

THE ANTIRADICAL, ANTI-INFLAMMATORY AND ANTI-GENOTOXIC POTENTIAL OF HERBAL PREPARATION CHLAMYFIN

Andreja Leskovic¹, Gordana Joksić¹, Igor Pašti², Tamara Lazarević-Pašti¹,
Branislav Nastasijević¹, Sandra Petrović¹

¹*Vinča Institute of Nuclear Sciences, University of Belgrade,*

M. Petrovića Alasa 12-14, 11001 Belgrade, Serbia

²*Faculty of Physical Chemistry, University of Belgrade,*

Studentski trg 12-16, 11158 Belgrade, Serbia

andreja@vinca.rs

Herbal preparation Chlamyfin was investigated for its total polyphenol content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, and anti-inflammatory and genotoxic properties. A high total polyphenol content provided evidence of high DPPH radical scavenging activity ($IC_{50} = 4.96 \pm 0.23 \mu\text{g/ml}$). Analysis of the electrochemical behavior of Chlamyfin indicated high reducing ability, i.e., high antioxidant capacity, in agreement with the DPPH test. Analysis of myeloperoxidase (MPO) inhibition by Chlamyfin suggested anti-inflammatory action (IC_{50} values of $5.40 \mu\text{g/ml}$ and $4.45 \mu\text{g/ml}$ for an incubation time of 10 and 30 min, respectively). For genotoxic assessment, oxidative stress was induced by irradiation of peripheral whole blood with gamma-radiation *in vitro*. In the presence of Chlamyfin, reduced incidence of micronuclei without disturbance to the proliferative potential of cells was evidenced in both irradiated and unirradiated samples, indicating its genoprotective properties. It was shown that Chlamyfin, in addition to its bactericidal effect, also possesses strong antioxidant, anti-inflammatory and anti-genotoxic properties.

Keywords: Chlamyfin; polyphenol; antiradical effects; anti-inflammatory potential; anti-genotoxic potential

АНТИРАДИКАЛСКИ, АНТИВОСПАЛИТЕЛЕН И АНТИГЕНОТОКСИЧЕН ПОТЕНЦИЈАЛ НА ХЕРБАЛНИОТ ПРЕПАРАТ ХЛАМИФИН

Хербалниот препарат Хламифин е испитуван во однос на вкупната содржина на полифеноли, антирадикалската активност на 2,2-дифенил-1-пикрилхидразил (DPPH), како и антивоспалителните и генотоксичните својства. Високата вкупна содржина на полифенолите ја потврдува високата антирадикалска активност на DPPH ($IC_{50} = 4,96 \pm 0,23 \mu\text{g/ml}$). Анализата на електрохемиското однесување на Хламифин покажа висока редуциона способност, т.е. висок антиоксидациски капацитет во согласност со тестот со DPPH. Анализата на инхибицијата на миелопероксидазата (MPO) со Хламифин укажува на антивоспалително дејство (IC_{50} вредности од $5,40 \mu\text{g/ml}$ и $4,45 \mu\text{g/ml}$ за време на инкубација соодветно од 10 и 30 min). За генотоксичната процена беше индуциран генотоксичен стрес со *in vitro* озрачување на периферната крв со гама радијација. Во присуство на Хламифин беше утврдена намалена појава на микројадра без нарушување на репродуктивниот потенцијал на клетките и кај озрачените и кај неозрачените примероци, покажувајќи генозаштитните својства. беше покажано дека Хламифин, покрај бактерицидното дејство, покажува и силни антиоксидациски, антивоспалителни и антигенотоксични својства.

Клучни зборови: Хламифин; полифенол; антирадикалско дејство; антивоспалителен потенцијал; антигенотоксичен потенцијал

1. INTRODUCTION

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen causing urogenital, ocular, and cardiovascular infections, with an estimated 92 million new cases occurring worldwide each year [1]. These infections frequently have a nonspecific clinical picture and atypical symptoms, which become the source of more or less serious complications. There is growing evidence that the production of reactive oxygen species (ROS) is rapidly elevated during infection, serving to facilitate pathogen clearance as well as contributing to the signaling cascades associated with inflammation, cell proliferation and immune responses [2]. However, it has been reported that late-stage chlamydial infection induces significant damage to the infected cells, as evidenced by increased lactate dehydrogenase release, reactive oxygen species production and a reduced ATP level [3]. At least six months antibiotic therapy without a break is needed to treat infections by *Chlamydia* species. Patients undergoing therapy are often immunosuppressed and susceptible to opportunistic infections, so proper nutrition is an imperative [4]. Natural remedies which have antioxidant potential could therefore be very useful, especially during or after antibiotic therapy.

