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Genotoxic Effects of *Hypericum heterophyllum* Vent. in Human Lymphocytes Cultures

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Abstract *Hypericum* species, growing wild in Anatolia, widely used in traditional medicine in Turkey. In order to determine the genotoxic effect of *Hypericum heterophyllum*, human lymphocytes were incubated with the aqueous extracts. The increasing extract concentrations of *Hypericum heterophyllum* induced micronucleus. The rise of micronucleus shows that *Hypericum heterophyllum* at high concentrations may become carcinogenic and genotoxic. The positive correlation was observed between micronucleus and age. Also, the positive correlation was observed between micronucleus rates of female and male. Further studies will be needed to determine the effects of the main bioactive components isolated from this species on micronucleus.

Keywords Hypericum, Genotoxic, Micronucleus

1. Introduction

Traditional oriental herbal prescriptions have become popular over the past decade; they are widely used for the treatment and prevention of various diseases due to their effectiveness[1].

The genus *Hypericum* L. (St. John's Wort, Hypericaceae) includes at the most recent count, 484 species that are either naturally occurring on, or which have been introduced to, every continent in the world, except Antarctica. These species occur as herbs, shrubs and infrequently trees, and are found in a variety of habitats in temperate regions and in high mountains in the tropics, avoiding only zones of extreme aridity, temperature and salinity[2]. *Hypericum heterophyllum* Vent., an Endemic Turkish species, is a source of medicinal compounds and well known with its antifungal activity[3].

A micronucleus (MN) is a small extra nucleus separated from the main one, generated during cellular division by late chromosomes or by chromosome fragments. Because of its association with chromosomal aberrations, MN has been used since 1937 as an indicator of genotoxic exposition based on the radiation studies conducted by Brenneke and Mather[4]. Investigations on MN frequencies support the widely accepted assumption that MN is a product of early events in human carcinogenic processes[5].

Evaluation of the genotoxic potential is one of the most important nonclinical safety studies required for registration and approval for marketing of pharmaceutical products. Furthermore, studies on the genotoxicity of medicinal plants used by the population are needed to identify those which pose mutagenic and carcinogenic risks. In the present work, we attempted to evaluate the genotoxic effects of *Hypericum heterophyllum* extracts used in traditional medicine in Turkey. For this purpose, the extracts were assessed by the micronucleus (MN) on human peripheral blood lymphocytes.

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2. Materials and Methods

2.1. Preparation of the Extracts

Aqueous extracts (AE) (decoction) were prepared by boiling the air-dried aerial parts of the plants grounded by mechanical mill in water at 100℃ for 5 min in the case of decoction. Preparations were sterilized through a filter and stored at + 4℃.

2.2. Chemicals

PB karyotyping medium (Biological Industries, Israel), colcemid (Sigma, Germany), cytochalasin B (Sigma) and giemsa stain (Merck, Germany) were used in peripheral blood cultures. PB karyotyping medium is based on RPMI-1640 basal medium supplemented with L-glutamine, foetal bovine serum, antibiotics (gentamycin) and phytohemagglutinin.

2.3. In Vitro Micronucleus Assay

After getting approval from Yozgat Government Hospital, heparinized blood samples (0.4 mL), obtained from ten healthy donors, were placed in sterile culture tubes containing 5 mL of PB karyotyping medium. Then, AE were added

to obtain the four final concentrations (0.05, 0.1, 0.5 and 1 mg/mL). After mixing the contents of each culture tube by gently shaken, the culture tubes were incubated in a slanted position at 37℃ for 72 h. Cytochalasin B was added at 44 h of incubation at a final concentration of 5 µg/mL to block cytokinesis. After 72 h of incubation, the cultures were harvested and stained according to Fenech and Morley[6].

2.4. Statistical Analysis

The computer software program SPSS 10.0 was used to analyze the data. The statistical significance of the effects of *Hypericum heterophyllum* on the MN was assessed using repeated measures of the analysis of variance (ANOVA) and the differences between groups were determined by the Tukey-Kramer test with $p < 0.01$ and $p < 0.05$ were considered significant. Correlation and regression coefficients were calculated between two parameters (MN and doses, MN and age, MN male and MN female).

