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Yellow gentian root extract provokes concentration- and time-dependent response in peripheral blood mononuclear cells

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Yellow gentian (*Gentiana lutea* L.), a medicinal plant widely used in traditional medicine, displays multiple biological effects, ranging from beneficial to toxic. Since many promising applications have been reported so far, our aim was to evaluate its potential concentration- and time- dependent cytotoxic and genotoxic effects *in vitro*. To that end we exposed human peripheral blood mononuclear cells to 0.5, 1, and 2 mg/mL of yellow gentian root extract (YGRE) to determine its effects on oxidative stress parameters [pro/antioxidant balance (PAB) and lipid peroxidation], DNA damage (alkaline comet assay and chromosome aberrations), and cell viability (trypan blue exclusion test). Cell viability decreased with increasing concentrations and treatment duration. Only the lowest YGRE concentration (0.5 mg/mL) increased oxidative stress but produced minor DNA damage and cytotoxicity. At higher concentrations, redox parameters returned to near control values. The percentage of chromosome aberrations and percentage of DNA in the comet tail increased with increased YGRE concentration after 48 h and declined after 72 h of treatment. This points to the activation of DNA repair mechanism (homologous recombination), evidenced by the formation of chromosomal radial figures after 72 h of treatment with the highest YGRE concentration of 2 mg/mL. Our results suggest that YGRE, despite induction of cytotoxic and genotoxic effects, activates cell repair mechanisms that counter oxidative and DNA lesions and induce cell death in highly damaged cells. Therefore, observed protective effects of yellow gentian after longer exposure could be a result of activated repair and removal of cells with irreparable damage.

KEY WORDS: cytotoxicity; genotoxicity; *Gentiana lutea* L.; homologous recombination; redox parameters

List of abbreviations

CA – chromosome aberrations; DAPI – 4′,6′- diamidino-2-phenylindole; DMSO – dimethyl sulphoxide; DSBs – double-strand breaks; DPPH – 2,2-diphenyl-1-picrylhydrazyl; EDS – energy dispersive spectroscopy; FESEM – field emission scanning electron microscopy; GSH – glutathione; HNE – 4-hydroxyalkenals; HR – homologous recombination; HRP – horse radish peroxidase; LMPA – low melting point agarose; LPP – lipid peroxidation products; MDA – malondialdehyde; NMPA – normal melting point agarose; PAB – pro/antioxidant balance; PBMC – peripheral blood mononuclear cells; PBS – phosphate buffer saline; ROS – reactive oxygen species; SOD – superoxide dismutase; TB – trypan blue; TMB – 3,3',5,5'-tetramethylbenzidine; UPLC – ultra performance liquid chromatography; YGRE – yellow gentian (*Gentiana lutea* L.) root extract;

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Yellow gentian (*Gentiana lutea* L.) belongs to the *Gentianaceae* family, which is common in the mountainous regions of Europe, Southeast Asia, and North America (1). Virtually all plant parts (leaves, flowers, rhizomes, roots) or whole plants are used in traditional medicine. The root has been used to mitigate gastrointestinal disorders for thousands of years. It is also used to support gallbladder and liver functions and treat dyspepsia, anorexia, fever, sore throat, and rheumatic pain (2). It has a bitter taste due to the presence of triterpenoids, amarogentin and gentiopicroside in particular. Its extract contains many other bioactive compounds, such as xantones, flavonoids, and iridoids (1, 3), some of which exhibit beneficial anti-inflammatory, antioxidative, radioprotective, immunomodulatory, and antimicrobial effects (2, 4–7). Some reports suggest that the root extract has a potential to prevent and treat obesity, prevent insulin resistance and type 2 diabetes mellitus, and to mitigate cardiovascular complications, including atherosclerosis (8–10).

Therefore, it is hardly surprising that yellow gentian extract has been the subject of numerous pharmacological studies in recent years. However, several have reported that crude extract can cause oxidative and genotoxic damage, disturb cell proliferation, and trigger cell death (11–13). Its

cytotoxic effects have also been demonstrated against malignant cell lines, suggesting that yellow gentian extract has a potential for anticancer treatment (14, 15).

Taking into account multiple biological effects of yellow gentian, widespread traditional use and high potential for applications, evaluation of its cytotoxicity and genotoxicity are essential.

The aim of this study was to evaluate *in vitro* effects of yellow gentian root extract (YGRE) applied at different concentrations on redox parameters (pro/antioxidant balance and lipid peroxidation), DNA damage, and survival of human peripheral blood mononuclear cells after 48 and 72 h of treatment.

