

CAROB FIBRE – FUNCTIONAL EFFECTS ON HUMAN COLON CELLS



Stefanie Klenow, Michael Glei, Bernd Haber¹, Beatrice L. Pool-Zobel

Department of Nutritional Toxicology, Institute for Nutrition, Friedrich Schiller University, Dornburger Str. 25, D-07743 Jena, Germany

¹Nutrinova Nutrition Specialties & Food Ingredients GmbH, Industriepark Höchst, 65926 Frankfurt/Main, Germany

INTRODUCTION: Carob fibre is a food ingredient from the mediterranean carob pod (*Ceratonia siliqua L.*). During a mild procedure soluble carob constituents (sugar, tannins) are removed by water extraction and the insoluble dietary fibre (e.g. lignin, cellulose, and hemicelluloses) is retained in the residue, in addition to tannins and other polyphenols. Polyphenols potentially reduce colon cancer risk via iron chelation and scavenging of free radicals.

AIM: Carob fibre was investigated for its ability to modulate a variety of different cellular parameters of chemoprotection using two human colon cell lines.

METHODS: An aqueous extract of the carob fibre (100 g carob fibre / 1 l cell culture medium) was prepared (Figure 1) and added to human colon carcinoma HT29 cells or to LT97 human adenoma colon cells in culture at different concentrations. After 24, 48 and 72 hours treatment growth and survival of the cells was measured by determining metabolic activity (reduction of resazurin), cell number (intercalation of dye DAPI into DNA of remaining cells) and rate of DNA-synthesis (BrdU incorporation). The Comet Assay was used to determine DNA damage, or rather more the carob-mediated inhibition of DNA damage induced by genotoxic risk factors. For this HT29 cells were first treated with subtoxic amounts of carob extract for 24 hours to stimulate possible stress responses. Subsequently, the cells were then challenged with genotoxic agents, namely Fe-NTA (Fe³⁺) and FeSO₄ (Fe²⁺), both at 500 and 1000 µM. Alternatively, cells were incubated with mixtures of carob extract and both iron sources for 15 minutes, to assess a possible interaction of the components that could reflect scavenging of reactive intermediates.



