dyspepsia, jaundice, toothache and epilepsy (Haghparast et al., 2008). They also have stomachic, diuretic, carminative, emmanogogic, antispasmodic, antimicrobial and hypoglycaemic properties (Allahghadri et al., 2010).

*F. vulgare* is a widespread perennial umbeliferous (Apiaceae) herb, native to the mediterranean area but naturalized elsewhere as well. Fennel possesses a plethora of gastrointestinal effects that, antispasmodic action). It is particularly used in the treatment of mild digestive disorders (Picon et al., 2010). Fennel is also highly recommended for diabetes, bronchitis and chronic coughs, and for the treatment of kidney stones. Anti-inflammatory, analgesic, antibacterial, diuretic and antioxidant activity of Fennel have also been reported (Kaur and Arora, 2009). The beneficial effects of Fennel are attributed to its volatile essential oils, a mixture of at least one dozen of different chemicals. They comprised almost 50 to 70% trans-anethole, 20% fenchone, and 9% estragole. Other compounds are present in concentrations that are usually less than 1% (limonene, camphor, and  $\alpha$ -pinene) (Picon et al., 2010).

*Sollievo* Bio is intended to be taken to maintain, support and optimize the gastrointestinal physiological transit function within the limits of its homeostatic condition. In fact, *Sollievo* Bio tablets are laxative, carminative, and eupeptic. The laxative action is due to Senna leaves and Aloe, which stimulate colon motility resulting in propulsive contractions. The word carminative derives from carmine (= purify) and indicates removing gases accumulated in the stomach and intestines. Carminative herbs act on the bowel wall and vessels of the same, improve the tone of smooth muscle thus facilitating the expulsion of gas and reducing the tendency to spasmodic events. Among carminative herbs are a large number of plants rich in essential oils such as Caraway, Cumin, and Fennel. A eupeptic action is characteristic to herbs that stimulate digestion, usually identified as the so-called bitter herbs, a term that includes all herbs that stimulate acid and bile secretions such as Dandelion and Chicory.

The aim of the present study was to gather further knowledge regarding *Sollievo* Bio to determine its potential to induce cytotoxicity and/or genotoxicity, using a continuous human colorectal adenocarcinoma cell line (HT-29); cytotoxicity was determined using the lactate dehydrogenase (LDH) leakage assay, whereas genotoxicity was evaluated using the cytokinesis-block micronucleus (CBMN) test. In addition, antigenotoxic effects of *Sollievo* Bio against mitomycin C (MMC) - induced genotoxicity were evaluated using the CBMN test. HT-29 cell line was selected as a colon tissue

model for its ultrastructural features that include microvilli, microfilaments, large vacuolated mitochondria with dark granules, smooth and rough endoplasmic reticulum with free ribosomes, lipid droplets, few primary and many secondary lysosomes (Zweibaum et al., 1985).

## MATERIALS AND METHODS

### Reagents and culture media

All reagents used were of analytical grade. Acetic acid, dimethyl sulfoxide (DMSO), Giemsa stain solution, methanol, potassium chloride (KCl), disodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) and monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) were purchased from Carlo Erba Reagenti, Milan, Italy. MMC was obtained from Sigma-Aldrich Srl, Milan, Italy. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), cytochalasin B, trypsin-EDTA, L-glutamine, antibiotics (penicillin and streptomycin), and Dulbecco's phosphate-buffered saline, pH 7.4 (PBS) were purchased from Invitrogen, Milan, Italy. Conventional microscope slides and coverslips were supplied by Knittel-Glaser, Braunschweig, Germany. Eukitt was from O. Kindler GmbH, Freiburg, Germany. Distilled water was used throughout the experiments. Lactate dehydrogenase (LDH) cytotoxicity detection kit was purchased from Takara Bio Inc. (Kyoto, Japan).

### Sample preparation

*Sollievo* Bio tablets were manually crushed using a ceramic mortar to obtain a fine powder. The solution used in this experimental approach was obtained by dissolving *Sollievo* Bio powder in DMSO at a concentration of 80 mg/ml. To ensure optimal solubilization the *Sollievo* Bio solution was subjected to sonication, and prior to use, the solution was centrifuged to remove the coarse woody debris.