MATERIALS AND METHODS

Yellow gentian root extract (YGRE) preparation

Yellow gentian root (serial number 03970219) was purchased from the Institute of Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia (CAC/RCP 1-1969, rev.4-2003). Before extraction, the root was ground with a hand grinder. In order to optimise and standardise the extraction technique, ground particles were imaged after each stage of extract preparation, namely powder, lyophilisate, and dissolved lyophilisate (Figure 1), with a field emission scanning electron microscope (FESEM) with energy dispersive spectroscope (EDS) (FEI SCIOS 2, Thermo Fisher Scientific FEI Company, Tokyo, Japan) at 350x magnification and their size determined with SemAfore 5.2 software (Insinööritoimisto J. Rimppi Oy, Ojakkala, Finland).

The obtained aqueous extract was placed in 25 mL acrylic chambers, transferred in the freezer at -18 °C and allowed to freeze for 24 h. Pre-frozen supernatant was then vacuum freeze-dried under vacuum pressure of 400 Pa for 48 h.

Before dissolving, lyophilised powder was kept in a desiccator at 4 °C and then dissolved in 50 % ethanol to obtain the concentration of 50 mg/mL. Particle size measurements showed that lyophilised particle aggregates consisted of soluble compounds. Dissolved lyophilisate was filtered through a 0.2 µm pore diameter Minisart® filter (Sartorius, Göttingen, Germany) in order to eliminate the remaining non-soluble particles and sterilise the extract solution. The filtrate was to determine the concentration of secoiridoids in the extract. The final extract concentrations in cell cultures were 0.5, 1, and 2 mg/mL.

Ultra performance liquid chromatography (UPLC)

Chromatographic separations of YGRE constituents were done by ACQUITY UPLC system (Malvern Panalytical Ltd, Malvern, UK) with a photodiode array (PDA) detector and a LUNA 3u, C18(2), 3 µm, 100 mm × 2 mm Phenomenex column as a stationary phase (Phenomenex, Torrance, CA, USA). All analyses were done under the gradient condition (Table 1), with a mobile phase consisting of solvent A (0.1 wt % HCOOH in water) and solvent B (0.1 wt % HCOOH in methanol) at a constant flow rate of 0.3 mL/min. Autosampler was maintained at 4 °C. 3D chromatograms were recorded in the wavelength range of 210–450 nm. The run time was 6 min, and the injection volume 3.5 µL.

Isolation and cultivation of human peripheral blood mononuclear cells

Peripheral blood samples were collected in Li-heparin vacutainers (2 x 10 mL) from three healthy donors aged 20 to 40 years. All subjects signed informed consent, and the study was approved by the Ethics Committee of the Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia.

Peripheral blood mononuclear cells (PBMC) were isolated in Ficoll™ medium (Thermo Fisher Scientific, Waltham, MA, USA) and resuspended in RPMI 1640 medium supplemented with 1 % penicillin–streptomycin and 10 % foetal bovine serum (FBS) at a concentration of 1×10^6 viable cells/mL. The obtained cell cultures were then incubated with root extract at 37 °C for 48 and 72 h.

Trypan blue exclusion test

Trypan blue was used to evaluate cell viability as described elsewhere (17). Briefly, equal volumes of PBMC suspension and 0.4 % Trypan blue dye were mixed and applied to a haemocytometer (Cambridge Instruments Inc., Buffalo, NY, USA). Live, unstained cells were counted in four sets of 16 squares. Viable cell counts per mL represent the mean of each set, multiplied by 10^4 and by 2 as dye dilution correction factor.

Chromosome aberrations assay

To establish cell cultures, 0.5 mL of heparinised whole blood was added in RPMI 1640 medium supplemented with 1 % penicillin–streptomycin, 10 % FBS, and phytohaemagglutinin and treated with YGRE to obtain final concentrations of 0.5, 1, and 2 mg/mL. All chemicals used for cell culture were purchased from Capricorn Scientific GmbH (Ebsdorfergrund, Germany). Harvesting was performed 48 and 72 h after culture initiation. Three hours before harvesting, colchicine (Sigma-Aldrich Co., St. Louis, MO, USA) was added (in the final concentration of 2.5 µg/

Table 1 Gradient condition details used for UPLC separation.

