Investigating The Apoptotic Effect of Leaf Extracts on Colon Cancer Cells

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BACKGROUND

- Colorectal cancer ranks 2nd in global cancer-related mortality.
- Early detection and treatment are important for improved prognosis as early-stage symptoms may be subtle.
- Targeting apoptosis markers can reveal the potential therapeutic effects of different leaf extracts on colorectal cancer cells.

OBJECTIVES

- Determine the protein concentration and expression of Caspase-3 in CACO2 cells treated with different concentrations of leaf extracts and a positive control (Curcumin 20μM) using the Western Blot method.

METHODS

1.PREPARE CELL LYSATE

- Treat CACO2 cell line with different concentrations of leaf extracts and positive control (Curcumin (20μM)), and incubate for 48 hours.
- Lyse cell membranes using RIPA buffer to prepare cell lysate.

2. DETERMINATION OF TOTAL PROTEIN IN CELL LYSATE

- Prepare standard (bovine serum albumin) panel and curve with different given concentrations.
- Prepare 5-time diluted samples with the unknown solutions of cell lysate.
- Add BCA reagent to the standard and samples, incubate for 30 mins at 37°C, then read the absorbable at 562nm.
- Calculate the unknown protein concentration of the samples based on the BSA standard curve

3. CALCULATE REQUIRED VOLUMES OF EACH SAMPLE

- Form sample solutions with 20μg of protein and add equal volumes of sample buffer (Laemmli buffer).
- Heat the sample at 95°C for 5 mins to denature the proteins.

4. LOAD SAMPLES AND PERFORM POLYACRYLAMIDE GEL ELECTROPHORESIS

- Separates proteins based on size & charge.

5. PERFORM A SEMI DRY MEMBRANE TRANSFER

6. BLOCK THE MEMBRANE

- Blocking buffer TBST in (1:10) milk
- Reduces non-specific binding

7. IMMUNOBLOTTING WITH PRIMARY ANTIBODY (CASPASE 3)

- Detects caspase 3 protein

8. IMMUNOBLOTTING WITH SECONDARY ANTIBODY (GOAT ANTI RABBIT)

- Detects primary rabbit antibody for human caspase 3.

9. DETECTION OF CASPASE 3

- With HRP enzyme (catalyst) and Enhanced Chemiluminescence (ECL)

RESULTS

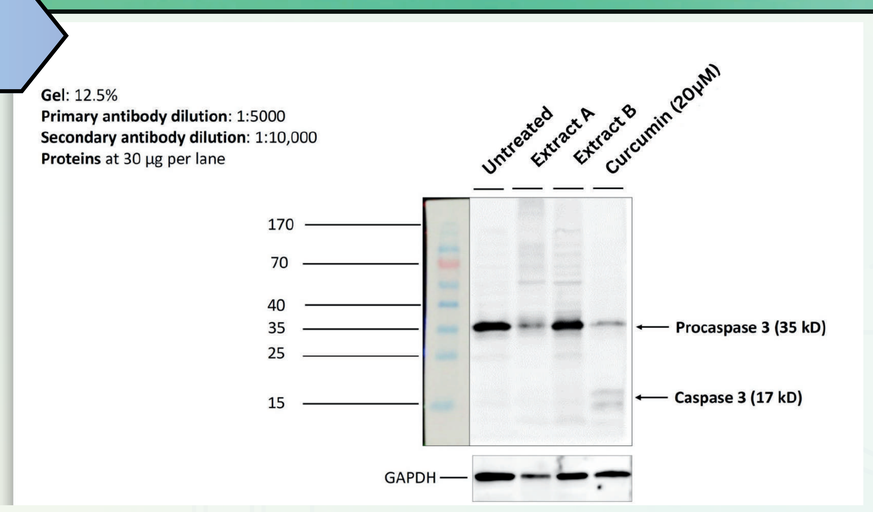

FIGURE 1. WESTERN BLOT ANALYZING PRESENCE OF CASPASE-3 IN TREATED CACO2 CELLS WITH EXTRACT A, B, AND CURCUMIN (POSITIVE CONTROL)

- The western blot analyzes the protein concentration and presence of Caspase-3, a cysteine protease that induces apoptotic cell death.
- The results show whether there is the presence of Procaspase-3 and, if cleaved, Caspase-3 in Leaf Extracts A, B, and Curcumin (20μM).
- **All three extracts had Procaspase-3 at 35 kD** with the greatest molecular presence being shown by Extract B.
- **Curcumin (20μM) had the most prominent presence of cleavage and Caspase-3 at 17kD, however, Extract A and B had minimal presence of Caspase-3.**
- These results suggest that **Curcumin has greater potential in inducing apoptosis in CACO2 cells** and with further analysis, it is expected to show similar apoptotic features in these cells.

EXPERIENCES & REFLECTION

Through this two-week program, we were exposed to, able to experience and learn diverse biomedical techniques utilized in developing therapies for colorectal adenocarcinoma.

We were introduced to the remarkable environment within a medical research career and gained better insight on cell behavior and processes involved in pharmaceutical therapy development. We immensely enjoyed this experience, the lab environment, and deeply appreciate the support of the research team.

Experiences & Skills Learnt:

- Western Blot
- Cell Culture
- Cell Counting
- Flow Cytometry
- Photo Cytometry
- Pipetting