Myeloperoxidase (MPO) plays a very important role in neutrophil microbicidal action. It is a heme enzyme that is present in large amounts in neutrophils and is also found in monocytes [5, 6]. In the presence of halide and pseudohalide thiocyanate ions, MPO catalyzes the oxidation of these substrates with hydrogen peroxide, producing oxidizing and halogenating agents. Halogenating agents, especially hypochlorous acid (HOCl), promote the oxidative killing of micro-organisms by neutrophils, but also the destruction of healthy tissue in inflammatory diseases [7, 8]. However, MPO is relatively nonspecific with respect to its reducing substrates, some of which inhibit its activity. Suicide substrates undergo a structural change during the course of catalysis that results in ir-

reversible binding to the enzyme, inactivating it [9]. In contrast to these substrates, numerous non-steroidal and anti-inflammatory drugs, anilines and phenols inhibit MPO reversibly [10, 11].

Herbal preparation Chlomyfin is a herbal mixture that contains extracts of medicinal plants (*Arctostaphylos uva-ursi* (family: Ericaceae), *Plantago lanceolata* (family: Plantaginaceae), *Thymus serpyllum* (family: Lamiaceae), *Juniperus communis* (family: Cupressaceae) and *Syzygium aromaticum* (family: Myrtaceae)) that have for decades been used in traditional medicine to treat infections and diseases of the urogenital tract, especially *Chlamydia* infection, nonsurgical sterility and prostate disease [12–16]. To date, bactericidal effects of Chlomyfin have been demonstrated for *Chlamydia trachomatis*, *Escherichia coli*, *Mycoplasma hominis* and *Ureaplasma urealyticum*, which is mainly attributed to the antibacterial effects of *Arctostaphylos uva-ursi*, *Juniperus communis* and *Syzygium aromaticum* [17–19]. Chlomyfin has been recommended in the treatment of urinary tract infections at doses of four capsules per day (one capsule is equivalent to 0.08 mg/ml). As a complex herbal mixture, effects other than anti-bacterial ones might also be operative in Chlomyfin, possibly providing joint action when treating chlamydial infections. An interdisciplinary approach was used in this study to investigate the total polyphenol content, antiradical capacity, anti-inflammatory properties (by examination of the inhibitory effects on MPO activity) and anti-genotoxic effects of Chlomyfin herbal preparation.

2. EXPERIMENTAL

2.1. Plant material and extract preparation

Chlomyfin[®] capsules were obtained from Medical F International, Novi Sad, Serbia. One capsule contained 400 mg of lyophilized herbal extracts consisting of *Arctostaphylos uva-ursi* 141.60 mg, *Plantago lanceolata* 35.60 mg, *Thymus serpyllum* 141.60 mg, *Juniperus*

communis 70.80 mg, and *Syzygium aromaticum* 10.40 mg. The contents of the capsules were resuspended in distilled water, then sonicated for 5 min and centrifuged at 4000 rpm to remove insoluble additives. The supernatants obtained were diluted with distilled water to a concentration of 10 mg/ml.

2.2. DPPH radical-scavenging activity

The free radical scavenging capacity of the extracts, based on the scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, was determined using the method described by Brand-Williams [20]. DPPH (Fluka Chemie AG Buchs, St. Louis, USA) was dissolved in methanol to a final concentration of 78 µg/ml. The following concentrations of Chlamyfin were prepared: 1, 2.5, 5, 7.5 and 10 mg/ml. Then, 50 µl of these extracts were diluted with methanol up to 4 ml, and then mixed with 1 ml of DPPH (0.2 mM solution). After 30 min, absorbance was measured at wavelength of 517 nm, using a control prepared with water instead of herbal preparations, against methanol as a blank. The inhibition of DPPH was calculated as:

$$\text{Inhibition (\%)} = 100 \times \frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}}$$

where A_{DPPH} is the absorption of the DPPH control solution against the blank, and A_{sample} is the absorption of the extract against the blank. The extract concentration that yielded 50% of the antioxidant activity (IC_{50}) was obtained by plotting the DPPH inhibition against the plant extract concentration.

