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Published Black Raspberry Research at Ohio State University, 2007-present

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Plant and Horticultural Science Studies

Within the College of Food, Agricultural, and Environmental Sciences, investigators have shown that black raspberry cultivar, maturity, and production site can have an impact on the antioxidant capacity and phenolic levels (Ozgen 2008). In collaboration with the College of Education and Human Ecology, these growing conditions were shown to consequently impact biological activity (Johnson 2011) and should be controlled and optimized when conducting a clinical trial involving black raspberries.

Phytochemical Analysis and Product Development Studies

Analytical methods to quantify both phytochemicals in berries and biological tissues (once consumed) are essential to conduct well-controlled clinical trials. Analytical methods have been developed at investigators in the College of Food, Agricultural, and Environmental Sciences which allow quantification of the primary phytochemicals from black raspberries and their biological metabolites, including anthocyanins, ellagitannins, quercetin glycosides, myericetin glycosides, ellagic acid, and methyl ellagic acid derivatives (Tulio 2008, Zikri 2009, Wang 2011, Kamonpatana 2012, Mace 2014, Knobloch 2015). A NMR-based multivariate statistical model was also developed to determine biological activity of black raspberry compounds. This model has allowed the identification of new bioactive components and predicted their biological effect (Paudel 2014).

Investigators within the College of Food, Agricultural, and Environmental Sciences have designed and fully characterized black raspberry functional food products, with the intention of creating a product suitable for long-term cancer prevention studies. Both confections and nectar made with freeze dried black raspberry powder have been developed and were well accepted in sensory evaluations (Gu 2013). Confections retained >90% of anthocyanins and ellagitannin after processing, while nectars retained >69% of anthocyanins and >66% of ellagitannin. Confections with varying matrices were also developed to evaluate the release rate of black raspberry phytochemicals (Gu 2015). *In vitro* experiments showed that hard confections released phytochemicals most quickly (90 min), followed by pectin confections (150 min) and starch confections (540 min). Phytochemical release rate has potential to impact contact time with oral tissue. These black raspberry confections were then used in two clinical trials, one in healthy subjects to examine the dissolution rate in humans, and one in prostate cancer patients (publications pending).

Preclinical Evaluation and Mechanistic Studies

Oral and Esophageal

Within the College of Medicine and College of Public Health, esophageal tumors were induced in rats with the carcinogen NMBA, and rats were fed black raspberries either (1) before and during NMBA administration (modeling prevention), (2) immediately after a brief NMBA administration and after only preneoplastic esophageal lesions have formed (modeling very early therapy), or (3) after NMBA administration and after tumor development (modeling therapy after full disease). Black raspberry was effective in prevention of esophageal tumors and as a very early therapy but was not effective as a therapeutic agent once the full disease had developed (Stoner 2007). The black raspberry diet also influenced several genes in the healthy rat esophagus, including those associated with cellular matrix, signaling cascades, transcription regulation, apoptosis, metabolism, contraction, cell regulation, signal transduction, and metabolism (Lechner 2008). The black raspberry diet also returned many genes that were negatively impacted by the NMBA carcinogen back to normal levels (Stoner 2008). In additional collaboration with the College of Food, Agricultural, and Environmental Sciences, the cancer fighting properties of black raspberry was confirmed when an ethanol extract of black raspberries and two component anthocyanins (cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside) were effective in inhibiting growth and inducing apoptosis of a highly tumorigenic rat cell line (Zikri 2009).

More recently, within the Colleges of Public Health, Medicine, and Dentistry, the hamster cheek pouch model used to evaluate the effect of topical black raspberry on cancer development in high at-risk oral mucosa (HARM). A 12-week topical application of a black raspberry suspension resulted in reduced squamous cell carcinoma multiplicity, reduced tumor incidence, reduced proliferation rate, and increased retinoblastoma gene (*RB1*) expression (but had no significant impact on gene expression of other genes related to oral carcinogenesis) compared to the control. These findings support future research to study topical black raspberry application in humans at risk of oral cancer (Warner 2014).

Skin

Led by investigators in the College of Medicine, hairless mice were subjected to ultraviolet B rays and immediately treated topically with either black raspberry extract or a control solution. Mice treated with the black raspberry extract developed significantly fewer and smaller tumors, and had lower biomarkers of inflammation, compared to mice treated with the control. This encourages further investigation of use of black raspberry extract in prevention of skin cancers in humans (Duncan 2009).

Female Reproductive System

Preliminary studies at OSU (College of Public Health and College of Medicine) have suggested that black raspberries may be effective in some cancers of the female reproductive system. An ethanol black raspberry extract inhibited growth and induced apoptosis in three cervical cancer cell lines (Zhang 2011), and a vulvar squamous cell carcinoma cell line incubated with black raspberry extracts had higher caspase-14 levels, indicating a protective effect (Joehlin-Price 2014). This encourages further investigation on the use of black raspberry extracts for these types of cancers.

