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Thesis title: *In vitro* evaluation of selected flavonoids on Colon Cancer Cells via targeting its Signaling Cascade

### Findings

Under the present work we tried to explore the effectiveness of Naringenin (NR) and Diosmin (DS) and the combination of the two as well as the 5-fluorouracil (5-FU), a conventional drug for colon cancer. Apoptosis and inflammatory pathways were the two mechanistic approach that were undertaken for the study. The expression of markers of these two pathways were studied.

The present work encloses the following findings: We primarily treated the colon cancer cells with our flavonoids and the drug individually and calculated IC<sub>50</sub> of each on HCT116 and SW480. The combinations NR+DS and 5-FU+NR has greater inhibitory effect as compared to their individual treatments. The cytotoxic effect of the combined agents i.e NR+DS and FU+NR were assessed on HEK293 cells which showed negligible toxicity on normal cell line HEK293. TM he combination index of the combinations NR+DS and FU+NR was calculated by the CompuSyn software which showed that the combinations NR+DS and FU+NR act synergistically in both HCT116 and SW480 cell lines as represented by combination index (CI) values in a wide range of concentrations. The combination therapy was significantly found to enhance apoptosis in HCT116 and SW480 cells as observed through DAPI assay, DNA fragmentation, Annexin V/FITC apoptosis assay, apoptotic protein expression assay and mRNA expression through real time PCR. Expression of Bax, Caspase 3, caspase 8, caspase 9 and p53 were increased while Bcl-2 decreased in both NR+DS treatment and 5FU+NR treated cells as compared to other treatments. The effect of the combinations on cell cycle were also evaluated and we observed that the combination NR+DS and 5FU+NR caused cell cycle arrest in the G0/G1 phase in both cell line HCT116 and SW480.

The strongest impact of downregulation of inflammatory gene NF- $\kappa$ B was observed in NR+DS. The genes considered to be the activators of NF- $\kappa$ B; IKK- $\alpha$  and IKK- $\beta$  were also found to be least expressed in NR+DS and 5FU+NR and appear to have a lesser inflammatory role. Similar expression pattern were observed at protein level and mRNA level.

Finally, it can be concluded that the combination of NR and DS can act as a more effective drug than the two compounds, taken individually. The combination 5-FU with NR was also found to be more potent as compared to 5-FU alone and the combination of 5-FU with DS.. Therefore, the combination of NR+DS and 5FU+NR has great potential to be developed as separate drugs against colon cancer.