2.3. Determination of total polyphenol content using the Folin–Ciocalteu method

The total polyphenol content was determined using a colorimetric method described by Singleton and Rossi [21]. Extracts were diluted to a concentration of 1 mg/ml. Oxidation was

performed by mixing of Folin–Ciocalteu (Appli-Chem-Biochemica, Darmstadt, Germany) reagent (500 µl) with 100 µl of each extract solution, followed by the addition of 2 ml of 15% Na_2CO_3 . Reaction mixtures were diluted up to 10 ml with distilled water. Absorption was measured after 2 h at a wavelength of 750 nm, against a blank and prepared with water, using the same procedures as for the samples. Quantification of total polyphenols in the extracts was made using gallic acid (Sigma-Aldrich, St. Louis, MO, USA) to establish a calibration curve, covering a range from 100 to 800 µg/ml. The results were expressed as mg of gallic acid equivalent per 1 g of dry weight of herbal extract.

2.4. Cyclic voltammetry analysis

Electrochemical measurements were performed using a conventional one-compartment three-electrode electrochemical cell with working glassy carbon (GC) disk electrode (base surface area 0.196 cm²). A saturated calomel electrode (SCE) and a large platinum plate were used as a reference and counter electrode, respectively. Solutions were purged with high-purity N_2 during the experiments. Electrochemical behavior was investigated in phosphate buffer solutions (pH 3, 7 or 9), with the addition of 0.05 M K_2SO_4 , in order to ensure good electric conductivity of the analyzed solutions. Chlamyfin was analyzed by diluting 1 cm³ of supernatant (Section 2.1.) in 10 cm³ of phosphate buffer solution. Between each measurement, the GC working electrode was thoroughly polished using diamond paste and washed with acetone and water. The measured current was expressed per unit mass of dry extract. The results were correlated to total polyphenol content and antioxidant capacity of the plant extracts.

2.5. Measurement of myeloperoxidase activity

Myeloperoxidase (MPO) from human neutrophils, purified to an $R_z (A_{430}/A_{280})$ of 0.84, was obtained from Planta Natural Products,

Vienna, Austria. Its concentration was calculated using $\epsilon_{430} = 91000 \text{ M}^{-1}\text{cm}^{-1}$ per heme [22].

O-dianisidine assay was used to investigate the influence of Chlamyfin on MPO activity. The experiments were performed by *in vitro* exposure of free enzyme (15 nM) to Chlamyfin in a concentration range from 1 to 80 $\mu\text{g/ml}$ in a final volume of 3 ml. The MPO and Chlamyfin was incubated for 10–30 min before the reaction was started using 0.15 mM hydrogen peroxide. An amount of 0.53 mM *o*-dianisidine (Sigma-Aldrich, St. Louis, MO, USA) was applied as an enzyme substrate and chromogenic reagent, and the formation of the product was monitored spectrophotometrically at 460 nm for 1 min. The control value was measured in the absence of Chlamyfin. The reaction was carried out in 50 mM phosphate buffer of pH 6.0 [23]. The spectrophotometric measurements were performed on a Perkin Elmer Lambda 35 UV-vis spectrophotometer, using a 1-cm path length cuvette. pH measurements were performed using a Metrohm pH meter, model 713, equipped with a glass electrode.

2.6. Cell cultures and irradiation procedure

Peripheral blood used in the experiment was obtained from three healthy, non-smoking young male donors in accordance with the current Health and Ethical regulations in Serbia [24]. Aliquots of heparinized whole blood were irradiated using a ^{60}Co γ -ray source (radiation dose of 2 Gy). Aliquots of unirradiated and irradiated whole blood (0.5 ml) were placed in cultures containing PB-max karyotyping medium (Invitrogen-Gibco, Paisley, UK) and treated with increasing doses of an aqueous solution of Chlamyfin (final concentrations of 0.08 mg/ml, 0.16 mg/ml, 0.32 mg/ml, 0.48 mg/ml and 0.64 mg/ml, respectively). Cell cultures grown in the absence of the herbal preparation served as a control.

