

Chemopreventive properties of young green barley extracts in *in vitro* model of colon cancer

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Abstract

Introduction and objective. Young green barley is the most valuable source of nutrients and bioactive substances. It has a broad spectrum of health-promoting properties such as antioxidant, anti-inflammatory, hypoglycaemic, anti-depressant, anti-atherosclerotic and anticancer. The presented study is an attempt to extend this knowledge with particular emphasis on the possibility of using green barley in colon cancer prevention.

Materials and method. Extracts were prepared on the basis of two commercial products: ground dried barley grass (YGB INT) and powder of young green barley juice (YGB GW). Their influence on colon epithelial cells (CCD841 CoN) viability and proliferation were analyzed by LDH and MTT assays. Anticancer properties of extracts were screened on colon cancer cell lines (LS180, HT-29) by MTT and BrdU assays. Changes in cells morphology induced by extracts were investigated after May-Grünwald-Giemsa staining.

Results. Tested extracts were not toxic against CCD841 CoN and did not affected their proliferation or morphology (LDH test, MTT test, microscopy observation). The MTT revealed that extracts significantly inhibited proliferation of colon cancer cells in a dose-dependent manner. Results of BrdU test confirmed antiproliferative properties of extracts, but opposite to MTT test, indicated YGB GW as a better anticancer agent. Light microscopy observation proved the data obtained from both MTT and BrdU tests and additionally suggested the ability of the extracts to induce necrosis in LS180 and HT-29 cells.

Conclusions. The study demonstrated that YGB extracts specifically inhibit proliferation of colon cancer cells without any undesirable effect on colon epithelial cells. Obtained results will provide a rationale for the future development of dietary supplements which could be beneficial in colon cancer chemoprevention.

Key words

Hordeum vulgare, barley, colon cancer, chemoprevention

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the first plants cultivated by humans. The domestication of this cereal took place in the Middle East about 10,000 years ago [1]. Barley yield is around 30% of the total worldwide cereal production. [2]. It is estimated that about 75% of the barley crop is destined for feeding animals, 20% is used in the production of alcoholic or non-alcoholic beverages, and other 5% is the food additive [3]. Barley is grown in many unfavourable areas thanks to its superior adaptability to diverse conditions and high resistance to environmental stress caused by climate changes [1]. These unique features, as well as low costs of cultivation, have made barley a paramount source of food in many countries and some areas of North Africa, the Middle East and the mountains of Central Asia [4].

Kazuhiko Kubota's studies have shown that young green barley is the most valuable source of nutrients and bioactive substances. The young green barley is defined as seedlings up to 200 hours from germination, with a height between 20–30 cm [5, 6]. Barley (grains and grass) contains significant amounts of amino acids, vitamins, minerals and trace

elements, insoluble and soluble dietary fibre, antioxidants and other bioactive substances, such as superoxide dismutase, catalase, 2-O-glycosyl isovitexin, peroxydase, carotenoids and chlorophyll [7, 8, 9, 10, 11]. Numerous studies have shown that the total content of nutrients and bioactive substances in young barley depends on the locus, soil type, rainfall, harvesting technology and plant age [11]. It was proved that the contents of vitamin C, polyphenols, phenolic compounds (ferulic acid), as well as amino acids, glucose and fructose, was inversely proportional to the age of the plant [9]. But not only the age of the plant affects on the amount of nutritious substances, the commercial form of barley is also important. Juice squeezed from barley leaves are a better source of easily digestible nutrients and/or bioactive substances than raw barley grass. Dehydrated juice has no moisture, the nutrients are intact and not broken down. As a result, the nutrients are retained in an excellent, assimilable food product which keeps well and is resistant to spoilage. [10].

Furthermore, young green barley has a broad spectrum of health-promoting properties, such as antioxidant [8, 9, 10, 12], anti-inflammatory [10, 13], anti-depressant [14], hypoglycemic [15, 16] and anti-atherosclerotic [17, 18, 19, 20]. Barley seedlings also may inhibit differentiation of osteoclasts and bone-resorbing activity in all the stages of osteoclastogenesis [21]. In that case, supplementation of green barley should be favourable for health and may

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prevent diseases caused by oxidative damage, such as neurodegenerative and cardiovascular diseases or various cancers [22].

The presented study investigates and compares the chemopreventive potential of young green barley extracts prepared on the basis of two different commercial products (ground dried barley grass from Intenson and powdered young green barley juice from Green Ways). Antiproliferative activity of the studied extracts was tested in human colon cancer cells. Furthermore, the impact of the extracts on viability and morphology colon epithelial cells in humans was also investigated.