3. Results and Discussion

Hypericum species, growing wild in Anatolia, are aromatic plants. In this study, genotoxic and clastogenic effects of *Hypericum heterophyllum* were investigated in cultured human lymphocytes. The peripheral lymphocytes are one of the best materials for the determination of cytogenetic effects. The MN technique has been proposed as a useful tool for measurement of genotoxicity *in vivo* and *in vitro* cultures. MN arises during cell division either from chromosomes that are lagging in anaphase or from chromosome fragments[6]. In living creatures, which are exposed to a mutagen factor, the probability of formation of mitotic and meiotic defects is increased and the rate of MN could increase due to this increase[7]. Alcohol consumption, smoking and viral infections increase MN rates in peripheral blood lymphocytes[8]. The donors chosen for this study did not smoke or consume alcohol. They had not been exposed to X-ray and gamma-ray and they did not have any viral infections.

Table 1. Micronucleus (%) (mean ± SDs) in human lymphocyte cultures exposed to extracts of *Hypericum heterophyllum*

Donor		Concentrations (mg/mL)				
Gender	**Age**	**Control**	**0.05**	**0.1**	**0.5**	**1**
Female	18	0.8	1.4	1.4	2.4	2.4
Female	25	0.8	1.2	1.6	2.2	2.0
Female	30	1.0	1.4	1.6	2.2	2.6
Female	40	1.0	1.2	1.2	1.6	2.4
Female	50	1.4	2.0	2.4	3.0	3.2
Male	22	0.4	1.0	0.8	1.8	1.8
Male	27	0.6	1.0	1.4	2.0	2.2
Male	38	0.6	1.2	1.0	1.8	2.4
Male	56	1.2	1.2	1.8	2.4	2.8
Male	73	1.2	2.0	1.8	2.2	2.6
MN Mean ± SDs		**0.90 ± 0.32**	**1.36 ± 0.36**	**1.50± 0.45***	**2.16 ± 0.40****	**2.44 ± 0.40****

* $p<0.05$, ** $p<0.01$ (significantly different from control)
SS: Standard Deviation

The results of MN test are given in Table 1. When MN formation was analysed after treatment with different concentrations of the extracts of *Hypericum heterophyllum*, significant changes in the percentage of MN were detected for 0.1, 0.5 and 1 mg/mL ($p < 0.05$) and for 0.5 and 1 mg/mL ($p < 0.01$). Other extract concentration (0.05 mg/mL) did not induce any change in MN frequencies compared to untreated group (control) ($p > 0.01$ and $p > 0.05$). An increase in MN may result from interactions of a great variety of cytotoxic and genotoxic agents with DNA. MN is an extremely valuable and highly relevant endpoint for the detection of potential carcinogens. Our results show an increase in the percentage of MN (Table 1), suggesting a strong interaction between extracts (0.5 and 0.1 mg/mL) of *Hypericum heterophyllum* and DNA, which could be responsible for the observed genotoxicity.