time (min)	% B
0 → 2	10 → 28
2 → 3.5	28 → 30
3.5 → 5	30 → 55
5 → 5.1	55 → 10
5.1 → 6	10 →

mL) to collect metaphase cells. Cell cultures were then centrifuged and treated with hypotonic solution (0.56 % KCl) at 37 °C for 20 min. The supernatant was removed and cells fixed in Carnoy's fixative solution (3:1 methanol-to-glacial acetic acid ratio), washed three times in fixative, and pipetted on clean microscopic slides. Slides were dehydrated with increasing concentrations of ethanol (70, 95, and 100 %), stained with 4′,6′- diamidino-2-phenylindole (DAPI)-containing Vectashield solution (Vector Laboratories Ltd, Peterborough, UK), and analysed under a Zeiss-Axioimager A1 microscope (Carl Zeiss, Jena, Germany), with the ISIS imaging software package (MetaSystems Hard & Software GmbH, Altlussheim, Germany). At least 200 complete metaphase spreads per sample were analysed, whereas the scoring criteria included determination of karyotype and indicators of chromosome damage (chromosome breaks, dicentric and ring chromosomes, acentric fragments, and radial figures) (18, 19).

Alkaline comet assay

The alkaline comet assay was an adaptation of the method described by Singh et al. (20). Briefly, PBMC treated for 48 h were washed in phosphate buffer saline (PBS), resuspended in 1 % low melting point agarose (LMPA) in PBS at 37 °C, and placed on microscopic slides precoated with a thin layer of 1 % normal melting point agarose (NMPA). Slides were allowed to settle at 4 °C for 10 min and then immersed in a lysis solution at 4 °C for 1 h. Electrophoresis was performed at the same temperature and voltage of 25 V (1 V/cm, 300 mA) for 20 min. Slides were then washed three times in 0.4 mol/L Tris-HCl, pH 7.5, at 4 °C, air dried, and counterstained with a DAPI-containing Vectashield solution. At least 300 cells were evaluated for each slide under a Zeiss-Axioimager A2 microscope with automated Metafer CometScan software (MetaSystems). The results are presented as the percentage of DNA in the comet tail (% DNA).

Prooxidant-antioxidant balance (PAB) assay

The assay was done as described by Alamdari et al. (21), with minor modifications. This method is based on two concomitant oxidation-reduction reactions in the same sample. In the first reaction, catalysed by horse radish peroxidase (HRP), chromogen 3,3',5,5'-tetramethylbenzidine (TMB) is oxidised to a coloured cation. In the second reaction, this coloured cation is reduced by antioxidants to a colourless compound.

Standard solution was prepared by mixing different proportions (0–100 %) of 1 mmol/L H_2O_2 with 6 mmol/L uric acid in 10 mmol/L NaOH. To prepare solution of TMB cation, a mixture of 0.1 mL TMB/DMSO (6 mg/mL), 5 mL acetate buffer (0.05 mmol/L, pH 4.5) and 17.5 µL freshly made chloramine T (100 mmol/L), was mixed, shacked for 1 h at 37 ºC, and incubated in a dark place. Then 2.5 U of enzyme solution HRP was added to this mixture. TMB solution II was made of 0.1 mL TMB/DMSO (6 mg/mL) and 5 mL acetate buffer (0.05 mol/L, pH 5.6).

In each well of reaction plate, 10 µL of sample/standard/blank was mixed with 180 µL of working solution, freshly made from 0.5 mL of TMB cation and 5 mL TMB solution II that were mixed for 6 min at room temperature in the dark. Then, the plate was incubated for 12 min at 37 °C, in a dark place. The reaction was stopped by adding 40 µL of 2 mol/L HCl to each well.

The difference between these parallel reactions was measured as optical density (OD) at 450 nm wavelength, with a reference wavelength of 570 nm on absorbance microplate reader Sunrise (Tecan Group Ltd, Männedorf, Switzerland). The PAB values were calculated from the standard curve that represents the percentage of hydrogen peroxide in the standard solution and were expressed in arbitrary Hamidi-Koliakos (HK) units.

Lipid peroxidation product (LPP) assay

Lipid peroxidation product (LPP) assay used in this study is based on the reaction of chromogen *N*-methyl-2-phenylindole with products of lipid peroxidation, namely malondialdehyde (MDA) and 4-hydroxyalkenals (HNE) (22, 23).