MATERIALS AND METHOD

Reagents. Unless otherwise indicated, the chemicals used in the study were purchased from Sigma-Aldrich Co. LLC

Preparation of young barley extracts. 1) Ground barley grass meal (INTENSON, Całowanie 94G, 05-480 Karczew) was purchased in a local market. 2.5 g of grass powder were suspended in 100 ml of pure sterile water and extracted on a magnetic stirrer for 24 h (20°C). After this time, the solution was centrifuged (10 min, 4075 x g, 20°C), filtered, and vacuum evaporated (vacuum rotary evaporator type 350, UNIPAN, Warsaw, Poland). Stock solution (100 mg/ml) was prepared by dissolving the crystallized form of the barley in PBS (phosphate-buffered saline). In the current study, this extract was designated YGB INT.

2) Powdered juice from young barley leaves (GREEN WAYS, Ovocný trh 572/11, 110 00 Prague 1, Czech Republic) was obtained from a distributor. 3.3 grams of powder were suspended in 100 ml of PBS and extracted on a magnetic stirrer for 24 h at 20°C. The solution was then centrifuged (10 min, 4075 x g, 20°C). The obtained supernatant was filtered in sterile conditions, while precipitate was collected, dried and weighed in order to assess the amount of substance that actually went into solution during the extraction. The precipitate weighed 1.218 grams and the resulting extract which contained 2.082 grams of substance was stock solution (100%; ~21 mg/ml). This extract was designated YGB GW.

Nutritional values of young barley commercial products used for the preparation of the studied extracts is shown in Table 1. Comparison of young barley extracts preparation is shown in Figure 1.

Stock solutions of YGB INT and YGB GW extracts were stored at 4°C. Stock solutions were prepared once and used in all described experiments. The duration of the study from the beginning to the end was 6 months. During this period, no disturbing changes were observed in the stock solutions, i.e. precipitation, colour change, or development of microorganisms (microscopic observations). Stability of biological activity of the extracts was vitrified by MTT test in LS180 cells at the end of study, as described below. Changes between results of MTT test carried out at an interval of 6 months were not statistically significant. Working solutions of both extracts were prepared immediately before use by dissolving an appropriate stock solution in a culture medium. Studies were conducted using YGB INT at concentrations: 25, 50, 75, 100, 250 µg/ml, and YGB GW at concentrations: 16, 32, 63, 158, 315, 630, 1050, 1575, 2100 µg/ml.

Table 1. Nutritional values of young barley commercial products used for the preparation of YGB INT and YGB GW extracts. Nourishing value was given per 100 g dry weight of the product. Prepared based on the manufacturer's information

	Intenson ground dried barley grass	Green Ways powdered young green barley juice
Energy	1143 kJ / 275 kcal	1340 kJ / 319 kcal
Fats, of which:	3.2 g	3.9 g
saturated fatty acids	0.7 g	1.1 g
monounsaturated fatty acids	not specified	0.2 g
polyunsaturated fatty acids	not specified	2.6 g
Carbohydrates, of which:	16 g	39 g
sugars	11 g	23 g
Fiber	44 g	6.9 g
Protein	24 g	28 g
Salt	0.3 g	2.9 g

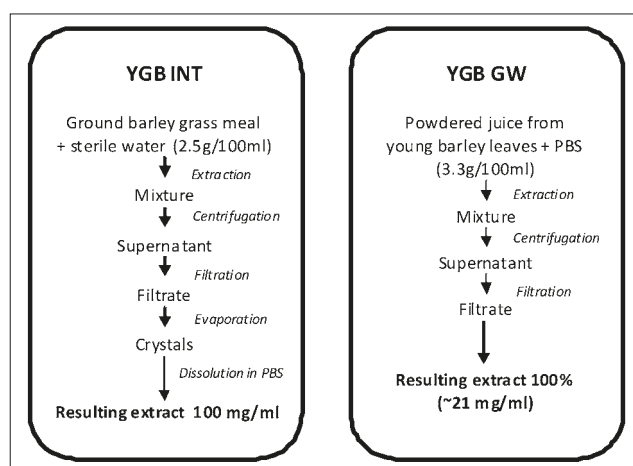


Figure 1. Differences in preparation of young green barley (YGB) extracts. YGB INT – extract of young green barley prepared on the basis of a product from Intenson. YGB GW – extract of young green barley prepared on the basis of a product from Green Ways.

Cell lines. The human colon adenocarcinoma cell lines LS180 and HT-29 were obtained from ECACC (European Collection of Cell Cultures, Center for Applied Microbiology and Research, Salisbury, UK). The human colon epithelial cell line CCD 841 CoN was purchased from the ATCC (American Type Culture Collection, Menassas, VA, USA).