2.7. Micronucleus assay and slide scoring

For micronuclei (MN) preparation, the cytokinesis block method was followed [25]. Cytochalasin B (Sigma-Aldrich, St. Louis, MO, USA) was added to each culture 44 h after incubation in order to inhibit cytokinesis. The lymphocyte cultures were incubated for a further 28 h. Cells were collected by centrifugation and treated with hypotonic solution (0.56% KCl+0.90% NaCl, mixed in equal volumes) at 37°C. Cell suspension was fixed in methanol/acetic acid (3:1), washed three times with fixative and dropped onto clean slides. Slides were air-dried and stained in alkaline Giemsa.

All slides were analyzed with an Axio-Imager A1 microscope (Carl Zeiss, Jena, Germany). A minimum of 1000 binucleated cells were scored to evaluate the percentage of cells with one, two three, four, or more than four micronuclei.

A cytokinesis-block proliferation index (CBPI) was calculated using the equation: $\text{CBPI} = [\text{MI} + 2\text{MII} + 3(\text{MIII} + \text{MIV})]/\text{N}$, where MI-MIV represents the number of cells with one to four nuclei, respectively, and N is the number of cells scored [26].

2.8. Statistics

A statistical analysis was carried out using the statistical software package Statistica 7.0 and OriginPro 8 for Windows Vista. A Student's *t* test was used to evaluate the parameters under consideration. A P value < 0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

Herbal preparation Chlamyfin was investigated for antiradical activity and total polyphenol content, MPO-inhibition activity and genotoxic effects.

3.1. DPPH radical-scavenging capacity and total phenolic content

The scavenging activity of the DPPH free radical revealed that Chlamyfin herbal preparation has a high antiradical activity (Table 1). Since Chlamyfin is in fact a mixture of several plants in different proportions, it is difficult to determine which of them has the greatest impact on the overall antiradical activity. The antiradical scavenging activity of this herbal preparation is comparable to the scavenging activity of individual polyphenol compounds, such as quercetin, known to be highly efficient radical scavengers [27] (Table 1), or herbal extracts and herbal mixtures [28]. The rather high total phenolic content of Chlamyfin was determined using the Folin–Ciocalteu method (Table 1). According to the applied analytical procedure, this value was derived from all the flavonoids, anthocyanins and nonflavonoid phenolics present in the sample.

Table 1

Antiradical activity of herbal preparations and quercetin (standard) obtained by the DPPH test and total polyphenol content of these extract^a

| Herbal preparation | DPPH IC ₅₀ inhibition (µg/ml) | Total polyphenol content ^b (mg/g) |
|--------------------|--|--|
| Chlamyfin | 4.96±0.23 | 268.4±3.8 |
| Quercetin | 0.91±0.04 | – |

^aData are represented as means ± standard deviation of three independent experiments

^bTotal phenolic content was expressed as mg of Gallic acid equivalents per 1 g dry weight of extracts.

Extracts of certain constituents of Chlamyfin have been analyzed in terms of phenolic profile and antioxidant activity. For example, Beara *et al.* [29] analyzed methanol/water extract (4:1) from *P. lanceolata*, identifying the number of phenol compounds, as well as estimating the antioxidant activity. The authors

determined IC₅₀ values for the scavenging of DPPH[•], HO[•], O₂^{•-} and NO[•] to be 4.20±0.19 µg/ml, 236.12±9.35 µg/ml, 23.85±0.79 µg/ml and 0.19±0.02 mg/ml, respectively. Komes *et al.* [30] demonstrated the antioxidant capacity of different *Thymus serpyllum* extracts using a ferric reducing/antioxidant power (FRAP) assay and an ABTS radical cation decolorization assay. Elmestas *et al.* [31] demonstrated a strong *in vitro* antioxidant activity of water and the ethanol extracts of *Juniperus communis*. These authors have shown that both extracts display free radical scavenging activity, superoxide anion radical scavenging and hydrogen peroxide scavenging activity, as well as metal chelating activities. An aroma extract of *Syzygium aromaticum* was analyzed by Lee and Shibamoto [32], who identified 22 compounds using gas chromatography and gas chromatography/mass spectrometry. The antioxidant activity of the extract itself, as well as its major components, was confirmed by analysis of malonaldehyde formed from cod liver oil at various levels. In addition, Makchuchit *et al.* [33] analyzed the DPPH scavenging activity and inhibition of NO production by ethanolic *Syzygium aromaticum* extract. The authors determined IC₅₀ values amounting to 6.57 ± 0.31 and 81.3 ± 2.6 µg/ml.

