# Colon-available raspberry extract exhibits anti-cancer effects on *in vitro* models of colon cancer

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## INTRODUCTION

- Colorectal cancer (CRC) is the second most common cancer in the Western world[1]
- Consumption of fruit (including berries) is associated with a decreased risk of developing cancers of epithelial origin[2]
- Animal and *in vitro* studies suggest berry constituents exert an anti-cancer effect[3-5]
- Limited information exists on the role of berries and berry extracts in colon cancer[6]

## AIM

- Examine the anti-cancer properties of a colon-available raspberry extract (CARE) on a range of biomarkers biologically relevant to CRC
- The biomarkers chosen represent *in vitro* models of the key stages in carcinogenesis including initiation, promotion and invasion[7], represented in figure 1

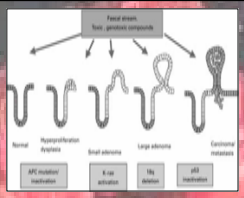

Figure 1: Representation of colorectal cancer development

## METHODS

***IN VITRO* DIGESTION: to produce colon-available raspberry extract (CARE)**

Gastric digestion: Incubation: pH 2, 37°C, 2 hrs, pepsin
Pancreatic digestion: Diffusion of bicarbonate out of dialysis tubing to bring pH to 7.5, 37°C, 2 hrs, pancreatin and bile salts

***IN VITRO* STUDIES: assessment of anti-cancer activity of CARE in the following models at the dose range 0 - 50 µg/ml GAE* as described previously[8].**

Initiation:
- Comet assay – anti-genotoxic potential of CARE (figure 2)

Promotion:
- Flow cytometry – effect of CARE on cell proliferation
- Transepithelial resistance – effect of CARE on tumour promotion (figure 3)

Invasion:
- Matrigel invasion assay – anti-invasive activity of CARE (figure 4)

Statistical analysis:
- Each data set is the mean of 3 replicate experiments. Analysis of variance by ANOVA and Dunnett T-test was carrier out where significance was accepted at $p<0.05$

* Gallic acid equivalents

Figure 2: Images obtained by comet assay

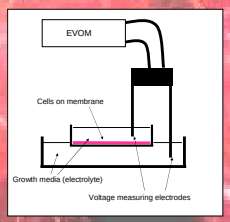


Figure 3: schematic of TER assay

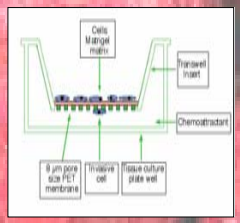


Figure 4: schematic of invasion assay

## RESULTS

*In vitro* digestion: Figures 5&6
- CARE is depleted in anthocyanins and ellagitannins when compared to the original raspberry juice. Polyphenols and polyphenol breakdown products more stable to digestion are present in CARE but not original juice

Comet assay: Figure 7
- Anti-genotoxic dose-dependent effect observed after 24 hr pre-incubation with CARE
- Significant from 3.12 – 50 µg/ml GAE (*$p<0.001$)

Cell cycle:
- A significant ($p=0.024$) decrease in G1 population of HT29 cells was observed after 24 hr pre-incubation with 50 µg/ml GAE CARE (data not shown)

Transepithelial resistance:
- Barrier function was unaffected by any concentration of CARE, measured by recording transepithelial resistance of CACO2 cells (data not shown)

Matrigel invasion assay: Figure 8
- CARE significantly decreased invasion of HT115 cells in a dose response manner

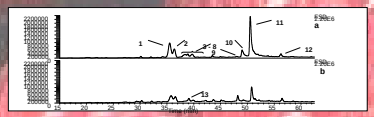

Figure 5:Comparison of raspberry extract (a) and digested sample (b)

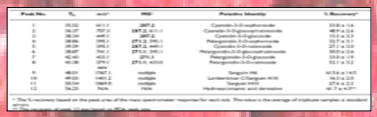

Figure 6: Peak identities

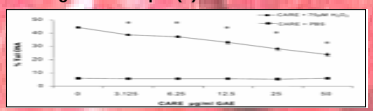

Figure 7: Effect of CARE on tail DNA

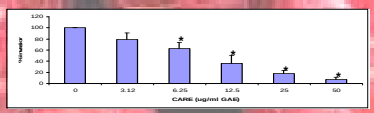


Figure 8: Effect of CARE on invasion rates

## CONCLUSIONS

- CARE exerted a range of effects on colon cell cultures (significant 6.25 – 50 µg/ml GAE, *$p<0.05$) indicative of anti-cancer activity, but did not inhibit tumour promotion.
- Results indicate beneficial modification of CARE at various stages of carcinogenesis:

**- GENOTOXICITY - CELL CYCLE - INVASION**

- The *in vitro* anti-cancer activity of CARE supports the limited data on the protective effects of berries in colon cancer

## REFERENCES

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