The LS180 and HT-29 cells were grown in a mixture of Dulbecco's Modified Eagle's Medium (DMEM) and nutrient mixture F-12 Ham (1:1). The CCD 841 CoN cells were cultured in Eagle's Minimum Essential Medium (EMEM). All media were supplemented with 10% FBS (foetal bovine serum), 100 units/ml penicillin and 100 µg/ml streptomycin. Cells were maintained in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C.

Studies were conducted in cell lines derived from female donors in order to bypass the gender-specific differences [23, 24].

LDH assay – extract cytotoxicity assessment. CCD 841 CoN cells were plated on 96-well microplates at a density of 1×10^5 cells/ml. On the following day, the growth medium was removed and the cells were exposed to a series of tested extracts dilutions. The extracts solutions were prepared in a medium containing 2% FBS. After 24 hours of exposure,

the culture supernatants were collected and transferred to new 96-well plates which were used to execute the LDH assay, according to the manufacturer's instructions (In vitro Toxicology Assay Kit, Lactate Dehydrogenase Based). Absorbance was recorded on a microplate reader (BioTek ELx800, Highland Park, Winooski, Vermont, USA) at 450 nm wavelength. The findings were presented as a percentage of LDH released from the cells treated with barley extracts versus cells grown in the control medium (indicated as 100%).

MTT assay – cell viability/proliferation assessment. CCD 841 CoN, LS180 and HT-29 cells were plated on 96-well microplates at a density of 3×10^4 cells/ml. The following day, the growth medium was replaced by a fresh one, and the cells were exposed to series of dilutions of tested extracts (YGB INT 25–250 µg/ml; YGB GW 16–2100 µg/ml) prepared in a medium containing 10% FBS. After 96 hours of incubation, the cells were incubated with MTT solution (5 mg/ml) for 3 h. Formazan crystals were solubilized overnight in SDS buffer pH 7.4 (10% SDS in 0.01N HCl). After this time, the colour product was quantified spectrophotometrically by measuring the absorbance at 570 nm wavelength using a microplate reader (BioTek ELx800). Cell proliferation (%) was expressed as a percentage relative to the untreated control cells.

BrdU assay – cell proliferation assessment. LS180 and HT-29 cells were plated on 96-well microplates at a density of 5×10^4 cells/ml. The next day, the growth medium was replaced by a fresh one and the cells exposed to series of dilutions of tested extracts (YGB INT 25–250 µg/ml; YGB GW 16–2100 µg/ml) prepared in the medium supplemented with 10% FBS. Cell proliferation was quantified after 48 hours of treatment, according to the manufacturer's instructions, Cell Proliferation ELISA BrdU (Roche Diagnostics GmbH, Penzberg, Germany). The absorbance was measured at 450 nm wavelength using a microplate reader (BioTek ELx800). The influence of the barley extracts on DNA synthesis was presented as a percentage of BrdU incorporation versus control cells (indicated as 100%).

Morphological examination. To assess changes in cell morphology, May-Grünwald-Giemsa staining was used. Cells were plated on Lab-Tek Chambers Slide (Nunc) at a density of 0.5×10^5 cells/mL. Next day, the culture medium was removed and the cells were exposed to serial dilutions of the tested extracts (YGB INT 50, 10, 250 µg/ml; YGB GW 58, 1050, 2100 µg/ml) in culture medium supplemented with 10% FBS. After 48 hours, the cells were stained by the May-Grünwald-Giemsa method. The stained cells were observed by light microscope (Olympus BX51 System Microscope, Olympus Optical Co., Japan).

Statistical analysis. The data were presented as the mean value and standard error of the mean (SEM). The data were analyzed by one-way ANOVA test, Tukey's Multiple Comparison Post-test. Significance was accepted at $p < 0.05$. The IC_{50} value (concentration causing proliferation inhibition by 50%, compared to control) was calculated according to the method of Litchfield and Wilcoxon [25].

RESULTS

Influence of barley extracts on human colon epithelial cells viability and proliferation. The cytotoxicity of the extracts and impact on the proliferation of human colon epithelial cells (CCD 841 CoN) were examined by LDH and MTT tests after 24 hours and 96 hours of cells treatment, respectively. The study showed that YGB INT and YGB GW extracts were not toxic against human colon epithelial cells in the whole range of analyzed concentrations. Furthermore, YGB INT at concentration 100 µg/ml significantly reduced LDH release from the treated cells (Fig. 2, upper panel). Results of MTT assay showed that the investigated extracts did not affect colon epithelial cells proliferation in the whole range of tested concentrations (Fig. 2, lower panel).