After treatment, cells were washed and lysed in 0.9 % NaCl in freeze-thaw cycles. An aliquot of the samples was used for protein determination. The reaction mixture contained 140 µL of sample/standard/blank, 455 µL of reagent diluted in acetonitrile/methanol (3:1) to a final concentration of 10 mmol/L, and 105 µL of methanesulphonic acid containing 34 µmol/L Fe(III). It was added to microcentrifuge tubes, vortexed, and incubated at 45 °C for 60 min. Samples were centrifuged at 15000x*g* for 10 min to obtain a clear supernatant.

The product of this reaction is a stabile chromophore, whose OD value was measured at 586 nm on the Sunrise absorbance microplate reader (Tecan). The values are presented as nmol of LPP per mg of protein using a standard curve of 1,1,3,3-tetramethoxypropane.

Statistical analysis

All experiments were done in duplicate and repeated three times. The results are presented as means ± standard error (SE). ANOVA test and Pearson correlation coefficient were used for statistical analysis. The level of significance was set at $p<0.05$. All statistical analyses were run on the SPSS 10 for Windows (IBM, Armonk, NY, USA).

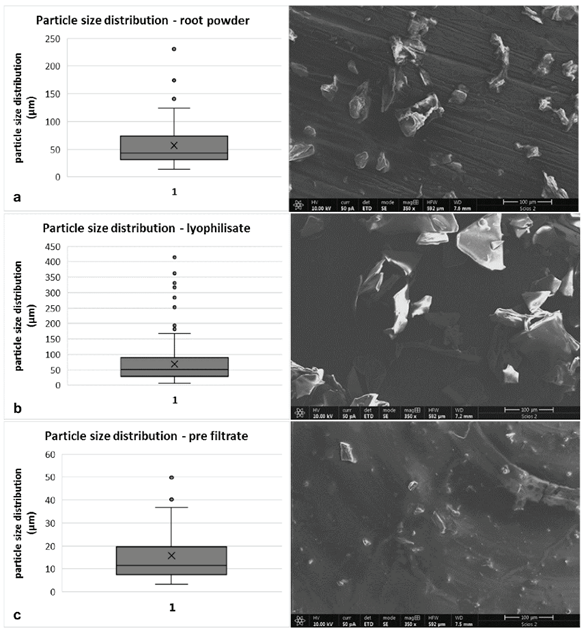

Figure 1 Particle size distribution of dispersed particles in (a) yellow gentian root powder; (b) lyophilisate; and (c) dissolved lyophilisate (pre-filtrate). Scale bar – 100 µm

RESULTS

Characterisation of yellow gentian root extract (YGRE) particle sizes by Field Emission Scanning Electron Microscopy (FESEM) with energy dispersive spectroscopy (EDS)

Analysis of FESEM photomicrograph showed that the particle size in dispersed root powder was 53.9±2.95 µm (Figure 1 a). After filtration and lyophilisation, the particles displayed laminar or plate-like structure: smaller particles formed aggregates, whose average dimension was 68.4±3.6 µm (Figure 1 b). The aggregates were mostly composed of soluble compounds, since particles observed in the lyophilisate dissolved in 50 % ethanol (concentration of 50 mg/mL) were of a much smaller dimension – 15.8±1.3 µm (Figure 1 c).

Ultra performance liquid chromatography (UPLC) with PDA detector

Analytical standards of selected compounds were used to identify some of the most intensive peaks in chromatogram. Detailed data are presented in the report by Valenta Šobot et al. (24). Figure 2 shows UPLC chromatograms of YGRE ethanolic solution exported from 3D chromatograms at three wavelengths, revealing a high content of gentiopicroside and high stability of other compounds during treatment.

Cell viability

Figure 3a shows results of cell viability assay. All treatments reduced cell viability to a certain extent ranging from mild (91.84 % for 0.5 mg/mL, 48 h treatment) to more noticeable (59.54 % for 2 mg/mL, 72 h treatment, $p<0.001$). Cell viability decreases with increasing YGRE concentration, at both exposure times. Statistically significant reduction was observed for concentrations of 1 and 2 mg/mL for both 48 ($p<0.05$ and $p<0.01$, respectively) and 72 h treatment ($p<0.01$ and $p<0.001$, respectively, Figure 3a).

