Oregano Essential Oil and Carvacrol reduce Cryptosporidium parvum infectivity of HCT-8 Cells

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Abstract

Infection Immunodetection

Abstract

Introduction

Undernutrition (UN) is defined as the outcome of insufficient food intake (quantity and quality) and repeated infectious diseases (UNICEF). It affects close to 1 in 2 children around the World, resulting in unmeasurable loss of 3 million children lives every year. In India alone, 60 million children suffer from UN and are either underweight or stunted. UN is a key underlying factor of child mortality and has severe consequences on academic performance, future productivity, potential income and susceptibility to chronic diseases. Lipid-based nutrient supplements (LNS), a type of nutrient and caloric dense fortified food products, are widely used to address the problem of UN. Although, existing LNS are efficient in providing adequate nutrition, limited evidence exists on tackling diseases from bacterial and parasitic infection, enteropathy, and its characteristic chronic inflammation. Infection with enteric parasites results in reduced nutrient digestion and absorption, chronic gut inflammation, iron deficiency anemia, protein-energy malnutrition, reduced growth and reduced cognitive development in children. Therefore, LNS based interventions focused only on providing nutritional foods to children are not sufficient to tackle the multi-etiological problem of undernutrition.

Cryptosporidiosis, a parasitic disease caused by a protozoan of the genus Cryptosporidium, is the second largest cause of diarrheal disease and death in infants after rotavirus. Current strategies to address parasitic infections, such as antibiotic treatments (abendazoles and mebendazoles), have resulted in limited success due to increasing resistance, severity of side effects, and reemergence of parasites in treated patients. Thus, it is the need of the hour to develop new natural treatments to address such problems. Oregano Essential oil (OEO), also known as the "Medicinal miracle", elicits antibacterial, antiviral, antifungal, antiparasitic, and antioxidant activities. The principal components responsible for the bioactivity of OEO are carvacrol (CV) (LSD 980 mg/kg) and thymol. Although there is vast literature on the effectiveness of OEO and its constituents against bacteria, evidence on its effectiveness and efficacy against gut parasites is limited.

Methodology

Aim: Assess the effectiveness of oregano essential oil and carvacrol on preventing C. parvum infectivity of HCT-8 cells in vitro at several concentrations (0 µg/mL – 250 µg/mL).

Hypothesis: Oregano essential oil and carvacrol will reduce C. parvum invasion and infection at concentrations well tolerated by the HCT-8 cell monolayers (97% viability).

Results: Infectivity Assay

Carvacrol

IC50 = 25.25 µg/mL
After log transformation a = -48.34
b = -17.80

Oregano Essential Oil

IC50 = 65.84 µg/mL
After log transformation a = -39.90
b = -14.42

Figure 4. Dose-response relationship to calculate IC50 of Carvacrol and Oregano Essential Oil using the Modality 2 cell assay. Data points represent Means ± SD from three experimental replicates × 3

Figure 5. Montages showing C. parvum infection of HCT-8 cell monolayers and patterns of infectivity after treatment with bioactives

Figure 2. C. parvum lifecycle

Figure 3. Holtrophic design to assess the invasion and growth inhibition of C. parvum.

Table 1: Treatment with carvacrol and oregano essential oil did not reduce cell viability at any concentration. Inhibition of cell viability was observed at 250 µg/mL of CV and OEO.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of replicates</th>
<th>0 µg/mL</th>
<th>25 µg/mL</th>
<th>50 µg/mL</th>
<th>100 µg/mL</th>
<th>200 µg/mL</th>
<th>250 µg/mL</th>
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<tbody>
<tr>
<td>CV</td>
<td>3</td>
<td>69.64%</td>
<td>69.64%</td>
<td>69.64%</td>
<td>69.64%</td>
<td>69.64%</td>
<td>69.64%</td>
</tr>
<tr>
<td>OEO</td>
<td>3</td>
<td>69.64%</td>
<td>69.64%</td>
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Figure 3. HCT-8 cell viability as observed using phase-contrast microscopy. Green represents live cells and red represents dead cells.

Conclusions

From Modality 1 Assays

• No difference was observed in infectivity of HCT-8 cells treated with several doses of carvacrol or oregano essential oil (p>0.05), indicating that there was no effect of bioactives on C. parvum in vitro.
• Carvacrol (IC50 = 25.25 µg/mL) and oregano essential oil (IC50 = 65.84 µg/mL) reduced C. parvum infectivity (p<0.05).
• Decontamination of cell monolayer and loss of cell viability was observed at 62.5 µg/mL carvacrol, whereas no changes in cell viability were observed in cells treated with oregano essential oil at dosages as high as 125 µg/mL.
• Complete loss of cell viability was observed at doses higher than 250 µg/mL for both carvacrol and oregano essential oil.
• Mechanism of action of carvacrol or oregano essential oil on C. parvum growth and infection is unknown. Some evidence points to the promotion of oxidative stress, inhibition of phospholipases, and modification of calcium signaling; all of which modulate C. parvum invasion and infection.

References


Acknowledgements

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Figure 1. Interrelationship of inadequate nutrient intake and repeated parasitic infections

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