Antiproliferative effect of barley extracts on human colon cancer cells. The antiproliferative effect of barley extracts was assessed on human colon adenocarcinoma cell lines LS180 and HT-29 by MTT and BrdU tests after 96 hours and 48 hours of cells treatment, respectively.

MTT test revealed that YGB INT and YGB GW extracts inhibited proliferation of colon cancer cells; nevertheless, only YGB GW induced changes in dose-dependent manner (Fig. 3). YGB INT extract elicited a more significant antiproliferative effect compared to YGB GW, in both cell lines. YGB INT at the highest tested concentration 250 µg/ml decreased cell proliferation to 23.7% in LS180 and 43.9% in HT-29, while YGB GW induced a similar effect at a concentration of around 2,100 µg/ml (LS180) and 630 µg/ml (HT-29). The IC_{50} value was as follow: 62 µg/ml (YGB INT; LS180), 144 µg/ml (YGB INT; HT-29), 1239 µg/ml (YGB GW; LS180), 294 µg/ml (YGB GW; HT-29).

In a more specific antiproliferative test, BrdU showed that both extracts inhibited DNA synthesis in treated colon cancer cells (Fig. 4). The YGB INT reduced proliferation of the LS180 cells in a dose dependent manner. YGB INT at 250 µg/ml decreased BrdU incorporation in the treated cells by 22.9%. The HT-29 cells were much less sensitive to the tested extract. Significant inhibition of DNA synthesis in HT-29 cells (11.2% vs control) was observed only after the cells were treated with YGB INT at the highest tested concentration (250 µg/ml). YGB GW more effectively inhibited DNA synthesis in LS180 cells than YGB INT; nevertheless, the observed changes were not dose dependent. The antiproliferative effect observed in cells treated with the extract at concentrations of 16 and 315 µg/ml (17.1% and 18.0%) was similar, and corresponded with results obtained after exposure of the cells to YGB INT at concentration 100 µg/ml (16.1%). HT-29 cells were also more sensitive to YGB GW than YGB INT. BrdU test revealed significant dose-dependent inhibition of HT-29 cells proliferation after exposure to YGB GW. The pronounced effect was observed in cells treated with 32 µg/ml YGB GW, which reduced BrdU incorporation to DNA to 85.9%, which exceeds the range of changes reported after cells exposure to 250 µg/ml YGB INT (88.8%). The strongest antiproliferative effect in HT-29 cells was observed after exposure of the cells to 2,100 µg/ml YGB GW, which inhibited DNA synthesis by 51.6%, compared to control.

Induction of morphological changes on human colon epithelial cells and human colon cancer cells by barley extracts. Changes in morphology of CCD 841 CoN, LS180