Incidence of chromosome aberrations

The results of chromosome aberrations assay showed that incidence of chromosomal breaks increased with the increase of YGRE concentration after 48 h of treatment, from 0.8 % (0.5 mg/mL) to 8.11 % (2 mg/mL) (Figure 3 b). Chromosome aberrations were also analysed after 72 h to evaluate the impact of prolonged incubation on DNA damage repair. The trend of increasing frequency of breaks was noticed after 72 h treatment for concentrations of 1 and 2 mg/mL (0.93 and 5.1 %, respectively), however the percentage of breaks was reduced comparing to 48 h treatment (Figure 3). The concentration of 0.5 mg/mL did not induce chromosomal breaks after 72 h. Figure 4 shows the appearance radial figures between homologous chromosomes at 2 mg/mL of YGRE, which are indicators of double-strand break (DSB) repair.

As expected, the percentage of chromosome breaks inversely correlated with viability assay for both treatment times ($p<0.01$, $r=-0.67$ for 48 h treatment and $p<0.01$, $r=-0.89$ for 72 h treatment).

Comet assay

All tested YGRE concentrations increased the percentage of DNA in the comet tail compared to control after 48 h of treatment and the difference was significant for the two higher concentrations ($p<0.001$) (Figure 5). Again, as expected, the parameter percentage of DNA in the comet tail inversely correlated with cell viability ($p<0.01$, $r=-0.797$).

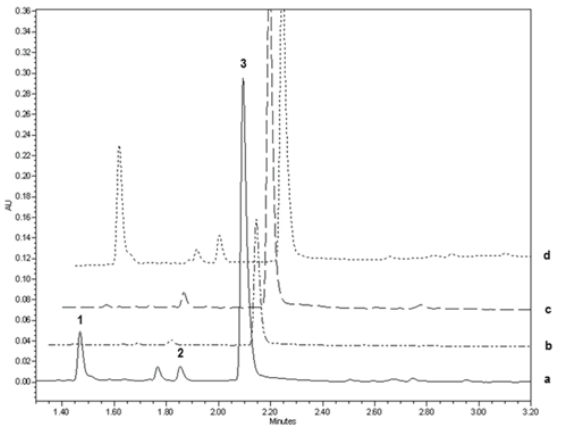

Figure 2 UPLC chromatograms of yellow gentian root extract in ethanol solution exported at 254 nm (a); 300 nm (b); 270 nm (c); and 240 nm (d). 1 – loganic acid; 2 – swertiamarin; 3 – overlapping gentiopicroside and sweroside peaks

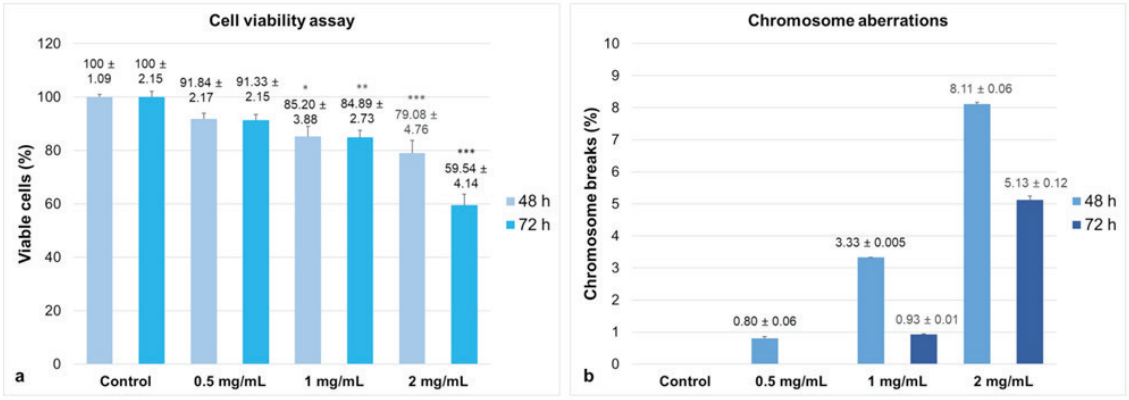

Figure 3 Cell viability expressed as percentage of control (a) and percentage of chromosome breaks (b) at different concentrations of yellow gentian root extract after 48 and 72 h treatment. *p<0.05, **p<0.01, ***p<0.001

