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# Effects of *Hypericum Perforatum* L. and *Matricaria Chamomilla* L. Extracts on the Human Chromosomes

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**Abstract:** The cytogenetic effects of the *Hypericum perforatum* L. and *Matricaria chamomilla* L. extracts have been studied in cultured human lymphocytes. The extracts were prepared by perculation method using 70% ethanol as a solvent. The effects of hydroquinone and ethanol were also investigated as positive and negative controls, respectively. The cytogenetic abnormalities detected were dicentric chromosome, chromatid breaks and polyploidy. The *Hypericum perforatum* L. (at final concentration of 0.4 μg $mL^{-1}$ hypercin) and *Matricaria chamomilla* L. (at final concentration of 50 μg $mL^{-1}$ chamazulenel) extracts, significantly increased the frequency of abnormal metaphases (For extract of *Hypericum perforatum* L. OR = 6.04, 95% CI: 3.11-12.1; For extract of *Matricaria chamomilla* L. OR = 6.22, 95% CI: 3.21-12.2).

**Key words:** Herbal medicine, *Hypericum perforatum* L., *Matricaria chamomilla* L., human chromosome, aberration

## Introduction

Public interest in all things natural and organic including, especially, drugs and medicines, continues to increase at an unprecedented rate. It has developed from ancient civilizations that used parts of plants and animals to concoct various points to eliminated pain, control suffering and counteract disease. A number of the drugs used by the ancients are still employed in much the same manner by today's medical practitioners (Tyler *et al*., 1988; Wheaton *et al*., 2005).

The dried, flowerings, aeril parts of *Hypericum perforatum* L. (Fam. Guttiferae), St. John's Wort, are described in the British Herbal Pharmacopia (BHP). The drug acts as a sedative and astringent. It has antibacterial properties and is reported to be used in the former USSR for the treatment of infections, in the USA as a food preservative and in Germany for making soft drink (Evans, 1996).

Chamomile [*Matricaria chamomilla* L. (Fam. Compositae)] is extensively cultivated in Europe, where it is widely utilized in folk medicine for its carminative, spasmolytic and anti-inflammatory effects (Evans, 1996; Tyler *et al*., 1988). In Iran it is used as a vegetable and medicine. The most common from of the drug is a tea, but various extracts and volatile-oil-containing preparations are also available. In fact, chamomile is so highly regarded and so extensively used that it might be labeled ginseng of Europe (Evans, 1996; Tyler *et al*., 1988). Although the chamomile and *Hypericum perforatum* L. have become one of the most popular herbal medicine in several populations, those mutagenecity effect is still unknown. In the present study, the cytogenetic effects of the *Hypericum perforatum* L. and *Matricaria chamomilla* L. extracts have been studied in cultured human lymphocytes.

## Materials and Methods

*Subjects and Cell Culture*

Blood samples were obtained from five healthy subjects and collected into heparinized tubes. None of the individuals had confounding factor(s) (e.g. current illness, on chemotherapy, exposure to radiation and chemicals, cigarette smoking). Lymphocytes were cultured for 72 h at 37°C in RPMI-1640 supplemented by 15% heat inactivated fetal calf serum, 30 μg $mL^{-1}$ Streptomycine and 0.2 $mL^{-1}$ of phetohemaglotinin-M. Chromosomes were conventionally stained with Giemsa. The metaphases were analyzed for the number and type of chromosome aberrations. Chromosome aberrations were classified to dicentric chromosomes, chromatid breaks and polyploidy.

The extracts were prepared using perculation method. The extracts of *Hypericum perforatum* L. and *Matricaria chamomilla* L. contain 0.2 μg $mL^{-1}$ hypericin and 2 μg $mL^{-1}$ chamazulene, respectively. Considering that it is reported that the hydroquinone induced chromosomal and DNA damage on human cells (Saadat *et al*., 1998) hydroquinone at final concentration of 25 μmol was used as positive control. A culture without any extracts was used as a negative control. In order to exclude the effect(s) of ethanol on chromosome damage, one percent of ethanol was added to medium (as another negative control).