### Cell line and culture conditions

The human HT-29 cell line (ATCC HTB-38™) was derived from colon adenocarcinoma of a female Caucasian (Fogh and Trempe, 1975) and was obtained from the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia, Italy.

The cells were grown as monolayer cultures in DMEM with L-glutamine, with the addition of 10% FBS and 1% penicillin/streptomycin. Cell stocks were incubated at 37°C in a humidified atmosphere containing 5%  $\text{CO}_2$ . Under these conditions, doubling time for HT-29 cells was about 24 h. All experiments were performed on HT-29 cells at passages between 36 and 38. Cell stocks were routinely frozen and stored in liquid nitrogen.

### Lactate dehydrogenase (LDH) leakage assay

Cytotoxicity induced by *Sollievo* Bio tablets was assessed by LDH release into the culture medium. The HT-29 cells were seeded ( $2.5 \times 10^4$ /well) in flat bottomed 96-well tissue culture plates (Orange Scientific, Braine-l'Alleud, Belgium) and were allowed to attach for 24 h before treatment with *Sollievo* Bio. The medium was then removed and replaced by fresh DMEM containing *Sollievo* Bio at concentrations ranging from 0.0021 to 0.8 mg/ml (cytotoxicity, exposure protocol) and cells were incubated for 24 h. Following treatment, plates were centrifuged at 250 x g for 10 min in order to obtain a cell free supernatant fluid and the culture medium was

aspirated. The activity of LDH in the medium was determined using Takara's LDH Cytotoxicity Detection Kit according to manufacturer's instructions. LDH leakage assay is based on the conversion of lactate to pyruvate in the presence of LDH with parallel reduction of NAD<sup>+</sup> and on a coupled reaction which converts a yellow tetrazolium salt into a red, formazan-class dye. The formation of formazan from the above reaction results in a change in absorbance at 492 nm. Absorbance was recorded using a Tecan Sunrise microplate reader (Tecan Italia S.r.l., Milan, Italy). *Solllievo* Bio solutions did not interfere with the release of LDH within the range of concentrations assayed. The experiments were performed in triplicate to confirm the accuracy of the results. The results of this assay determined the concentration (0.2, 0.4 and 0.8 mg/ml) of *Solllievo* Bio to be evaluated for the genotoxicity and antigenotoxicity protocols.

### Cytokinesis-block micronucleus (CBMN) test

HT-29 cells were grown in 6-well tissue culture plates (Orange Scientific, Braine-l'Alleud, Belgium) at an initial concentration of  $5 \times 10^5$  cells/well (5.0 ml/well). After 24 h of incubation, the medium was removed from each well and was replaced by fresh complete growth medium. To assay genotoxicity and antigenotoxicity properties of *Solllievo* Bio, two different treatment procedures were employed (exposure and co-exposure protocol, respectively).

In the first set of experiments (exposure protocol), the cells were treated with three different concentrations of *Solllievo* Bio (0.2, 0.4 and 0.8 mg/ml) for 24 h (37°C, 5% CO<sub>2</sub>). In the second set of experiments (co-exposure protocol), the cells were simultaneously exposed to *Solllievo* Bio (the same concentrations as above) and 3 µg/ml MMC for 24 h at 37°C (5% CO<sub>2</sub>). The concentration of MMC was determined on the basis of a positive control dose range-finding study (0.1 to 6 µg/ml, data not shown). The dose-dependent increase in levels of MN was recorded and finally the dose that induced a detectable level of MN in HT-29 cells (about 20 to 30 micronuclei in 1000 binucleated cells) was selected.