3.2. Cyclic voltammetry measurements

Cyclic voltammograms of Chlamyfin displayed pH-dependent electrochemistry and irreversible behavior with poorly resolved voltammetric peaks (Figure 1). The pronounced onset potential shift toward lower potentials upon increasing the pH value of the solution indicated that the oxidation process was accompanied and facilitated by deprotonation, as being characteristic of polyphenolic compounds [34]. This indicates that the effectiveness of Chlamyfin as a potential antioxidant (see below for details) under physiological conditions might be highly dependent on the pH of the environment.

As Chlamyfin is a complex mixture, it was not possible to make a detailed analysis of the obtained cyclic voltammograms. However, its electrochemical behavior can be correlated with antioxidant activity and total polyphenol content. It has previously been demonstrated that the electrochemical response at low potentials, in terms of passed charge, is important for estimating the antioxidant capacity of a complex mixture (assessed by the DPPH scavenging test), and can be correlated rather well to the total polyphenol content [28].

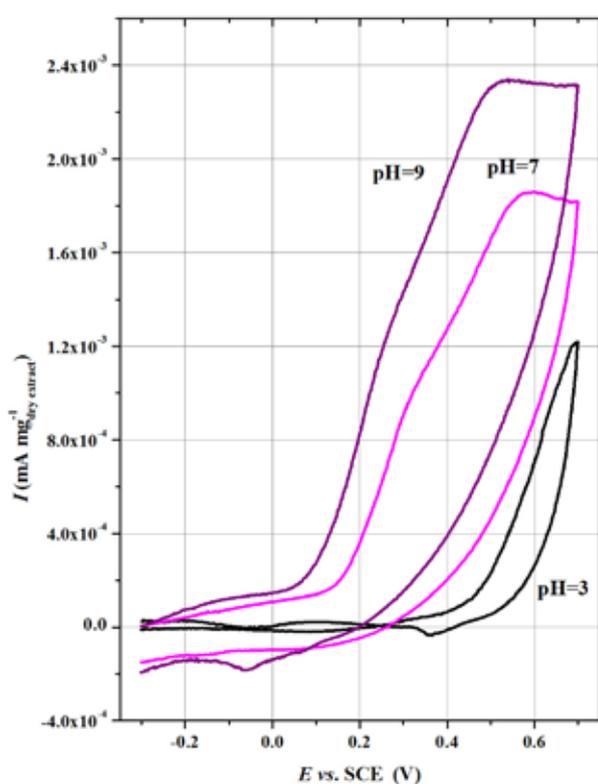


Fig. 1. Cyclic voltammograms of Chlamyfin solutions at pH = 3, 7 and 9. Measured current is expressed per unit mass of dry extracts

The obtained results indicated high response and low oxidation onset potentials (E_{onset}) for Chlamyfin. E_{onset} was determined to be 0.321, 0.114 and 0.039 V vs. SCE, at a pH of 3, 7 and 9, respectively. When compared to recently reported *Gentiana lutea* extracts [28], it can be seen that, in near neutral solutions, E_{onset} was lower by at least 100 mV. Bearing in mind

the rather good correlation of E_{onset} with DPPH IC_{50} values [28], the obtained results indicated a high DPPH radical scavenging activity as well as high total polyphenol content. This conforms excellently with the results of independent analyses of total polyphenol content and antioxidant capacity regarding DPPH radical scavenging activity (Section 3.1, Table 1). Compared to the recently reported analysis of *Gentiana lutea* extracts [28], the total polyphenol content of Chlamyfin is up to five times higher, while its DPPH IC_{50} value is lower by one order of magnitude. The low E_{onset} for Chlamyfin solutions can be linked to the high radical scavenging activity for free radicals other than DPPH, such as the peroxy radicals [35] that naturally occur under physiological conditions, while an analysis of the antioxidant activity of extracts of individual components of Chlamyfin (Section 3.1.) confirms that this herbal preparation should display a strong antioxidant activity towards different naturally occurring reactive species.