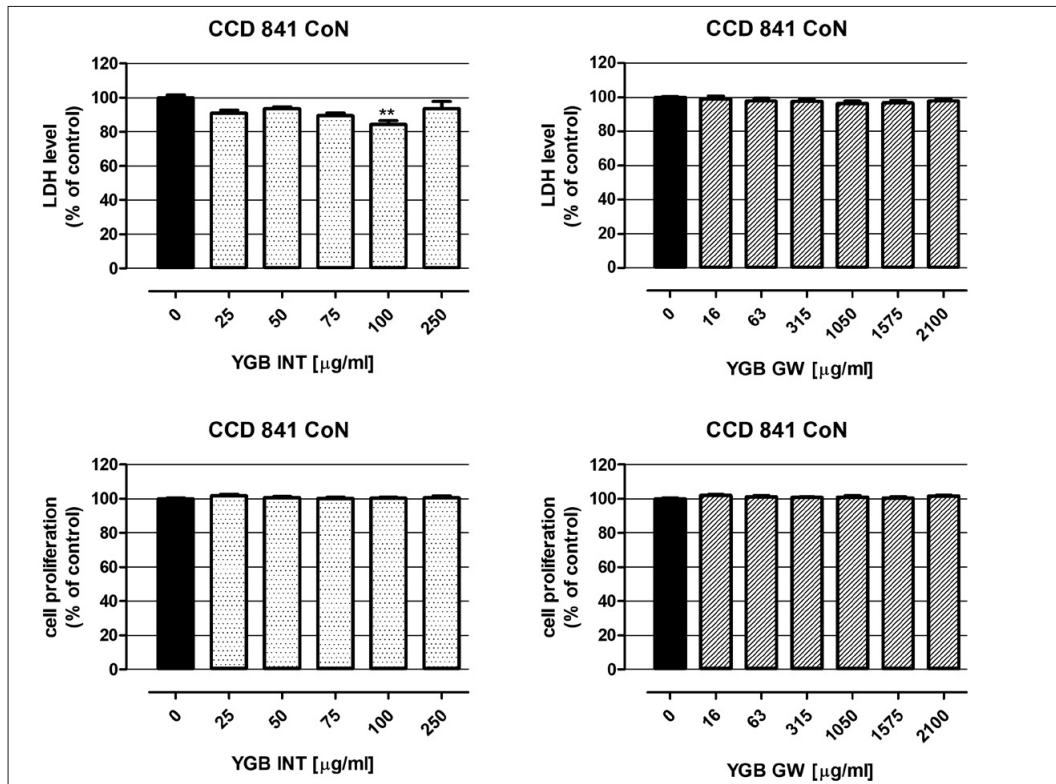


Figure 2. Impact of YGB INT and YGB GW extracts on human colon epithelium cell line CCD 841 CoN viability and proliferation. Cells were exposed to culture medium alone (control) or extracts in concentrations ranging from 25–250 µg/ml YGB INT – 16–2100 µg/ml YGB GW for 24 h. Extracts cytotoxicity was measured photometrically by LDH assay (upper panel). Cell proliferation was measured photometrically by MTT assay (lower panel). Results presented as mean ± SEM of 5 measurements.

* $p < 0.05$ vs. control; ** $p < 0.01$ vs. control, one-way ANOVA test; post test: Tukey.

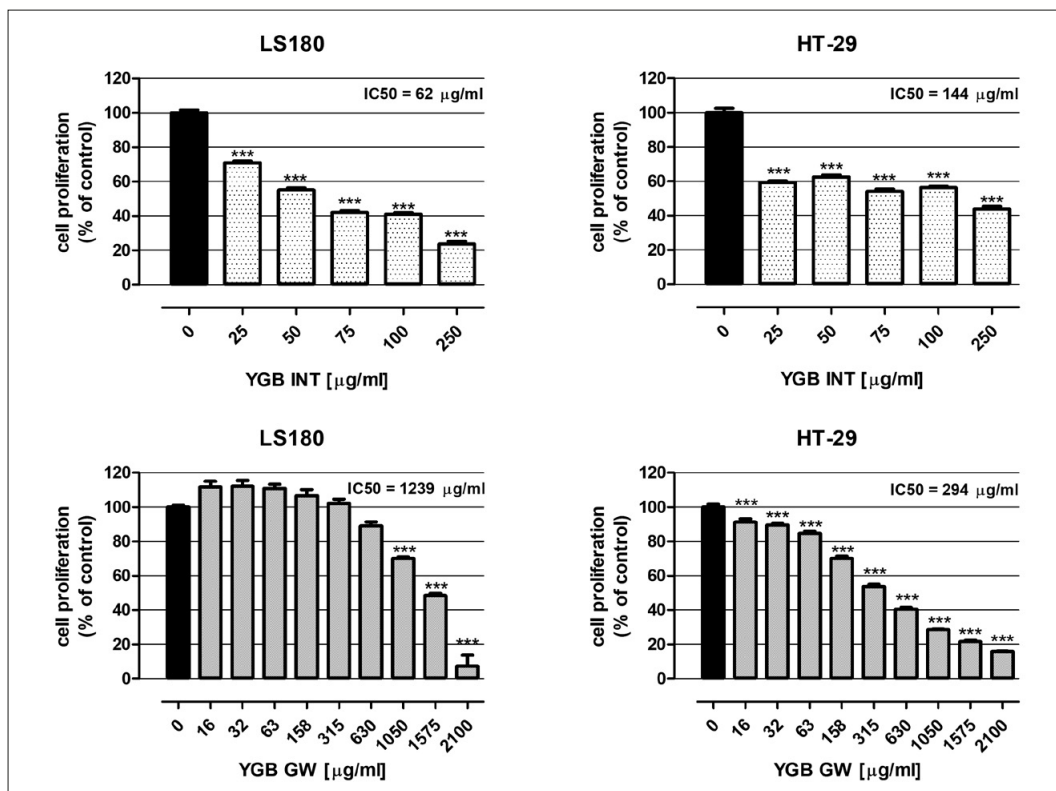


Figure 3. Antiproliferative effect of YGB INT and YGB GW extracts in human colon adenocarcinoma cell lines LS180 and HT-29. Cells were exposed to culture medium alone (control) or extracts in concentrations ranging from 25–250 µg/ml YGB INT – 16–2100 µg/ml YGB GW for 96 h. Cell proliferation was measured photometrically by MTT assay. Results presented as mean ± SEM of 6 measurements.

* $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control, one-way ANOVA test; post test: Tukey.