The CBMN test was performed according to the original method (Fenech, 2007), with marginal modifications for adaptation to HT-29 cells. Immediately after the *in vitro* treatment, the medium was removed and replaced by fresh DMEM containing cytochalasin B (final concentration 6 µg/ml) to inhibit cell division after mitosis. The cells were then incubated for 30 h. After that the cells were washed twice with PBS, detached by trypsinization (300 µl of 0.05% trypsin-EDTA, 5 min) and centrifuged for 10 min at 720 x g. The pellets were then resuspended in hypotonic solution (3 ml of 0.56% KCl) at room temperature and fixed with 3 ml of Carnoy's reagent (methanol:glacial acetic acid - 5:1 v/v). The cell suspensions were centrifuged again for 10 min at 720 x g and resuspended in 6 ml of fixative. Next, the tubes were centrifuged for 10 min, the supernatant discarded and the cell suspensions dropped on glass slides (two slides per concentration). After drying, the slides were stained with 4% Giemsa in phosphate buffer (0.06 M Na<sub>2</sub>HPO<sub>4</sub> and 0.06 M KH<sub>2</sub>PO<sub>4</sub>, pH 6.8) for 7 to 8 min, washed with distilled water, air-dried and finally mounted with Eukitt. Cells were examined for MN at 400 x magnification according to previous reports (Fenech, 2007). All MN slide analysis was conducted under "blind-scoring" conditions. MN were scored in 1,000 binucleated cells (BNC) for each concentration of each repeated experiment. Positive (3 µg/ml, MMC) and negative (1%, DMSO) controls were included in each experiment. Three totally independent experiments were performed. At the end of the experiments, after the cells were detached, cell viability was evaluated by the trypan blue dye exclusion test using 0.2% trypan blue dye; live cells exclude the dye, whereas dead cells take it up into their cytoplasm. The number of viable (uncolored) and dead (colored) cells was counted using an automated cell counter (Countess<sup>®</sup>; Invitrogen, Carlsbad, CA). Viability % was calculated as the ratio of number of viable cells to all

cells.

To investigate the impact of *Solllievo* Bio on cell proliferation the nuclear division index (NDI) was determined using the formula (Eastmond and Tucker, 1989):

$$NDI = [1 \times N_1] + [2 \times N_2] + [3 \times N_3] + [4 \times N_4] / N$$

where  $N_1$ – $N_4$  represent the numbers of cells with 1 to 4 nuclei, respectively, and  $N$  is the total number of cells scored (1.000).

### Statistical analysis

Each result is expressed as the mean ± standard error (SEM) of three independent experiments. The Shapiro–Wilk test was used to determine the distribution of the data. Because the data were not normally distributed, Mann–Whitney  $U$  test was used when two groups were compared. For genotoxicity and antigenotoxicity experiments, the significance was calculated in comparison to the negative (DMSO) and positive (MMC) control, respectively. A value of  $p < 0.05$  was considered to be statistically significant for all the parameters evaluated. In the co-exposure protocol, the inhibition rate of genotoxicity (%) was calculated according to Waters et al. (1990) using the following formula:

$$\% \text{ Reduction} = 100 \times (A - B) / (A - C)$$

where A corresponds to the frequency of MN in the positive control; B to the frequency of MN in cells treated with *Solllievo* Bio plus the known mutagen (co-exposure) and C to the frequency of MN in the negative control. Spearman's rank correlation coefficients ( $r_s$ ) were calculated to investigate dose–response relationship in genotoxicity and antigenotoxicity testing. The SPSS version 10.0 (SPSS, Chicago, Illinois) statistical software program was used for the analyses.