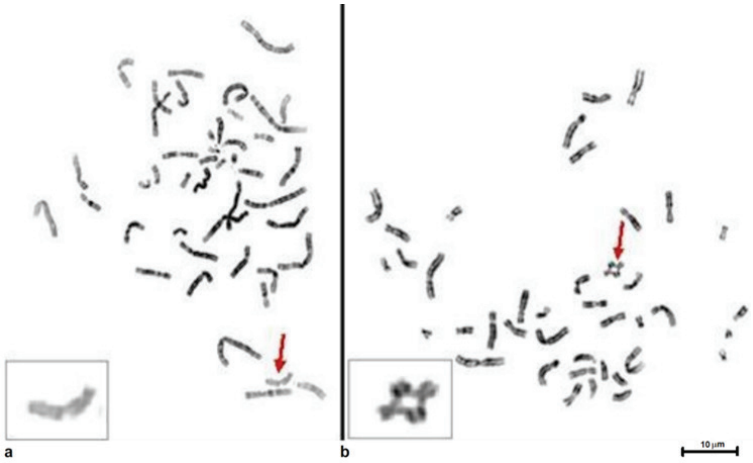

Figure 4 Photomicrograph of metaphase spreads after 72 h treatment with 2 mg/mL of yellow gentian root extract. Arrows mark chromatid break (a) and radial figure between two homologous chromosomes (17q) an indicator of homologous recombination - a mechanism of template-dependent DNA DSBs repair (b)

Oxidative stress

The levels of PAB and LPP were the highest (67.9 arbitrary HK units and 1.79 nmol/mg of proteins, respectively) and statistically significant for the lowest concentration of 0.5 mg/mL ($p<0.001$), while they dropped toward control levels at higher concentrations (Figure 6). PAB and LPP positively correlated ($p<0.01$, $r=0.894$).

DISCUSSION

Our results demonstrate concentration- and time-dependent reduction in survival of PBMC after exposure to increasing concentrations of YGRE. Similar results were reported after treatment of human lymphocytes with extracts from other plants of the *Gentianaceae* family, which have similar constituents, such as *Gentiana asclepiadea* and *Gentiana kurroo* Royle (25, 26). Concentration-dependent cytotoxic effects of YGRE were, likewise, observed in cancer cell lines, such as cervical, breast, prostate and colon cancer (14, 15). However, cytotoxic outcomes in those studies were observed at lower concentrations, most likely due to differences in the metabolism of cancer cells and terminally differentiated peripheral blood T-lymphocytes. Different response to yellow gentian between malignant and healthy tissue was already observed in a study of its radioprotective effects (7).

In view of the contradictory literature data about the effects of yellow gentian, we wanted to see whether its cytotoxicity was associated with redox parameters and DNA damage, and found that only the lowest YGRE concentration increased redox parameters, which, however, led to only a minor DNA damage and mild cytotoxicity. Over the decades many studies focused on the relationship between oxidative stress and DNA damage, evidencing strong interplay between reactive oxygen species (ROS) production and DNA alterations that trigger genomic instability and cell death (27, 28). Our results indicate that pro-oxidant state and elevated lipid peroxidation products observed after treatment with the lowest YGRE concentration did not lead to significant DNA damage. In contrast, higher extract concentrations only slightly reduced PAB and LPP levels, but provoked significant DNA damage seen as an increased percentage of DNA in the comet tail and frequency of chromosomal aberrations. This may be due to the capacity

of antioxidative defence, which is triggered only after a critical concentration of the tested compound is reached.

A comprehensive study by Kusar et al. (29) showed that YGRE displays strong antioxidative features by scavenging DPPH (2,2-diphenyl-1-picrylhydrazyl) and superoxide radical, but only at high concentrations of 19.0 mg/mL and 11.1 mg/mL. Similar findings were reported by several studies, but all of them were obtained in *ex vivo* experiments (4, 14, 30). The reported scavenging effect was probably due to the higher amount of constituents with antioxidant capacity, namely flavonoids, xanthones, and secoiridoids (31). Others (32, 33) demonstrated *in vivo* concentration-dependent antioxidative activity of *G. cruciata* and *G. asclepiadea* extracts rich in secoiridoids swertiamarin, sweroside, and gentiopicrin.

On the other hand, lower extract concentrations may favour pro-oxidant effects of other constituents, which leads to increased lipid peroxidation. The balance between extract constituents and their individual effects was well illustrated by Petrovic et al. (34), who found that a *G. dinarica* extract decreased lipid peroxidation products in peripheral blood lymphocytes, even though treatment with the same concentration of individual polyphenol compounds found in this plant generated opposite results. Isoorientin, isoorientin-4′-O-glycoside and norswertianin-1-O-primveroside had antioxidative, while norswertianin-8-O-primveroside and gentiacaulein displayed pro-oxidative effects. These results suggest that the cumulative outcome of plant extract mostly depends on the balance between its constituents and their individual pro-oxidative or antioxidative effects.

