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CYTOGENETIC EFFECTS OF *Myrtus communis* AND *Plantago albicans* INFUSIONS ON BEAN ROOT-TIP AND MICE BONE-MARROW CELLS

[48]

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ABSTRACT

In Libya the plants *Myrtus communis* and *Plantago albicans* are commonly used in folk medicine to treat various diseases. However, there are a large number of plant compounds which can cause many aberrations in genetic material. This study was carried out to investigate any possible cytotoxic and mutagenic effects for *M. communis* and *P. albicans* on mitotic criteria of cell cycle and chromosomes. Two infusion concentrations were prepared for each plant, one of them was used as folk medicine (o.25 mg/ml) and other as to 10 times of this value (2.5 mg/mL). Bean (*Vicia faba,* L.) root-tip cells (RTC) and Balb / C mice (*Mus musculus*) bone-marrow cells (BMC) were used as test systems. The *M. communis* infusions at both concentrations and the *P. albicans* infusion at the lower concentration had no statistically significant depressive effect on mitotic criteria of RTC. Where as significant depressive effect on these criteria of RTC was found for the high concentration of *P. albicans* as compared with a negative control. No significant effect on the induction of chromosome aberrations and the rate of mitotic index of BMC was found by using infusions of *P. albicans* and *M. communis* as compared with control.

Key words: Cytogenetic, *Myrtus communis, Plantago albicans,* Infusions.

INTRODUCTION

In Libya, the majority of the population uses traditional natural preparations derived from plant material for treating a variety of disease, and because of this it is extremely important that genotoxicity tests are applied to the active ingredients of these preparations in order to assess their mutagenic potential. The plants *Myrtus communis* (Myrtaceae) and *Plantago albicans (*Plantaginaceae) are commonly used in popular Libyan medicine.

The fresh and dry leaves of *M. communis* are used as an infusion to lower the blood glucose level in type-2 diabetic patients and to treat ulcers and

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gastritis **(Sepici *et al* 2004)**. All myrtle extracts were very rich with polyphenols, flavonol glycosides and hydrolysable tannins (galloyl-glucosides, ellagitannins, galloyl-quinic acids) **(Romani *et al* 2004).**

All plant parts except of *P. albicans* are used as an infusion in folk medicine for treating infectious diseases related to the respiratory, urinary and digestive tracts **(Chiang *et al* 2003).** The tissues of this plant contain the phenolic compounds, especially caffeic acid **(Chiang *et al* 2002).** The research reported in this paper used bean (*Vicia faba*) root-tip cells and Balb/C mice (*Mus musculus*) bone-marrow cells assays to assess whether these plants have any effect on mitotic index or the occurrence of chromosome aberrations.

MATERIAL AND METHODS

Plant materials

Myrtus communis and *Plantago albicans* were obtained from the flora of Wadi Al-Koof, Al- Jabal Al-Akhdar, AL-Baida,Libya. Infusions were prepared in the same way as is normally done when they are used by the general population. The *M. communis* or *P. albicans* leaves (*in natura*) were boiled for five minutes in tap water, and the infusion was covered and allowed to cool. The infusions from both plants were prepared at two concentrations, one corresponding to that normally used by the general population and the other at a concentration ten times higher by evaporator of stock solution, *i.e.* 0.25 and 2.5 mg/mL for each plant.

Root-tip cells

Bean legumes were placed on filter paper inside Petri-dish containing aerated water at room temperature until rooted, After which root samples were taken to act as time-zero (t zero) controls. Some legumes were then placed in the infusions prepared above (controls were put in water) for 24 h, after which more roots were removed and the legumes were returned to water for 24 to observe if there was recovery from any possible damage. The roots were fixed and stained using the Feulgen reaction and permanently mounted on slides. The slides were examined by using an optical microscope with a 40X objective. For each legume 1000 cells were analyzed, *i.e.* a total of 5000 cells each for the control, treatment and recovery groups. Cells with morphological structural alterations were recorded and the mitotic index (MI) of the cells was calculated. The statistical evaluation was performed using the χ^2 test at a probability level of 0.05.

Bone marrow cells

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Balb / C mice with a body weight (b.w.) of about 23-27g were obtained from the Biology Department, Faculty of Science, University of Omar El-Mukhtar, Three male and three female mice being used for each group (treatments and control). For each group, live mice were injected intraperitoneally for 24 h with 1 mL of one of the infusions prepared above, positive control animals being treated with 20 mg of cyclophosphamide (CP)/kg b.w. All mice were injected with 0.5 mL/100 g b.w. of a 0.16% colchicine solution an hour-and-a-half before sacrifice, bone marrow cells being obtained by modification of the method of **Ford and Hamerton (1956).** Chromosome analysis was carried out using optical microscopy and a 100X immersion lens. For each mouse, 100 metaphases were examined 500 metaphases each for the control and treatment groups. Mitotic index values were calculated for the 5000 cells by sex (a total of 10000 cells/group). Statistical evaluation was performed using he χ^2 test at a probability level of 0.05.