3.3. Influence of Chlamyfin on MPO activity

The influence of Chlamyfin on MPO activity was examined for concentrations of Chlamyfin ranging from 1 to 80 $\mu\text{g/ml}$ in phosphate buffer at pH 6. The effect was monitored through the oxidation of *o*-dianisidine in the presence of H_2O_2 . The concentration of oxidized *o*-dianisidine is a measure of MPO activity and was determined spectrophotometrically, as described earlier (Section 2.5.).

Primarily, the optimal concentration of MPO for the inhibition test was determined. It was found that MPO activity increased slowly until 13 nM enzyme was reached, and then began to grow rapidly. A value of 15 nM was chosen as the optimal MPO concentration for the inhibition test. At that point, MPO activity increased dramatically compared to lower concentrations. High MPO activity is very important for proper and reliable data acquisition. On the other hand, for the test to

be cost-effective, it was necessary to choose the minimal MPO concentration that would yield satisfactory results. An MPO concentration of 15 nM satisfied all these criteria.

Furthermore, the inhibition of the 15 nM MPO activity induced by Chlamyfin in a concentration range from 1 to 80 $\mu\text{g/ml}$ was evaluated. The incubation time for MPO and Chlamyfin was 10 and 30 min, respectively. As can be seen from the inhibition study (Table 2), MPO activity depended on the Chlamyfin concentration and was time-dependent. Activity of MPO is expressed as the mean value of % of MPO activity relative to the corresponding control value in the absence of OPs. It was determined that, upon increasing the incubation time between the enzyme and Chlamyfin from 10 to 30 min, the MPO activity decreased by 10 to 20%. It was also found that Chlamyfin leads to complete inhibition of 15 nM myeloperoxidase after 10 min of enzyme and Chlamyfin (20 $\mu\text{g/ml}$) incubation.

MPO inhibition curves (Figure 2) displayed a characteristic sigmoidal shape. The effects of inhibition time diminished at Chlamyfin concentrations above 8 $\mu\text{g/ml}$, indicating that

higher concentrations of Chlamyfin quickly and effectively inhibit MPO. IC_{50} values for MPO inhibition by Chlamyfin were determined from the inhibition curves. If the incubation period was 10 min, then the IC_{50} value was 5.40 $\mu\text{g/ml}$. In the case of the 30-min incubation time, the IC_{50} value was 4.45 $\mu\text{g/ml}$. There is therefore a clear difference between them, but it is not so significant, in accordance with earlier observations.

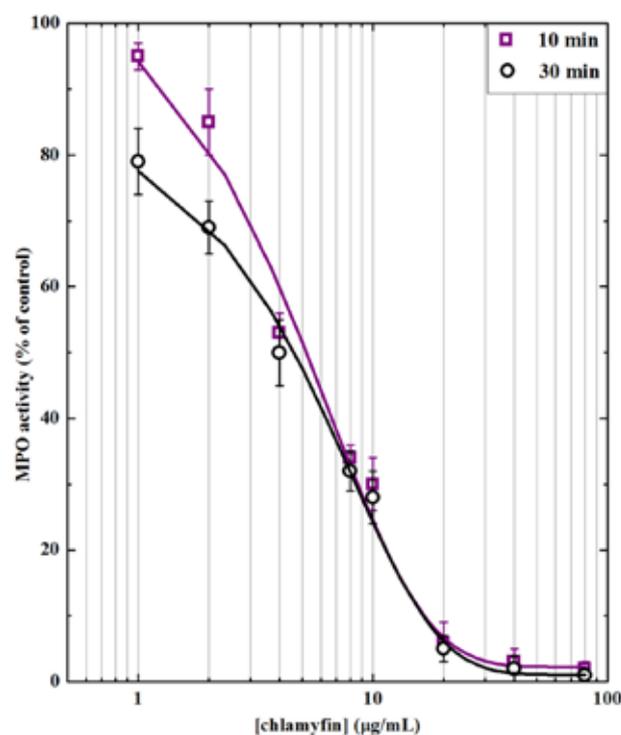


Fig. 2. Inhibition of MPO activity in the presence of Chlamyfin

Table 2

Dependence of MPO residual activity on Chlamyfin concentration (incubation time between enzyme and Chlamyfin 10 and 30 min)