However, since higher extract concentrations induced significant DNA damage and reduced cellular survival, it is more likely that increasing YGRE concentrations provoke higher production of ROS, which consequently activate antioxidant enzymes such as SOD and catalase and endogenous antioxidant glutathione (GSH). Once activated, they could prevent oxidative damage within the cells to a certain extent, while a portion of cells is damaged beyond the repair capacity of terminally differentiated cells, as evidenced by diminished cell viability, and higher DNA damage. Treatment with 2 mg/mL of YGRE reduced cellular viability to 78.57 %, led to a significant increase of DNA % in the comet tail (from 6.41 to 32.98 %) and produced 8.11 % of chromosomal breaks after the 48-hour treatment, whereas PAB and LPP levels dropped to nearly control values. With longer exposure (72 h) cytotoxicity was even more pronounced, as cell viability decreased to 59.54 %.

Other research groups also reported genotoxic and cytotoxic effects of different concentrations of *Gentiana* extracts. For instance, *G. asclepiadea* root extract increased comet tail length in rats treated with 400 mg/kg of body weight and dose-dependently increased comet tail intensity in human peripheral blood lymphocytes (26, 33). *G.*

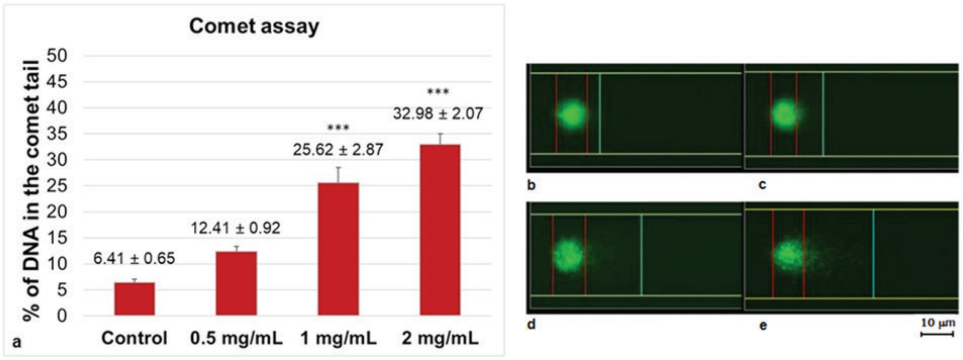

Figure 5 Graph (a) and photomicrograph (b-e) of YGRE-induced DNA damage established with the comet assay. b – control; c – 0.5 mg/mL; d – 1 mg/mL; e – 2 mg/mL; YGRE – yellow gentian root extract. Scale bar – 10 µm. ***p<0.001

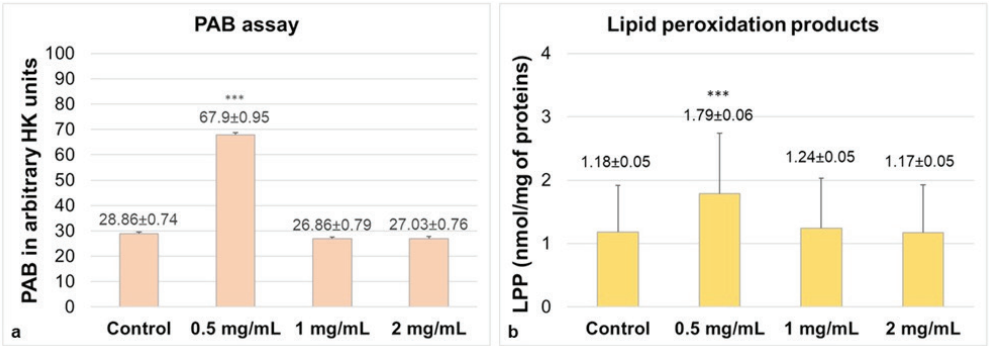

Figure 6 PAB levels presented as arbitrary HK units (a) and LPP levels expressed as nmol/mg of proteins (b) after treatment with increasing concentrations of yellow gentian root extract. ***p<0.001

dinarica extract elevated the frequency of micronuclei and enhanced apoptosis in peripheral blood lymphocytes (34). Similar results were found for many plant extracts known for their antioxidant activity and rich in polyphenols, such as black and green tea extract, suggesting that higher polyphenol concentrations could lead to spontaneous H_2O_2 generation, DNA damage, and cell death (35, 36). In line with this argument, the concentration determines whether certain compound or extract acts as anti- or pro-oxidant.