*Statistical Analysis*

The experiments were done in triplicate. Correlations between frequency of abnormal metaphases and concentrations of the studied extracts' were evaluated by the rank correlation test of Spearman. The Odds Ratio (OR) and its 95% Confidence Interval (CI) were calculated. An OR>1.0 shows an increase and OR<1.0 shows a decrease in frequency of abnormal metaphases in cultures treated with either positive control or the herbal extracts in comparison with the frequency of abnormal metaphases in cultures of negative control. To take into account the possibility of heterogeneity between individuals, a statistical test for heterogeneity was carried out based on the Q-statistic, in which a p-value greater than 0.05 suggested a lack of heterogeneity (DerSimonian and Laird, 1986). Pooled OR was calculated using random-effects model (if there was significant heterogeneity between individuals) and/or fixed-effects model (if there was no heterogeneity between individuals) (Mantel and Haenszel, 1959). A probability of $p<0.05$ considered statistically significant difference.

## Results and Discussion

Since no statistically differences were observed between the sets of negative controls, these two controls were pooled (data not shown). The Spearman's rank correlation coefficients between frequencies of abnormal metaphases and concentration of either hypericin or chamazulene were statistically significant at Rs = 1.0 (data not shown). High frequencies of abnormal metaphases with relatively small SD were noticed at final concentrations of 0.4 μg $mL^{-1}$ hypercin and 50 μg $mL^{-1}$ chamazulene. At both concentrations of 100 μg $mL^{-1}$ chamazulene and 0.8 μg $mL^{-1}$ of hypericin, the studied extracts showed cytotoxicity.

Results of chromosome analysis to evaluate the clastogenic potential activities of *Hypericum perforatum* L. (final concentration of 0.4 μg $mL^{-1}$ hypericin) and *Matricaria chamomilla* L. (final concentration of 50 μg $mL^{-1}$ chamazulene) are summarized in the Table 1. Positive control significantly increased the frequency of abnormal metaphases (pooled OR = 16.40, 95% CI: 9.58-35.7). In all of subjects chromosomal aberrations increased when the extracts of *Hypericum perforatum* L. (3.22-10.55 folds) and *Matricaria chamomilla* L. (4.09-9.41 folds) were used. There was no significant heterogeneity between the subjects (For hydroquinone Q-statistic = 2.778, df = 4, $p>0.05$; For *Hypericum perforatum* L. extract Q-statistic = 2.137, df = 4, $p>0.05$; For *Matricaria chamomilla* L.

Table 1: Abnormalities induced using the studies extracts

| Individuals | Metaphase No. | Abnormal metaphases | Percent | OR | 95% CI |
|---|---|---|---|---|---|
| No. 1 | | | | | |
| Negative control | 777 | 3 | 0.39 | 1.0 | - |
| Positive control | 229 | 12 | 5.28 | 14.27 | 3.72-64.2 |
| *Hypericum perforatum* L. | 229 | 9 | 3.49 | 10.55 | 2.60-49.5 |
| *Matricaria Chamomilla* L. | 341 | 12 | 3.52 | 9.41 | 2.46-42.2 |
| No. 2 | | | | | |
| Negative control | 712 | 4 | 0.56 | 1.0 | - |
| Positive control | 90 | 9 | 10.0 | 19.67 | 5.38-77.8 |
| *Hypericum perforatum* L. | 280 | 5 | 1.78 | 3.22 | 0.75-14.3 |
| *Matricaria Chamomilla* L. | 292 | 10 | 3.42 | 6.28 | 1.80-23.9 |
| No. 3 | | | | | |
| Negative control | 351 | 2 | 0.57 | 1.0 | - |
| Positive control | 121 | 10 | 8.26 | 15.72 | 3.10-105 |
| *Hypericum perforatum* L. | 183 | 5 | 2.73 | 4.90 | 0.84-36.8 |
| *Matricaria Chamomilla* L. | 218 | 5 | 2.30 | 4.09 | 0.70-30.7 |
| No. 4 | | | | | |
| Negative control | 417 | 2 | 0.48 | 1.0 | - |
| Positive control | 54 | 10 | 18.5 | 47.16 | 9.26-322 |
| *Hypericum perforatum* L. | 207 | 9 | 4.35 | 9.43 | 1.89-63.8 |
| *Matricaria Chamomilla* L. | 140 | 4 | 2.86 | 6.10 | 0.95-48.5 |
| No. 5 | | | | | |
| Negative control | 513 | 3 | 0.58 | 1.0 | - |
| Positive control | 165 | 8 | 4.85 | 8.66 | 2.07-41.7 |
| *Hypericum perforatum* L. | 163 | 5 | 3.06 | 4.28 | 0.80-24.3 |
| *Matricaria Chamomilla* L. | 158 | 4 | 2.52 | 4.42 | 0.83-25.1 |
| Total | | | | | |
| Negative control | 2770 | 14 | 0.50 | 1.0 | - |
| Positive control * | 659 | 49 | 7.44 | 16.40 | 9.58-35.74 |
| *Hypericum perforatum* L. ** | 1062 | 33 | 3.11 | 6.04 | 3.11-12.08 |
| *Matricaria Chamomilla* L.*** | 1149 | 35 | 3.05 | 6.22 | 3.21-12.23 |