## RESULTS

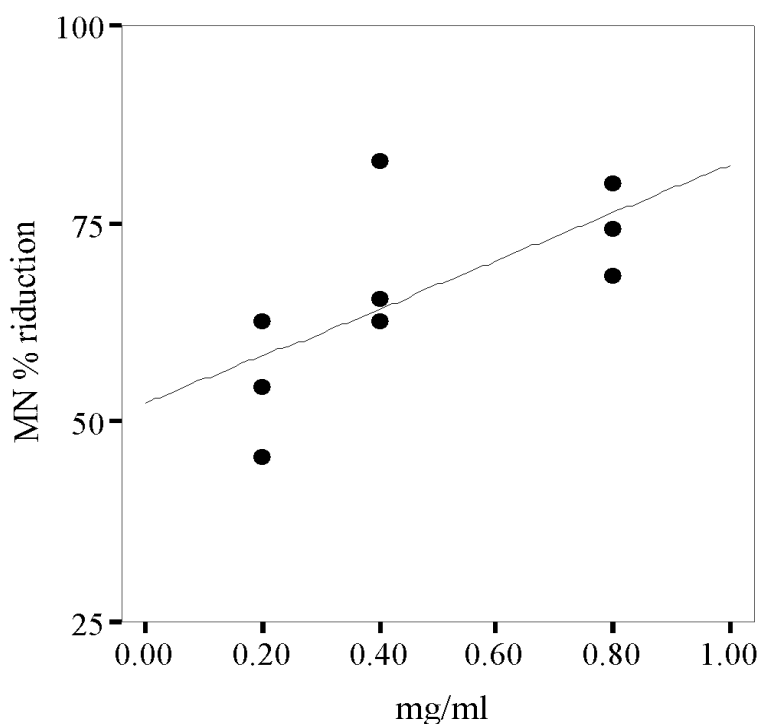
Prior to the genotoxicity and antigenotoxicity assays, *Solllievo* Bio at various concentrations that is (0.0021, 0.0063, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml) were evaluated for any cytotoxic effect against HT-29 cells. Twenty four-hour exposure to any of the *Solllievo* Bio concentrations tested did not induce any significant cytotoxicity and the reduction in cell viability was always lower than 5% (data not shown). Therefore, the three highest tested concentrations (0.8, 0.4 and 0.2 mg/ml) were chosen for further evaluation in the genotoxicity and antigenotoxicity protocols. The data on MN frequency and NDI in *Solllievo* Bio-treated HT-29 cells (exposure protocol) and in HT-29 cells simultaneously exposed to *Solllievo* Bio and MMC (co-exposure protocol) are shown in Table 2. *Solllievo* Bio did not induce any significant variation in the spontaneous MN levels ( $6.57 \pm 0.44$ ) at all the tested doses, whereas the positive control (MMC) caused significant MN levels ( $23.9 \pm 1.48$ ;  $p < 0.05$ ). In addition, MMC induced a significant reduction in NDI compared with the negative control (11.65%).

In the co-exposure protocol, the clastogenic activity induced by MMC was significantly decreased by *Solllievo* Bio. Almost all concentrations used (0.2, 0.4 and 0.8 mg/ml) were effective and the percentages of

**Table 2.** Micronuclei (MN) frequency and nuclear division index (NDI) in HT-29 cells treated with *Sollievo* Bio (exposure protocol) or simultaneously exposed to *Sollievo* Bio and MMC (co-exposure protocol). Results are expressed as mean  $\pm$  SE of data from three independent experiments with two replicates at each point.

Treatment	MN	NDI
DMSO (1%)	6.57 $\pm$ 0.44	1.70 $\pm$ 0.02
MMC (3 $\mu$ g/ml)	23.90 $\pm$ 1.47 <sup>†</sup>	1.50 $\pm$ 0.02 <sup>†</sup>
<i>Exposure protocol</i> (genotoxicity testing)		
<i>Sollievo</i> Bio 0.2 mg/ml	7.83 $\pm$ 0.17	1.72 $\pm$ 0.01
<i>Sollievo</i> Bio 0.4 mg/ml	6.33 $\pm$ 1.17	1.65 $\pm$ 0.02
<i>Sollievo</i> Bio 0.8 mg/ml	7.25 $\pm$ 0.63	1.74 $\pm$ 0.02
<i>Co-exposure protocol</i> (antigenotoxicity testing)		
<i>Sollievo</i> Bio 0.2 + MMC	14.50 $\pm$ 0.87 *	1.47 $\pm$ 0.01
<i>Sollievo</i> Bio 0.4 + MMC	11.67 $\pm$ 1.09*	1.43 $\pm$ 0.01*
<i>Sollievo</i> Bio 0.8 + MMC	11.00 $\pm$ 0.58*	1.38 $\pm$ 0.02*

<sup>†</sup>  $p < 0.05$  (Mann–Whitney  $U$  test) MMC vs DMSO, \*  $p < 0.05$  (Mann–Whitney  $U$  test) *Sollievo* Bio + MMC vs MMC.