Interestingly, in our study the frequency of chromosomal breaks was lower after 72 h of treatment for all tested concentrations. These and cell viability findings suggest that DNA repair mechanisms are activated in cells with aberrations that could be fixed, while the cells damaged beyond repair die. This is well illustrated by radial figures observed after 72 h of YGRE treatment, as they indicate homologous recombination, an important template-dependent DNA repair mechanism, specific for repair of double-strand breaks (DSBs). This is in accordance with the report by Patenković et al. (12), who demonstrated homologous mitotic recombination in *Drosophila* treated with YGRE (12). Even though homologous recombination could have negative effect due to loss of heterozygosity, activation of this repair mechanism is vital for the recovery from oxidative DNA damage (12, 37, 38). Upon oxidative DNA damage, complex protein network activates, including ATM, ATR, RAD50 and MRE11A proteins, leading to upregulation of RAD50, RAD51, XRCC2 and XRCC3 gene and protein levels, which contribute to the survival of affected cells (28, 37, 38).

CONCLUSION

The results of our study suggest that, although YGRE displayed concentration- and time-dependent cytotoxic and genotoxic effects, it activates repair mechanisms that counter oxidative and DNA lesions and induces cell death in cells damaged beyond repair. DNA fragmentation could serve to predict the cytotoxic effects of yellow gentian, as increased fragmentation was observed 24 h before significant increase in cell death. Considering these findings, it appears that the protective effects of yellow gentian result from the activation of cell defences and death of the cells beyond repair. However, since there is a clear correlation between concentration, treatment duration and toxic effects, and that these effects were observed at cell level, further studies are needed to get a better insight into the cyto/genoprotective potential of YGRE, and this compound should be used with caution.

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Conflict of interests

None to declare.

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Učinak ekstrakta korijena žute lincure na mononuklearne stanice periferne krvi ovisi o njegovoj koncentraciji i vremenu izlaganja

Žuta lincura (*Gentiana lutea* L.), ljekovita biljka koja se često koristi u tradicionalnoj medicini, pokazuje višestruke biološke učinke, od korisnih do toksičnih. Budući da je do sada zabilježeno mnogo mogućih primjena, cilj nam je bio procijeniti potencijalne citotoksične i genotoksične učinke ekstrakta korijena te biljke, koji ovise o njegovoj koncentraciji i vremenu izlaganja *in vitro*. Mononuklearne stanice ljudske periferne krvi izložili smo ekstraktu korijena žute lincure (YGRE), koncentracije 0,5 mg/mL, 1 mg/mL i 2 mg/mL, da bismo utvrdili njegove učinke na parametre oksidacijskoga stresa [pro/antioksidacijski balans (PAB) i peroksidacija lipida], oštećenja DNA (alkalni komet test i kromosomske aberacije) i preživljavanje stanica (tripan plavo bojenje). Preživljavanje stanica smanjivalo se s povećanjem koncentracije i trajanja izlaganja. Samo najniža koncentracija YGRE-a (0,5 mg/mL) dovela je do povećanja oksidacijskoga stresa, ali je proizvela manja oštećenja DNA i citotoksičnost. Pri višim koncentracijama, redoks parametri vratili su se blizu razine kontrolnih vrijednosti. Postotak kromosomskih aberacija i postotak DNA u repu kometa povećavao se s povećanom koncentracijom YGRE-a nakon 48 sati i smanjivao nakon 72 sata tretmana. To upućuje na aktiviranje mehanizma popravka DNA (homologna rekombinacija), što dokazuje prisutnost kromosomskih radijalnih struktura nakon 72 sata tretmana najvišom koncentracijom YGRE-a od 2 mg/mL. Naši rezultati pokazuju da YGRE, unatoč induciranju citotoksičnih i genotoksičnih učinaka, aktivira mehanizme popravka stanica koji suzbijaju oksidacijske i DNA lezije i induciraju smrt visoko oštećenih stanica. Zaključak je da uočeni zaštitni učinci dužeg izlaganja ekstraktu korijena žute lincure mogu biti rezultat aktivnoga popravka i uklanjanja stanica s nepopravljivim oštećenjima.

KLJUČNE RIJEČI: citotoksičnost; genotoksičnost; *Gentiana lutea* L.; homologna rekombinacija; redoks parametri