Antibacterial Potential of Quercetin against IBD Bacterial Isolates and Cytotoxicity against Colorectal Cancer

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ABSTRACT

Background: Quercetin is known for its vast pharmacological activities in recent years. It has shown beneficial effects in animal models of colitis. Purpose: The effectiveness of quercetin in human IBD is limited. Results from these pre-clinical studies indicate an opportunity for developing anti-colitic polyphenol treatments. Methods: In the present study, Quercetin was evaluated for its antimicrobial potential against clinical isolates of IBD patients (HM95, HM233, HM251, HM615). Cytotoxicity was determined against human colorectal adenocarcinoma cells (Caco2, COLO.205, HT.29), whereas, cytocompatibility against normal rat intestinal epithelial (IEC-6). Results: It showed an MIC value of 5 mg/mL and MBC value of 10 mg/mL against all the four bacterial isolates. A dose dependent response was observed against the cell lines. Conclusion: Antibacterial activity of Quercetin was found against the bacterial isolates from CD and UC patients used in the study. Cytotoxicity was observed against colorectal cancer cells with minimal effect on normal cells. However, further investigations are required to understand its precise mechanism. Key words: Polyphenol, Quercetin, Cytotoxicity, Inflammatory bowel disease, Colorectal cancer.

INTRODUCTION

Inflammatory Bowel Disease (IBD) comprises of chronic, relapsing, inflammatory disorders of the gastrointestinal tract which includes Ulcerative Colitis (UC) and Crohn’s Disease (CD).[1] It is characterized by diarrhoea, rectal bleeding, the urgency to have bowel movements, abdominal cramps, fever and weight loss.[2,3] IBD patients are at a greater risk of colorectal cancer due to prolonged exposure to chronic inflammation and immunosuppressive therapies.[4] The colonic mucosa of Crohn’s disease and mucosa of colon cancers specimens contain relatively abundant E. coli, compared to normal mucosa. These E. coli express hemagglutinins and adhere to intestinal epithelial cells are found in CD and UC patients.[5]

In the past IBD was considered as a disease of the western population, however new epidemiological studies in the last decade suggest rise in IBD patients are at a greater risk of colorectal cancer due to prolonged exposure to chronic inflammation and immunosuppressive therapies.[4] The colonic mucosa of Crohn’s disease and mucosa of colon cancers specimens contain relatively abundant E. coli, compared to normal mucosa. These E. coli express hemagglutinins and adhere to intestinal epithelial cells are found in CD and UC patients.[5]

In the past IBD was considered as a disease of the western population, however new epidemiological studies in the last decade suggest rise in cases throughout the world including the developing countries in Asia and Africa.[6] Its incidence and prevalence is increasing in India and estimated to have a high disease burden in the world with few resources to manage it.[7]

Quercetin has shown beneficial effects in animal models of inflammatory bowel disease, working through inflammatory pathways, oxidative stress reduction and altering gut microbiota composition.[6,8,9] It has also shown efficacy against human colon cancer cell lines. Although evidence for the effectiveness of polyphenols for IBD in humans remains very limited, results from these pre-clinical studies indicate an opportunity for developing anti-colitic polyphenol treatments.[10] In this study we aim at assessing the antibacterial activity of Quercetin against isolated four E. coli strains from inflammatory bowel disease patients, further evaluate cytotoxicity against 3 colorectal cancer cell lines.

MATERIALS AND METHODS

Test Materials and sample preparation

The polyphenol, Quercetin (R035PO) was obtained as gift samples from Pharmanza Herbal Pvt. Ltd.

Antibacterial activity

Bacterial isolates - *Escherichia coli* HM95 (AIEC), *Escherichia coli* HM615 (colonic mucosa associated. *E. coli*), *Escherichia coli* HM233 and *Escherichia coli* HM251 (colonic mucus associated patient strains) were received under Material Transfer Agreement with University of Liverpool, United Kingdom. The bacterial isolates were subcultured on MacConkey agar (HiMedia) plates and incubated aerobically at 37°C. The antibacterial activity of Quercetin was evaluated by agar well diffusion method and MIC (detected by broth dilution method) as previously reported with minor modifications.[11]