Immune

The human immune system and inflammatory pathways are closely linked to cancer development and progression. Led by the College of Medicine and in collaboration with the College of Public Health and College of Food, Agricultural, and Environmental Sciences, an ethanol black raspberry extract as well as two known metabolites of black raspberry (cyanidin-3-rutinoside and quercitin-3-rutinoside) were separately evaluated for their ability to modulate immune function *in vitro*. The black raspberry extract inhibited proliferation and viability of human T lymphocytes, reduced activation of STAT5 phosphorylation cells, and inhibited generation of myeloid-derived suppressor cells (MDSC), all of which reflect reduced cytotoxicity and reduced inflammation. The two metabolites that were evaluated modulated these markers of immune function in a similar manner. These studies suggest that black raspberries may be useful for both therapeutic intervention and as a potential source of compounds for drug development (Mace 2014).

Bioavailability and Metabolism in Humans

To study how black raspberry anthocyanins are absorbed and metabolized, investigators within the College of Food, Agricultural, and Environmental Sciences administered black raspberries to adult men and collected urine over the next 12 hours. Absorption and metabolism of anthocyanins was examined by analyzing anthocyanins and their known metabolites in urine samples. The majority of anthocyanins and metabolites were excreted in the urine between 4 and 8 hours after consumption, and it was found that methylation is the major metabolic pathway for anthocyanins. Individual metabolites are reported (Tian 2006).

The stability and transport of black raspberry anthocyanins into gastric and small intestinal tissues was investigated in a rat model within the College of Food, Agricultural, and Environmental Sciences and the College of Education and Human Ecology. Rats were fed a black raspberry extract and contents of the GI tract were analyzed at various time points over 3 hours. Anthocyanin glycosides were found to be relatively stable in the gastric and small intestinal lumens. Anthocyanin concentration decreased in the stomach over time and peaked in the small intestine at 120 min. Anthocyanins were absorbed into GI tissues but were not distributed into the blood or retained. The study contributes to a better understanding of the metabolism, absorption, and tissue uptake of anthocyanins (He 2009).

Within the College of Education and Human Ecology, College of Dentistry, and College of Food, Agricultural, and Environmental Sciences, anthocyanin extracts of different berries were incubated with saliva from healthy adults to evaluate anthocyanin degradation in saliva.

Degradation was impacted by anthocyanin structure and the presence of oral microbiota, suggesting that anthocyanin structure could impact efficacy (Kamonpatana 2012).

Human Clinical Intervention Studies

Mucoadhesive black raspberry gels were developed for use on oral tissue within the College of Dentistry (in collaboration with the College of Medicine and College of Food, Agricultural, and Environmental Sciences). Anthocyanins were stable at low pH and refrigeration temperature, and anthocyanins were absorbed into oral tissue within 5 minutes of application (Mallery 2007). This black raspberry gel was applied topically to premalignant oral lesions of human volunteers. The black raspberry gel modulated oral gene expression profiles, ultimately reducing epithelial COX-2 protein. It also reduced the loss of heterozygosity index, which reflects reduced risk of progression to oral cancer (Mallery 2008, Shumway 2008). While preliminary, these studies continue to lend promise to the therapeutic benefit of black raspberry gels for oral tumor prevention.

To study the effect of black raspberry consumption on biomarkers of colon and rectum tumor development, investigators fed patients with colorectal cancer freeze-dried black raspberry powder daily for up to 9 weeks (College of Medicine, College of Food, Agricultural, and Environmental Sciences). Black raspberry consumption modulated expression of genes associated with the *Wnt* pathway, proliferation, apoptosis, and angiogenesis, suggesting a protective effect of black raspberries on tumor development in the colon and rectum. While we would not recommend prescribing black raspberries for cancer treatment, these data warrant future studies on use of black raspberries for colorectal cancer prevention and in combination with traditional therapies (Wang 2011).

In collaboration between the College of Public Health, College of Medicine, and College of Food, Agricultural, and Environmental Sciences, patients with oral squamous cell carcinoma consumed a black raspberry troche daily for 2 weeks to evaluate the impact on gene expression biomarkers of inflammation and carcinogenesis. There were no major toxicities caused by the black raspberry treatment, and metabolites of compounds found in black raspberries were detected in oral tissues, showing that the bioactive compounds were reaching the tissue. Following administration of black raspberry, the expression of prosurvival genes and proinflammatory genes in oral squamous cell carcinomas were significantly reduced. These genes could serve as biomarkers for black raspberry consumption and efficacy in oral cancer (Knobloch 2015).

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