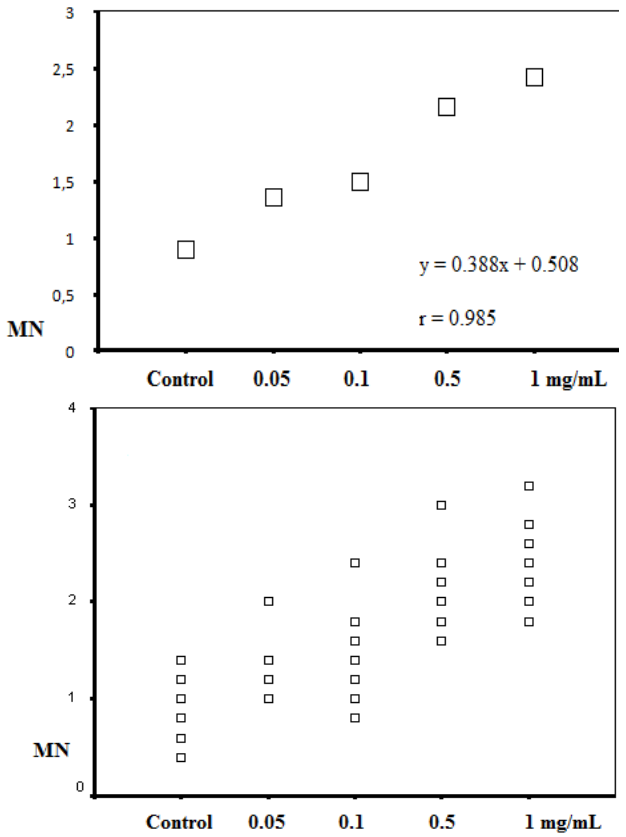

Figure 1. The positive correlations between micronucleus and the extract concentrations

Correlation and regression coefficients between MN and doses are given in Figure 1. According to Figure 1, the strong positive correlation was observed between MN and doses (r = 0.985). Also, the positive correlation was observed between MN and age (r = 0.613) (Figure 2). It was reported the positive correlation between MN and age[9,10]. The increase of MN with age is likely due to a combination of factors which include (i) the cumulative effect of acquired mutations in genes involved in DNA repair, chromosome segregation and cell cycle checkpoint and (ii) numerical and structural aberrations in chromosomes caused by exposure to endogenous genotoxins, inadequate nutrition, exposure to

environmental or occupational genotoxins, as well as a wide range of unhealthy lifestyle factors[11]. The moderate positive correlation was observed between MN rates of female and male (r = 0.447) (Figure 3). The MN rates of female were higher than male (Table 1). The increase in MN frequency in females can be accounted for by the greater tendency of the X chromosome to be lost as an MN relative to other chromosomes, and to the fact that females have two copies of the chromosome compared to only one in males [12,13].

In summary, it can be concluded that the *Hypericum heterophyllum* show considerable clastogenic and genotoxic effects as observed *in vitro* in human lymphocytes. Further studies will be needed to determine the effects of the main bioactive components isolated from this species on MN.

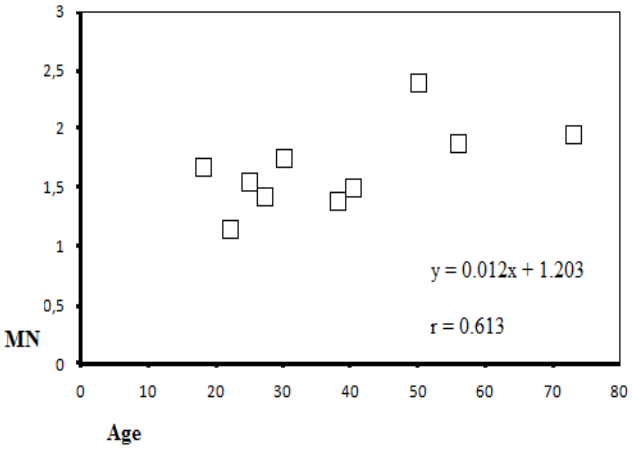

Figure 2. The positive correlation between micronucleus and age

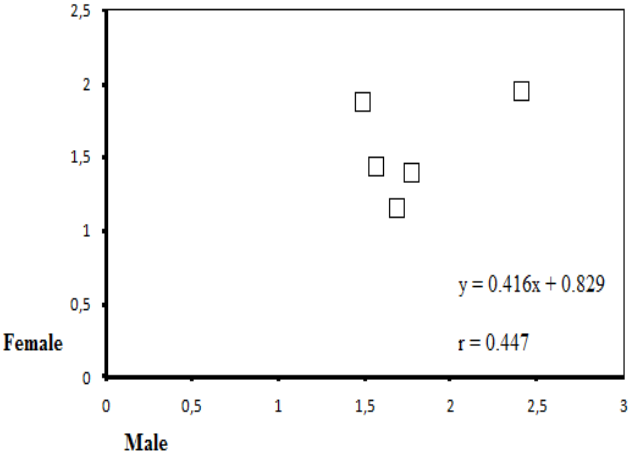

Figure 3. The positive correlation between MN rates of female and male

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