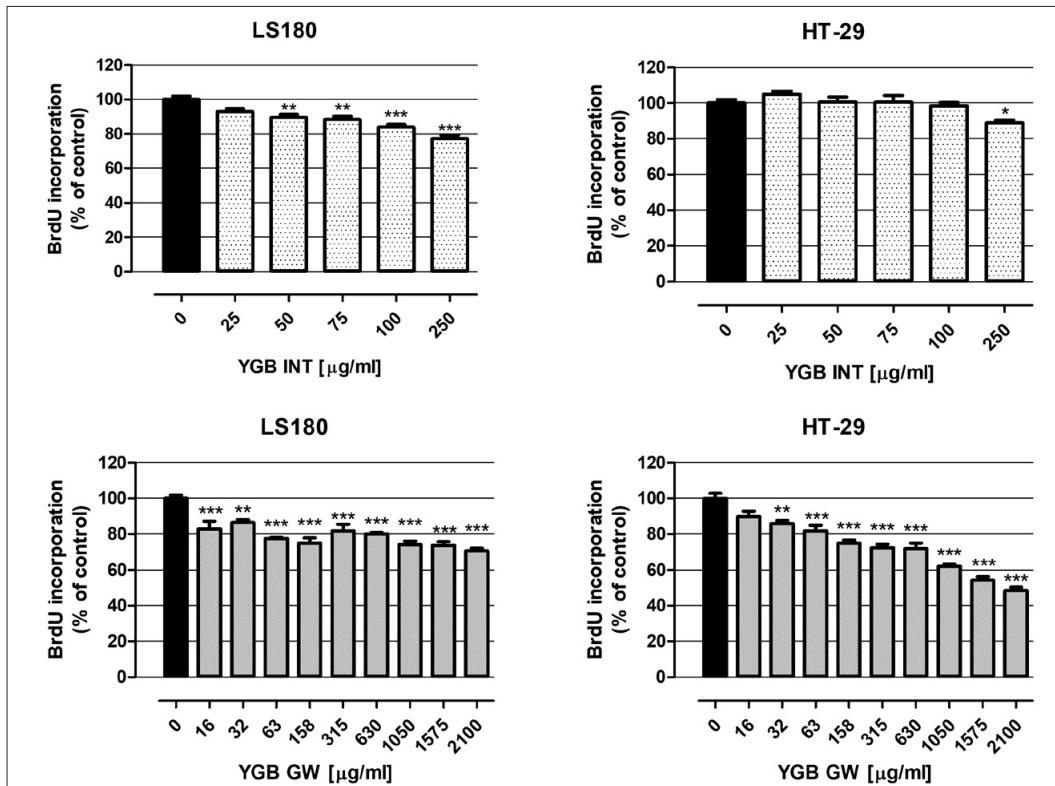


Figure 4. Antiproliferative effect of YGB INT and YGB GW extracts on human colon adenocarcinoma cell lines LS180 and HT-29. Cells were exposed to culture medium alone (control), or extracts in concentrations ranging from 25–250 µg/ml YGB INT – 16–2100 µg/ml YGB GW for 48 h. Antiproliferative activity was assessed by BrdU assay. Results presented as mean ± SEM of up to 6 measurements. * p< 0.05 vs. control; ** p< 0.01 vs. control; *** p< 0.001 vs. control, one-way ANOVA test; post test: Tukey.