Figure 1: From carob tree and carob pods to carob fibre and aqueous extract

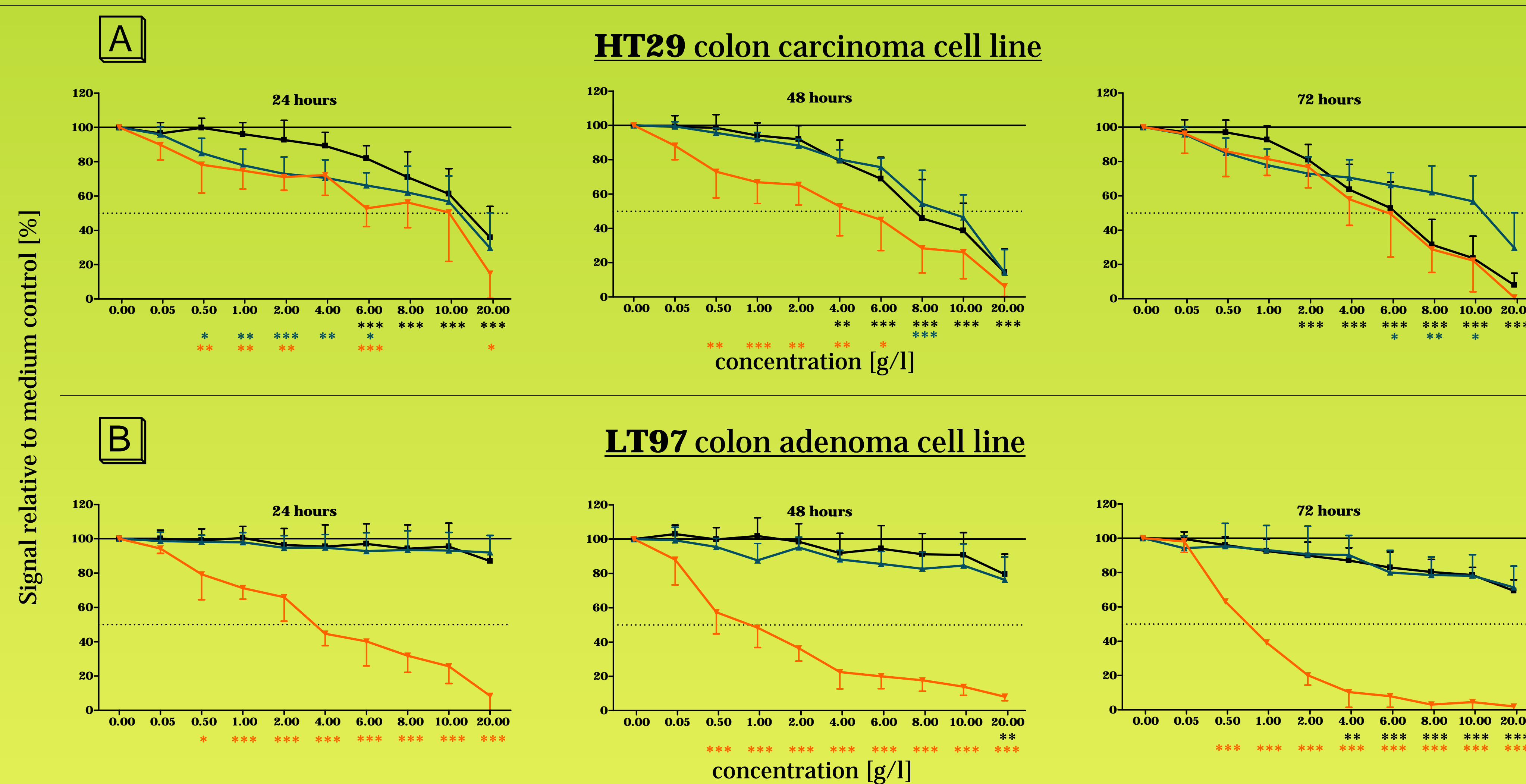
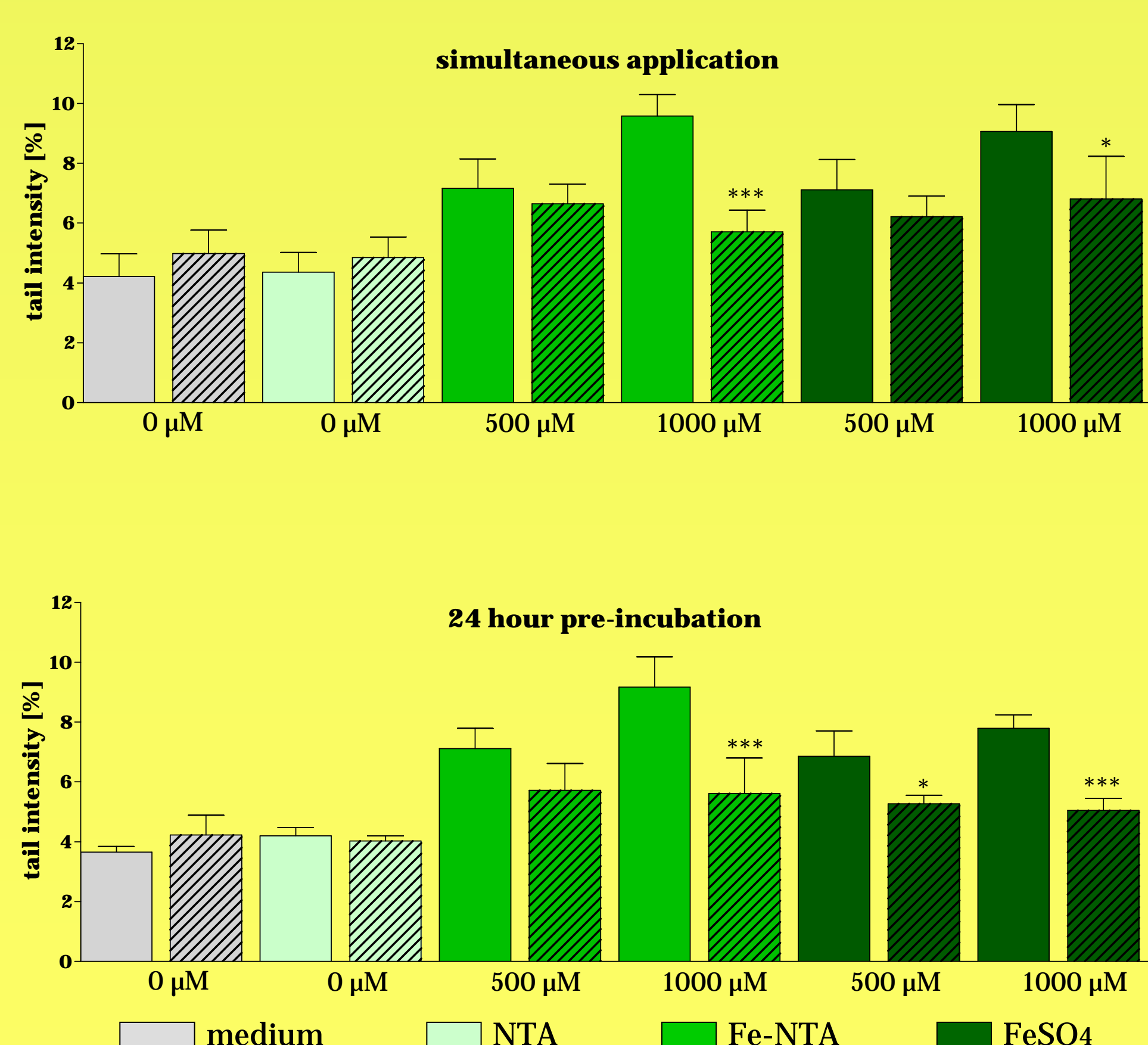