Determination of cytotoxicity and cytocompatibility

Human colorectal adenocarcinoma cells (Caco2, COLO.205, HT.29) and normal rat intestinal epithelial cells (IEC-6) were obtained from National Center for Cell Sciences, Pune-India. The cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) with two mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and supplemented with 10% FBS procured from Gibco Life Technologies,

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Bangalore-India. Viable cell suspension 50 µl with a density of 1 × 10⁶ cells/ml (determined by Trypan blue exclusion method) was seeded into each well in a 96 well micro titre plate and final volume made up to 150 µl with DMEM media. Test materials were diluted in DMEM media to obtain different concentrations. 100 µl of Quercetin (400 - 6.25 µg/ml) was added to the wells followed by incubation for 48 h in the presence of 5% CO₂ at 37°C into CO₂ incubator. After the incubation period, 20 µl of MTT reagent (3 - (4, 5-dimethylthiazol-2 - yl)-2, 5-diphenyltetrazolium bromide, 5 mg/ml in PBS) procured from HiMedia laboratories, was added to each well following 4 h incubation in dark. The supernatant was removed without disturbing the precipitated Formazan crystals. Formed crystals were dissolved by addition of 100 µl of DMSO and optical density (OD) was calculated at a wavelength of 492 nm. Cell-viability assays were conducted as per previously reported standard procedure. [12] The study was performed in triplicates and percent cell viability was calculated using equation -

\[
\text{Percent cell viability} = \frac{\text{OD of test material}}{\text{OD of control}} \times 100
\]

Statistical analysis
Statistical analysis was carried out using Graph Pad Prism 5.0 software.

RESULTS AND DISCUSSION

Antibacterial activity
The results of antibacterial effect of Quercetin against the four IBD clinical isolates are listed in Table 1. Quercetin is shown to modify bacterial diversity in Citrobacter rodentium induced colitis mouse model. Wherein it enhanced bacterial populations of Bacteroides, Bifidobacterium, lactobacilli and Clostridia and reduced Enterococcus and Fusobacterium. In this study quercetin showed MIC value of 10 mg/mL and MBC value of 5 mg/mL, against the four IBD strains used in the study. The highest zone of inhibition of 24.33 mm was observed against HM233. Use of antibiotics in IBD management has seen severe side effects and drug resistance cases due to need for prolonged or recurrent treatment. [13] This has stimulated the need for discovery of newer substances from natural origin, including medicinal plants as antimicrobial substances to attain efficacy and better tolerability. There is increasing evidence that the mucosa-associated microbiota, may be essential in the pathogenesis of the inflammatory bowel diseases: ulcerative colitis and Crohn’s Disease. [10] However, no study data has been published showing the antimicrobial activity of quercetin on IBD strains till date. Although a recent study demonstrated the bacteriostatic role of quercetin and the results of the study suggest that quercetin has potential as an alternative antibiotic feed additive in animal production. [14]

Cytotoxicity of Quercetin against Colorectal cancer cell lines
The percent cell viability decreased on 48 h exposure of colon cancer cells to quercetin at concentrations ranging from 400-12.5 µg/ml (Figure 1). The results are presented in Table 2 depicting IC₅₀ values. [15] IC₅₀ value was obtained to assess its inhibitory concentration that causes 50% cell viability. Previous studies suggest high toxic effect of quercetin against cancer cells, accompanied with little or no side effects or harm to normal cells. [16] Our results are in agreement with previous claims regarding cytotoxic effect of quercetin.

CONCLUSION
Antibacterial activity of Quercetin was found against the bacterial isolates from CD and UC patients used in the study. Cytotoxicity was observed against colorectal cancer cells with minimal effect on normal cells. However, further investigations are required to understand its precise mechanism.

ACKNOWLEDGEMENT
The authors are thankful to Prof. Barry J Campbell (Gastroenterology Research Unit, Institute of Translational Medicine, University of Liverpool, United Kingdom) for providing IBD strain through Material Transfer Agreement. The authors are also thankful to the KLE University, Belagavi for providing financial assistance towards the submitted research work.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

ABBREVIATIONS
AIEC: Adherent-invasive E. coli; IBD: Inflammatory bowel disease.

REFERENCES

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<th>Table 2: Cytotoxic effect of Quercetin on Colorectal cell lines. Cell line IC₅₀ Conc. (µg/mL)</th>
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Figure 1: Percent cell viability: Quercetin against Colorectal cancer and normal cells