* Q-statistic = 2.778, df = 4, p>0.05, ** Q-statistic = 2.137, df = 4, p>0.05, *** Q-statistic = 0.849, df = 4, p>0.05

extract Q-statistic = 0.849, df = 4, p>0.05). Using pooled data *Hypericum perforatum* L. (OR = 6.04, 95% CI: 3.11-12.1) and *Matricaria chamomilla* L. (OR = 6.22, 95% CI: 3.21-12.2) extracts significantly increased the frequency of abnormal metaphases.

Present data on *Hypericum perforatum* L. confirmed the previous study of other investigators, who used Ames test with two bacterial species named TA98 and TA100 (Moradian and Javadi, 2000). Also it is reported that *Hypericum perforatum* L. may lead to severe hematologic toxicity, with conditions involving bone marrow necrosis (Demiroglu *et al*., 2005).

It should be noted that *Matricaria chamomilla* L. showed genotoxic activity using the Somatic Mutation and Recombination Test in wings of *Drosophila melanogaster* (Romero-Jimenze *et al*., 2005). On the other hand, the inhibitory effect of *Matricaria chamomilla* L. essential oil on the sister chromatid exchanges induced by daunorbicin and methyl methanesulfonate in mouse bone marrow was reported (Hernandez-Ceruelos *et al*., 2002).

There is no information on the potential adverse effects of the studies herbal medicine extracts' on the human and/or experimental animal. The use of herbal medicines by specific populations, including children, is special concern (Lin *et al*., 2004; Woolf, 2003). Therefore, these extracts must be used cautiously in generally and particularly in pregnant women. Further experiments are necessary to clarify the significance of the present findings.

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**References**

Demiroglu, Y.Z., T.T. Yeter, C. Boga, H. Ozdogu, E. Kizilkilic, N. Bal, I. Tuncer and H. Arslan, 2005. Bone marrow necrosis: A rare complication of herbal treatment with *Hypericum perforatum* (St. John's wort), Acta Medica (Hradec Kralove), 48: 91-94.

DerSimonian, R. and N. Laird, 1986. Meta-analysis in clinical trials. Controlled Clin. Trials, 7: 177-188.

Evans, W.C., 1996. Trease and Evans Pharmacognosy. 14th Edn., WB. Saunders Company Ltd. London pp: 248.

Hernandez-Ceruelos, A., E. Madrigal-Bujaider and C. De La Cruz, 2002. Inhibitory effect of chamomile essential oil on the sister chromatid exchanges induced by daunorbicin and methyl methanesulfonate in mouse bone marrow. Toxicol. Lett., 135: 103-110.

Lin, Y.C., A.B. Bioteau, L.R. Ferrari and C.B. Berde, 2004. The use of herbs and complementary and alterative medicine in pediatric preoperative patents. J. Clin. Anesth., 16: 4-6.

Mantel, N. and W. Haenszel, 1959. Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst., 22: 719-748.

Moradian, R. and I. Javadi, 2000. Abstract Book of 1st National Congress on Human Genetics. Shahrekord, Iran, pp: 61.

Romero-Jimenez, M., J. Campos-Sanchez, M. Analla, A. Munoz-Serrano and A. Alonoso-Moraga, 2005. Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. Mutat. Res., 585: 147-155.

Saadat, I., A.A. Allameh and M. Saadat, 1998. DNA-repair capacity in Down's syndrome. Iran. Biomed. J., 2: 123-127.

Tyler, V.E., L.R. Brady and J.E. Robbers, 1988. Pharmacognosy. 9th Edn., Lea and Febiger, Philadelphia, pp: 466.

Wheaton, A.G., H.M. Blanck, Z. Gizlice and M. Reyers, 2005. Medicinal herb use in a population-based survey of adults: Prevalence and frequency of use, reasons for use among their children. Ann. Epidemiol., 15: 678-685.

Woolf, A.D., 2003. Herbal remedies and children: Do they work? Are they harmful? Pediatrics, 112: 240-246.