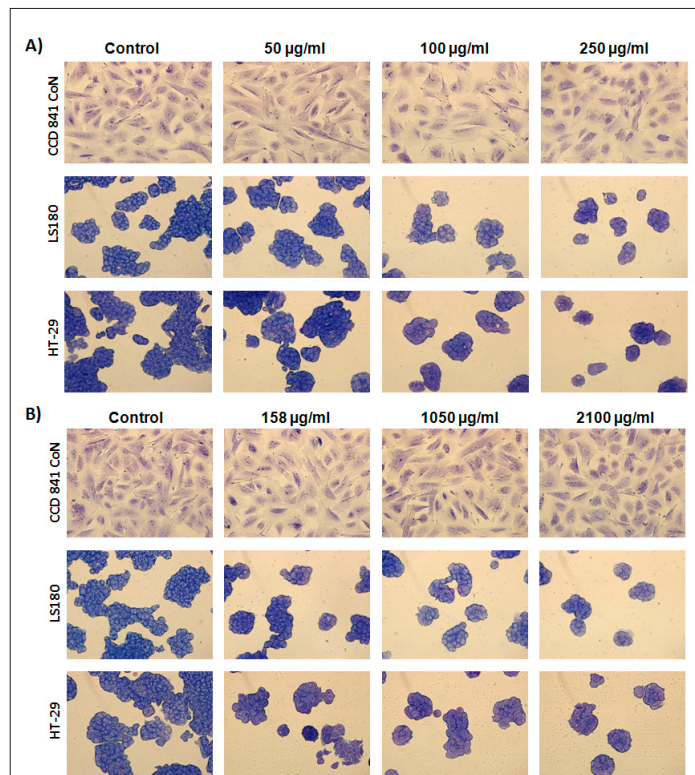


Figure 5. Impact of YGB INT and YGB GW extracts on morphology of human colon epithelial cell line CCD 841 CoN, and human colon adenocarcinoma cell lines LS180 and HT-29. Cells were incubated for 48 h alone or in the presence of YGB INT (A) or YGB GW (B). Micrographs show normal morphology of investigated cells – control, and changes of cell morphology after 48 h exposure to YGB INT at concentrations 50, 100 and 250 µg/ml (A) and YGB GW at concentrations 158, 1050 and 2100 µg/ml (B). Magnification 400x.

and HT-29 were investigated after 48 hours of cells treatment with barley extracts. After treatment, the cells were stained with May-Grünwald-Giemsa and induced alterations were visualized by light microscopy.

YGB INT did not affected morphology as well as growth pattern of colon epithelial cell line (Fig. 5A). At the same time, the tested extract inhibited proliferation of colon cancer cell lines LS180 and HT-29. Furthermore, YGB INT induced changes in the morphology of cancer cells. Cytoplasm swelling, disintegration of cell membranes and vacuolization were noticed and worsened in a dose-dependent manner.

YGB GW, similar to YGB INT, did not cause any negative changes in CCD841 CoN (Fig. 5B). On the contrary, the extract inhibited proliferation of LS180 and HT-29 cells and also induced changes typical for necrosis. Despite the fact that alterations in the morphology of colon cancer cells, induced by YGB GW, were similar to changes caused by YGB INT; nevertheless, both tested cell lines were less sensitive to this extract, and the observed effect was not dose dependent.

DISCUSSION

Young green barley is an important source of nutraceutical substances that can have an effect on biological functions to promote health and prevent disease, including cancer [2, 10, 26]. These nutritional values favoured of its success. In recent years, young green barley and preparations based on it (juice, tea, tablets or dried grass) have gained enormous popularity among dietary supplements and phytopharmaceuticals. Despite the immense popularity, there are few reliable and solid scientific research on the influence of young barley on the human body. Amazingly, even less is known about its direct effect on both normal and cancer cells. Thus, the presented study is one of the few attempts to extend this knowledge.

Cytotoxic studies revealed that both tested extracts did not affected the human epithelial cells (CCD841 CoN) viability, which corresponds with data presented by Czerwonka et al., which demonstrated that juice and young green barley water extract had a low cytotoxicity on CCD 841 CoTr (human colon epithelial cells) and HSF (human skin fibroblasts) [27]. Research conducted by Robles-Escajeda et al. also showed that aqueous green barley extract did not significantly affect the proliferation or viability of human foreskin fibroblasts (Hs27) [28]. Furthermore, in a whole range of investigated concentrations, the tested extracts did not affect colon epithelial cells proliferation.

The current study also revealed antiproliferative properties of the tested extracts in human colon cancer cell lines LS180 and HT-29, which corresponds with data obtained by Czerwonka et al. and Madhujith and Shahidi [27, 29]. As presented by Czerwonka et al., both water extract and juice obtained from young barley effectively inhibited HT-29 cells proliferation; nevertheless, their effectiveness – expressed as IC50 values – was worse than extracts tested in the current study, and amounted 0.7 mg/ml and 2.4 mg/ml, respectively [27]. On the contrary, the studies conducted by Madhujith and Shahidi showed that aqueous methanolic extracts of whole kernels from 6 different barley cultivars significantly inhibited proliferation of human colorectal adenocarcinoma cell line Caco-2. Extracts inhibited cancer cells proliferation

in the range of 29.3% – 51.2% at 0.5 mg/ml and 9.3% – 15.9% at 0.05 mg/ml after 96 hours of incubation [29].

All data cited above only suggest the possibility of using young barley extracts in the prevention and/or treatment of colon cancer. In this context, *in vivo* data provided by Kanauchi and Yao confirm the hypotheses [30, 31]. Kanauchi et al. showed that germinated barley foodstuff prevented the development of azoxymethane-induced colon cancers in rats, by reduction in the number of aberrant crypt foci [31]. On the contrary, Yao et al. revealed that the aqueous extract of fermented barley (prepared from barley-shochu distillery by-product) with *Lactobacillus plantarum* dy-1, protect mice from colon cancer development induced by HT-29 cells injection [30]. While discussing the antiproliferative properties of young barley extracts or products, it is also worth mentioning the research by Robles-Escajeda et al. They demonstrated that aqueous extract from barley leaves acts as effective inhibitors of divisions of human T-cell leukemia cells (Jurkat E6.1), human B-cell leukemia cells (NALM-6), and human Burkitt lymphoma B cells (BJAB) [28]. Interesting data was obtained by Meng et al., which revealed the ability of the methanol-soluble fraction from young barley leaves to decrease viability of murine melanoma cells (B16) through inhibition of melanin biosynthesis, which was correlated with the presence of tricin [32].