| Chlamyfin ($\mu\text{g/ml}$) | MPO activity (% of control) | |
|--------------------------------|-----------------------------|--------|
| | Incubation time | |
| | 10 min | 30 min |
| 1 | 95 | 79 |
| 2 | 85 | 69 |
| 4 | 53 | 50 |
| 8 | 34 | 32 |
| 10 | 30 | 28 |
| 20 | 6 | 5 |
| 40 | 3 | 2 |
| 80 | 2 | 1 |

Previous studies have demonstrated that some anti-inflammatory plants may inhibit MPO activity [28, 36]. Therefore, the inhibitory ability of Chlamyfin towards MPO leads to the conclusion that Chlamyfin can be considered to exert an anti-inflammatory effect. Closely connected to this conclusion is the work of Beara *et al.* [29] who confirmed the anti-inflammatory effect of *P. lanceolata*, a constituent of Chlamyfin, by means of inhibition of the cyclooxygenase-1 and 12-lipoxygenase enzymes, which are involved in the metabolism of arachidonic acid.

3.4. Analysis of genotoxicity

Genotoxicity was assessed on unirradiated and irradiated human lymphocytes treated with Chlamyfin *in vitro*. The increasing doses of an aqueous solution of Chlamyfin (10 mg/ml) were added to lymphocyte cultures to give the final concentrations from 0.08 mg/ml to 0.64 mg/ml. For induction of oxidative stress, peripheral whole blood was exposed to 2 Gy of gamma radiation *in vitro*. The interaction of ionizing radiation with living cells resulted in the generation of highly reactive oxygen species (hROS), which then attacked cellular macromolecules such as DNA, RNA, proteins and membranes, causing dysfunction and damage [37, 38]. Both the direct and indirect effects of radiation can initiate damage to DNA, leading to genomic instability, which may result in mutagenesis and carcinogenesis [39].

In unirradiated samples, recommended doses of Chlamyfin (0.08 mg/ml – 0.32 mg/ml) displayed protective properties, showing a significantly reduced incidence of micronu-

clei (46%–56%, Figure 3, left) compared to the control ($P < 0.05$), without disturbing the proliferative potential of the cells (Figure 3, right). At higher concentrations, no significant induction of micronuclei was observed. Similarly, in irradiated samples, also at the recommended doses, Chlamyfin displayed radioprotective properties with a significantly ($P < 0.05$) reduced incidence of micronuclei (up to 35%, Figure 4, left) with no influence on the proliferative capacity of cells (Figure 4, right). The results unambiguously demonstrated that biologically active compounds of Chlamyfin reduce genomic injuries.

Previous reports have shown that *A. uva-ursi* is generally considered to have anti-inflammatory, diuretic and anti-microbial activity [40], while *P. lanceolata* leaf extract and *Thymus serpyllum* oils possess anti-genotoxic properties and have a protective effect against oxidative damage [41, 42]. Furthermore, *Syzygium aromaticum* exhibits antioxidative activity and, due to its antimutagenic activity, acts as chemopreventive agent [32, 43]. It has been reported