Figure 2: Cell number, metabolic activity and cell cycle progression in HT29 cells (A) and LT97 cells (B) after treatment with different concentrations of aqueous extract. Signals were calculated to medium control. Stars indicate significant differences to the medium control [cell number, one-way ANOVA] or corresponding cell number [metabolic activity and DNA-synthesis, two-way ANOVA], respectively (Bonferroni post-test *p<0.05 **p<0.01 ***p<0.001), mean±SD, n=4-13 (A), n=3-7 (B)

The aqueous extract effectively modulated cell number and viability of HT29 cells (Figure 2). The effect followed dose and time dependent relationships. While 2 g carob fibre per litre significantly reduced HT29 cell number after 72 hours incubation, metabolic activity was affected already at lower concentrations (0.5 g/l). This effect was particularly apparent after 24 hours treatment, but was only temporary. In contrast, cell number of LT97 cultures was less impaired by the treatment with equal amounts of carob extract (Figure 2). In LT97 metabolic activity correlated highly with cell number. Nonetheless, the impact on DNA-synthesis in LT97 was even stronger than in HT29 cells and more pronounced with increasing incubation time.



Both, ferrous and ferric iron induced comparable levels of DNA-damage in HT29 cells (Figure 3). When applied simultaneously with the genotoxic iron preparations, carob extract significantly reduced the genotoxicity induced by the highest iron concentration (1000 µM). This finding indicated that carob-specific phenolic compounds may have chelating properties. Protective effects of equal magnitude were detected after treating the cells with the extract for 24 hours and then subsequently challenging them with Fe-NTA (Fe³⁺) and FeSO₄ (Fe²⁺). This finding pointed to protective effects resulting from an induction of stress response systems in the HT29 cells.

Figure 3: DNA-damage induced by 500 and 1000 µM iron (15 min) and effects of simultaneous and pre-incubation (24 hours) with aqueous extract of carob fibre (2 g/l, hatched) measured with Comet Assay. Stars indicate significant effect of carob fibre (two-way ANOVA, Bonferroni post-test *p<0.05 ***p<0.001), mean±SD, n=3

CONCLUSION: Carob modulates the growth of both colon carcinoma and colon adenoma cells. The impact seems to depend on growth kinetics of the cell line. Highly proliferating cells were more susceptible to the growth inhibitory properties of this plant extract, than cells with a lower cell turn over. In addition, carob may act protective in human colon cells by possible scavenging mechanisms and by inducing enzymes of the stress response. So far the available evidence points to an induction of the cellular defence systems, a finding that necessitates the identification of target genes and more in depth studies on how they may be altered by carob polyphenols.