

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 (0.25 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 gastritis (Sepici *et al* 2004). All myrtle extracts were very rich with polyphenols, flavonol glycosides and hydrolysable tannins (gal-

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loyl-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

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 the 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 | Meta-phase | Ana-phase | Telo-phase |
|--------------|----------------|---------------|------------|----------|------------|-----------|------------|
| 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 (0.25) | 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 |
| | Treated | 2.7 | 4864 | 98 | 32 | 2 | 4 |
| | Recovery | 3.4 | 4835 | 98 | 56 | 6 | 5 |
| Pa (0.25) | control | 5.2 | 4749 | 144 | 55 | 27 | 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.

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 *Pogostemon heyneanus* extracts in *A. cepa* root-tip cells (Dias and Takahashi, 1994) and glaucolide B extracted from *Vernonia 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 |
| Pa (2.5) | 2.6 | 0 | 0 | 0 | 0 | 0 |

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.

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] piren.

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 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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التأثيرات الخلوية الوراثية لنقيع نباتي المرسين *Myrtus communis* والايمنم

Plantago albicans على خلايا القمة النامية لجذور نبات الفول

وخلايا نخاع العظم للفار الأبيض

[٤٨]

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استخدم نظامي اختبار وهما فحص خلايا القمة النامية لجذور البصل (RTC) وخلايا نخاع العظم للفار الأبيض (BMC). ولقد بينت النتائج عدم وجود تأثير مثبت لنقيع نبات المرسين بكلا التركيزين وكذلك التركيز الأقل لنقيع نبات الایمنم على المعايير الانقسامية في اختبار RTC، في حين أظهر التركيز الأعلى لنبات الایمنم تأثيرات مثبطة معنوية على هذه المعايير في هذا الاختبار مقارنة بالقياسي السالب. كما أن نقيع النباتين لا يظهر تأثيرات معنوية في استحثاث التشوهات الكروموسومية و معدل الانقسام في اختبار BMC مقارنة بالقياسي.

في ليبيا يستخدم نباتي المرسين *Myrtus communis* والايمنم *Plantago albicans* بشكل واسع في الطب الشعبي لعلاج العديد من الأمراض. ومع ذلك فان هنالك عدد كبير من المركبات النباتية يمكن أن تسبب عدداً من الاختلالات في المادة الوراثية. أنجزت هذه الدراسة للتحري عن إمكانية وجود تأثيرات سامة خلوية أو مطفرة لنقيع نباتي المرسين والايمنم على المعايير الانقسامية للدورة الخلوية والكروموسومات. ولقد تم تحضير تركيزين من نقيع كلا النباتين، أحدهما يستخدم في الطب الشعبي (٠,٢٥ ملجم/مل) والآخر بتركيز مضاعف ١٠ مرات لهذا التركيز (٢,٥ ملجم/مل).

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