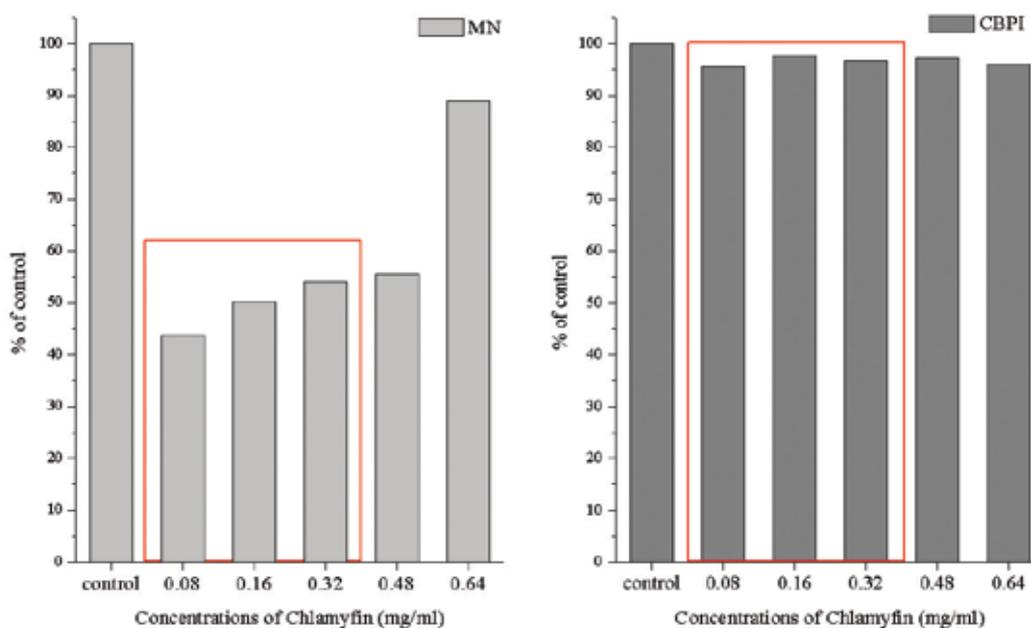


Fig. 3. Percentage of micronuclei (MN), and proliferative index (CBPI) in unirradiated human lymphocytes treated with different concentrations of Chlamyfin. Recommended doses are indicated in the figure

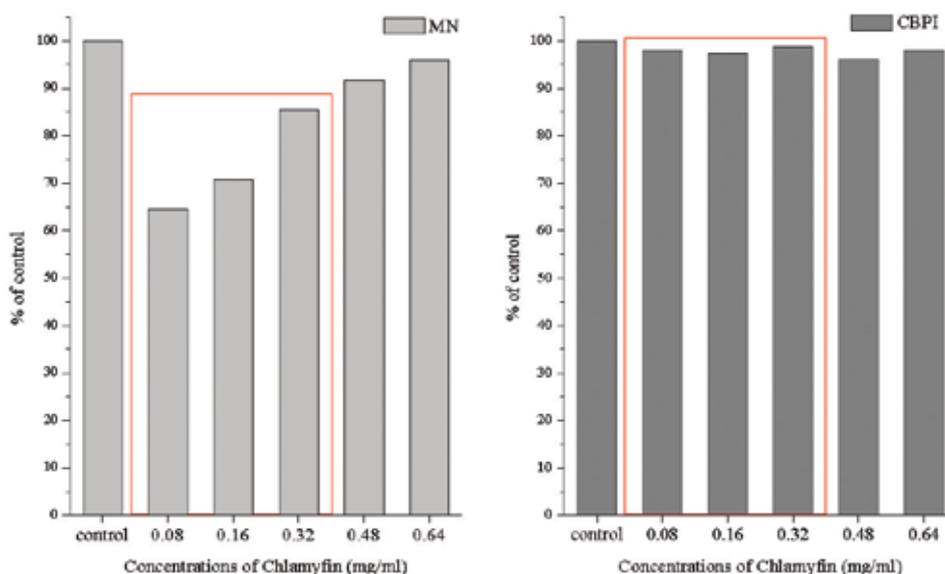


Fig. 4. Percentage of micronuclei (MN), and proliferative index (CBPI) in irradiated human lymphocytes treated with different concentrations of Chlamyfin. Recommended doses are indicated in the figure.

that *Juniperus communis* acts as an inhibitor of lipid peroxidation. Moreover, the various antioxidant mechanisms of juniper fruit extracts may be attributed to a strong hydrogen donating ability, metal chelating ability, and their effectiveness as good scavengers of hydrogen peroxide, superoxide, and free radicals [31]. In accordance with the aforementioned literature reports, we propose that the anti-genotoxic properties of Chlamyfin can be attributed to the high antioxidant capacity (Section 3.1.) of its constituents, the synergistic and/or balanced action of which reduces the level of genomic injuries, possibly by free-radical scavenging and/or by influencing the antioxidant enzyme activities.

4. CONCLUSION

Taken together, the results of the present study show that Chlamyfin, in addition to its bactericidal effect, also possesses strong antioxidant, anti-inflammatory and anti-genotoxic properties.

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