RESULTS AND DISCUSSION

In the case of *M. communis* the mitotic index of the root-tip cells decreased after 24 h in each concentration of extract, with a lower mean mitotic index occurring at the higher concentration (Table 1). This effect remaining even after the legumes was subjected to a 24 h recovery period in water, although the results were not significant according to the χ^2 test. For *P. albicans* the mitotic index of the root-tip cells also decreased after 24 h in each concentration of extract with the difference being statistically significant only for the higher concentration of 2.5 mg/mL ($\chi^2 = 5.97$). After recovery in water for 24 h there was a small increase in mitotic index, which although not significant, was well below the mitotic index of the t zero controls ($\chi^2 = 4.49$).

Table 1. Mitotic index of bean root-tip cells treated with *Myrtus communis* (Mc) and *Plantago albicans* (Pa) infusion

Groups	Treatment time	*Mitotic index*	*Interphase*	*Prophase*	*Metaphase*	Anaphase	Telophase
Control	control	10.1	4541	225	93	84	57
	Treated	9.3	4572	165	123	84	56
	Recovery	9.2	4576	242	93	60	29
Mc (o.25)	control	12.0	4461	319	104	80	36
	Treated	9.5	4564	263	98	48	27
	Recovery	7.9	4630	171	123	49	27
Mc (2.5)	control	13.0	4422	342	112	82	42
	Treated	5.1	4755	158	65	14	8
	Recovery	4.7	4774	121	75	20	10
Control	control	4.6	4780	144	39	16	21

Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 733-740, 2005

	Treated	2.7	4864	98	32	2	4
	Recovery	3.4	4835	98	56	6	5
Pa	control	5.2	4749	144	55	27	25
(0.25)							
	Treated	3.6	4823	101	61	14	1
	Recovery	4.2	4798	134	39	23	6
Pa(2.5)	control	8.7	4599	262	82	31	26
	Treated	0.8*	4957	22	14	4	3
	Recovery	1.9	4902	7	25	1	1

1. The number of cells analyzed in each group was 5000. concentration of infusion in mg/mL in parenthesis.
2. Treatment time: Control = 0 h (t zero), Treated = 24 h, Recovery = 24 h.

* Statistically significant.

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Neither of the *M. communis* infusions, nor the 0.25 mg / mL *P. albicans* infusion, produced a permanent significant depressive mitotic effect on the root-tip cells, although there was a statistically significant temporary inhibition of cellular division in the more concentrated *M. communis* infusion (2.5 mg/mL), but this effect was reversible.

In spite of the infusions having caused a decrease in cellular division in root-tip cells compared with non-treated controls only the higher concentration of *P. albicans* showed a significant inhibitory effect, although this cytotoxic effect was reversible with slight recovery in cell division after 24 h recovery in water. It is possible that a high concentration of any chemical will have an effect (inhibitory or stimulatory) on the cell cycle, as has been shown for caffeine in *Drosophila prosaltans* **(Itoyama *et al* 1997),** mefloquine in human blood lymphocytes **(Grisolia *et al* 1995),** *Alpinia mutans* and *Pogostemun heyneanus* extracts in *A. cepa* root-tip cells **(Dias and Takahashi, 1994)** and glaucolide B extracted from *Vemonia eremphila* Mart. in human lymphocytes **(Burim *et al* 1999).**

Mouse bone marrow cells

The results showed that there was no significant increase in the number of chromosome alterations in bone-marrow cells from animals treated with any of the infusions prepared from either *M. communis* or *P. albicans*, nor was there any alteration in the cellular division index as compared with the negative control (Table 2).

Table 2. Mitotic index and chromosome aberrations of bone-marrow cells of Balb/C mice treated with *Myrtus communis* (Mc) and *Plantago albicans* (Pa) infusion

Groups	Mitotic index	Total alteration	Chromatid gap	Chromosome gap	Chromatid break	Chromosome break
Control	2.2	1	1	0	0	0
Positive control	2.2	102	7	0	57	38
Mc (0.25)	2.9	2	1	1	0	0
Mc (2.5)	2.7	0	0	0	0	0
Pa (0.25)	2.6	3	3	0	0	0

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The number of cells analyzed in each group was 500. Positive control rats were injected with 20 mg cyclophosphamide/kg body weight. Concentration of infusion in mg/mL in parenthesis.