**Figure 1.** Correlation between *Sollievo* Bio concentrations and MN % reduction in HT-29 cells treated with MMC (co-exposure protocol).

genotoxicity inhibition (% reduction) increased with increasing *Sollievo* Bio concentrations (Table 2 and Figure 1). The treatment of HT-29 cells with the lowest dose of *Sollievo* Bio (0.2 mg/ml) in combination with MMC did not cause any statistically significant change in NDI, as compared with the positive control (MMC) alone.

In contrast, using the same experimental conditions, the other *Sollievo* Bio concentrations (0.4 and 0.8 mg/ml) in combination with MMC caused a further decrease in NDI values (4.79 and 8.34%, respectively), as compared with MMC alone.

Spearman's rank correlation coefficient ( $r_s$ ) was

calculated to investigate the possible correlation between *Sollievo* Bio concentrations and reduction of MN frequency in the co-exposure protocol. The results indicate a clear concentration-response correlation ( $r_s = -0.917$ ;  $p < 0.001$ ), with the highest concentration found to be the more effective and with gradual decreasing of *Sollievo* Bio concentrations resulting in a proportional decrease in the reduction of MMC-induced genotoxicity.

## DISCUSSION

In the context of cancer chemoprevention, defined as “a strategy of cancer control by administration of synthetic or natural compounds to reverse or suppress the process of carcinogenesis” (Sporn et al., 1976), the detection of protective effects against the action of DNA-damaging compounds can assume great relevance. As such, edible and medicinal plants (possessing pharmacological activities to treat diseases, as compared to edible plants that are used in daily life as a food) have all been suggested to potentially have an important role in cancer chemoprevention (Liu, 2004; Park and Pezzuto, 2002). According to De Flora (1998), the rationale implementation of chemoprevention strategies requires the assessment of the efficacy and safety of putative inhibitors.

The qualitative and quantitative composition of *Sollievo* Bio is well-known (Table 1) and the multiple constituents are responsible for its therapeutic effects. The different properties of the ingredients in *Sollievo* Bio tablets can be defined and grouped as follows: Senna and Aloe have a laxative action, Fennel, Caraway and Cumin have predominantly carminative action, Dandelion and Chicory assist all the digestive process that often is disturbed during episodes of constipation. This does not provide a complete picture of beneficial effects of *Sollievo* Bio, because its multiple phytochemicals constituents might have also other clinical relevant applications. Phytochemicals are defined as bioactive nonnutrient plant compounds that have been linked to a reduced risk for major chronic diseases, especially cancer and cardiovascular diseases (Liu, 2004). Numerous bioactive compounds have been isolated and identified and their potential health promoting effects have been evaluated both in *in vitro* and *in vivo* studies. Usually these active phytochemicals are carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds (Park and Pezzuto, 2002). As regard *Sollievo* Bio herbal constituents contain high levels of polyphenols, flavonoids, anthraquinones and other phytochemicals that are known to have antigenotoxic effect and can act as radical scavengers (Allahghadri et al., 2010; Loots Du, 2007).

In the present study, we evaluated the cytotoxic, genotoxic and antigenotoxic effects of *Sollievo* Bio tablets on HT-29 cells. The results from this study showed that *Sollievo* Bio was not cytotoxic or genotoxic in HT-29 cells

and these data support the safety of the product with regard to cytotoxicity and genotoxicity. The results also showed that *Sollievo* Bio plays a role in inhibiting the genotoxicity induced by MMC, in a concentration-dependent fashion. We have also observed that NDI, which is a measure of cytostasis (Fenech, 2007) was not affected by *Sollievo* Bio treatment, but it was decreased in cells treated with MMC alone or co-exposed to MMC and *Sollievo* Bio.

In conclusion, the present work indicates that *Sollievo* Bio does not induce MN in HT-29 cells, but shows a consistent antigenotoxic effect. About the possible mechanisms of action, the data suggest that compounds present in the *Sollievo* Bio formulation might act as scavenger and/or by inhibiting cell proliferation in cells with DNA damage induced by MMC. However, because the role of chemopreventive agents in the etiology of cancer is very complex and it involves several modes of action, and because our results are relative to *in vitro* experiments with a human cell line, additional animal and human studies involving different end points, should be addressed in order to clarify the antimutagenic potential of *Sollievo* Bio and the practical use of these compounds in chemoprevention.

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