Both MTT and BrdU tests conducted in the presented study revealed the different sensitivity of colon cancer cells to the investigated extracts. HT-29 cells were more susceptible to YGB GW, while in case of YGB INT, a better response was noted in LS180 cells. Due to the varying degree of differentiation of tested cells lines and their genetic profile, the above-mentioned observation may be a harbinger of the different molecular mechanisms underlying the discovered antiproliferative activities of the tested extracts [33]. However, the presented study did not allowed confirmation or denial of this hypothesis. It also has to be noted that the results of MTT test indicated YGB INT as a better inhibitor of mitochondrial activity in colon cancer cells, while to the contrary, BrdU test revealed remarkable efficiency in inhibiting DNA synthesis by YGB GW. This indicates that YGB GW should be considered as a preferable antiproliferative agent.

May-Grünwald-Giemsa confirm both the non-toxic effect of the investigated extracts against human colon epithelial cells (CCD 841 CoN), and the antiproliferative effect in the case of human colon cancer cells (LS180 and HT-29). Next to the significant reduction in the number of colon cancer cells, the changes in their morphology, like disintegration of cell membranes, cytoplasm swelling and vacuolization, were also noted. These changes also suggest ability of the extracts to induce cancer cells necrosis, nevertheless, this observation requires confirmation by other specific investigations, especially in the light of data collected by Woo et al. and well as Yao et al. [30, 34]. The former showed that barley grass extract induced apoptosis in human prostate cancer cells (DU 145) and human breast adenocarcinoma cells (MDA-MB-231) [34]. Proapoptotic properties of other products based on barley was also demonstrated by Yao et al., whose studies revealed that aqueous extract of fermented barley (prepared from barley-shochu distillery by-product) with *Lactobacillus plantarum* dy-1 could induce apoptosis in the subcutaneous transplantation of human HT-29 cells in nude mice [30]. It needs to be highlighted that despite the fact that the nature of the changes induced by the tested extract was similar, once

again their effectiveness was different. Both colon cancer cell lines were more sensitive to YGB INT than to YGB GW.

The differences in the extracts antiproliferative and pro-necrotic properties observed in colon cancer cell lines may be due to a few key differences in the extracts themselves. The most important seems to be the type of raw material. In GreenWays, leaves were the starting material, whereas in Intenson leaves and blades of grass were used. The method of dealing with the collected raw material was also different. In GreenWays, the juice was squeezed out of the leaves and then subjected to a drying process. In the case of Intenson, the harvested barley grass was dried and then ground up. Consequently, 2 different products were created: dried juice (GreenWays) and ground, dried grass (Intenson).

The procedures for the preparation of the extracts used in this study also differed. The first major difference was the proportion between the amount of raw material and the solvent, and the solvent used in order to prepare YGB INT; 2.5 g of raw material was suspended in 100 ml of water, in the case of YGB GW this proportion amounted to 3.3 g per 100 ml of PBS. If the first stages of extract preparation, i.e. extraction, centrifugation and filtration, were similar, the method for dealing with the obtained filtrates was different. In the case of YGB GW, the resulting filtrate was the stock solution used in the current study. However, in the case of YGB INT, the collected filtrate was evaporated and the resulting crystals dissolved in PBS. It needs to be noted that the high temperature accompanying the drying of the barley grass and the evaporation could have inactivated some of the bioactive molecules (enzymes) and changed their concentration in the YGB INT extract. This may explain the worse antiproliferative activity. On the other hand, the greater efficiency of necrosis induction in colon cancer cells by YGB INT could have been caused by a much higher fibre content in the product, on the basis of which the extract was made (44% compared 6.9% in the GreenWays product, according to the manufacturer's information).

CONCLUSIONS

The data obtained in the current study demonstrated that both young green barley extracts specifically inhibit proliferation of human colon cancer cells without any undesirable effect on human colon epithelial cells. Growth inhibition of cancer cells was closely associated with cell death induction by the tested extracts. Despite the very promising results, further studies are required to explain the molecular and cellular mechanisms of barley activity, the amount of consumption, possible adverse effects, and ideal methods of preparation. Additionally, identification and examination of the bioactive compounds present in barley extracts, responsible for the discovered anticancer properties, is also needed.

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