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Yen and Chen (1994) found that the principal components of tea leaves and their extracts are catechins, which seem to be responsible for antimutagenic activity, which varied from 4.3% in black tea to 26.7% for green tea. Green tea is produced from non-fermented *Thea sinensis* leaves (the most popular beverage in the orient) much used in Japan as an antipyretic, diuretic and antioxidant, and which has been shown to have antimutagenic and antitumor effects in vitro and in vivo **(Kada *et al* 1985; Shimoi *et al* 1986; Jain *et al* 1989 and Wang *et al* 1989).** They, showed that the mortality rate due to human cancers in areas of tea cultivation is significantly lower than in areas where tea is not grown **(Sasaki *et al* 1993),** and that the catechin present in green tea suppresses the action of many environmental mutagens **(Nakamura *et al* 1997)**. Black tea also has high antimutagenic activity in vitro, which varies according to the extent of fermentation of the tea during the manufacturing process **(Yen & Chen, 1994 and Apostolides *et al* 1996).** It has also been reported that black and green teas, without caffeine, have a chemo-preventive dose-dependent effect in preventing liver and lung cancer in rats **(Cao *et al* 1996).**

Horikawa *et al* (1994) assessed the activity of six Chinese medicinal herbs on *Salmonella* and found that tannin and catechin compounds were responsible for the inhibition of mutagenicity caused by benzo [a] pirene.

In a study involving the mutagenic effects of 2-(2-furyl)-3-(5-nitro-2-furyl) (AF-2), **Ohtsuka *et al* (1995)** investigated the antimutagenic effects of nine active compounds from the Chinese medicinal herb, and found that the main active antimutagenic compounds were the saponins and the flavonoids. According to **Bu-Abbas *et al* (1996)** the high concentration of flavonoids in green tea compared with black tea may mean that these compounds are one of those responsible for the antimutagenic and (possibly) anticarcinogenic properties of tea and its fermented products.

Plantago species exhibited cytotoxic activity, showing a certain degree of selectivity against the tested cells in culture. Since the flavonoids are able to strongly inhibit the proliferation of human cancer cell lines, we have identified luteolin-7-O-beta-glucoside as major flavonoid present in most of the Plantago species **(Galvez *et al* 2003).** Aqueous extract of Myrtus communis witch contain flavonoids exhibited anti-genotoxic and free-radical scavenging activities and the highest level of protection towards aflatoxin B1 (AFB1) and nifuroxazide was investigated in a bacterial assay system **(Hayder *et al* 2004).**

It may be considered that the presence of flavonoids, phenolics and tannins in *M. communis* and *P. albicans* was the

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reason why these medicinal plants did not show cytotoxic and clastogenic effects when tested in our system. It is also possible that extracts of these plants may have antimutagenic effects in different test systems, since the literature cited above indicates that plants containing flavonoids and tannin-like compounds can have such an effect. Our results indicate that the consumption of infusions made from *M. communis* and *P. albicans* can be continued, although they should be used with caution always exactly following the traditional methods of preparation, especially with regards to the concentration of the infusions and the duration of treatment, so that the infusions have the desired pharmacological effects without toxicity. Medicinal plants can be very useful, but it is still necessary for the general population to take care not to use such plants indiscriminately.

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Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 733-740, 2005

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التأثير المثبط والتثبيطي لمستخلص *Plantago* والآس *Myrtus communis* على نمو البكتريا المسببة لالتهاب الفم *albicans*

والخميرة المسببة لالتهاب الفم

]48[

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استخدمت طريقة التخفيف لمعرفة (RTC) والخميرة المسببة لالتهاب الفم (BMC). ولقد تم تحديد وجود تأثير مثبط للمستخلص المائي للآس على الكائنات الحية المجهرية، في حين أظهر التركيز RTC تثبيطاً قوياً على النبات تحت الدراسة، وهذه الأعمال أظهرت أن النبات يمتلك قابلية تأثير سلبي على نمو البكتريا وتأثيراً سلبياً على التكاثر والمتوسط والمقاومة BMC.

في هذه الدراسة استخدمت مستخلصات الآس *Myrtus communis* والأنين *Plantago albicans* لعلاج الأمراض الفطرية والبكتيرية. ومع ذلك فإن عدد كبير من المركبات النباتية يمكن أن تستخدم من التخفيفات الطبية المعدة للدراسة لتحديد تأثيرها على التكاثر والنمو وتأثير المستخلص المائي للنبات على المحتوى الغذائي المقاوم والكثافة والمقاومة المتوسطة والتركيز من النبات المستخلص لمدة يومين (0.25 ملجم/مل) بتركيز مرتين (25 ملجم/مل) لهذا التركيز 10 مرات مضاعف.

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