

## **Manilkara zapota (L.) P. Royen leaf water extract triggered apoptosis and activated caspase-dependent pathway in HT-29 human colorectal cancer cell line**

### **ABSTRACT**

Manilkara zapota (L.) P. Royen (Family: Sapotaceae), commonly called as sapodilla, has been applied as traditional folk medicine for diarrhea and pulmonary infections. Conventional therapy in colorectal cancer is not likely effective due to undesirable outcomes. The anti-colon cancer properties of Manilkara zapota leaf water extract have yet to be investigated thus far. Therefore, our present study aimed to evaluate the ability to induce apoptosis and the underlying mechanisms of Manilkara zapota leaf water extract against human colorectal cancer (HT-29) cells. The cytotoxicity of Manilkara zapota leaf water extract was screened in different cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) analyses. The morphological changes in HT-29 cell lines after exposure to Manilkara zapota leaf water extract were viewed under fluorescence and inverted light microscope. The apoptotic cell was measured by Annexin V-propidium iodide staining. The caspase-3 and -8 activities were assessed by colorimetric assay. Overall analyses revealed that treatment with Manilkara zapota leaf water extract for 72 h can inhibit the viability of HT-29 cells. Incubation with Manilkara zapota leaf water extract for 24, 48, and 72 h significantly increased ( $p < 0.05$ ) the total apoptotic cells compared to the control. Treatment with 21, 42, and 84  $\mu\text{g/mL}$  of Manilkara zapota leaf water extract for 72 h triggered both caspase-3 and -8 activities in a concentration-dependent pattern. We also found that the catalase level in the two treatment groups (21 and 42  $\mu\text{g/mL}$ ) was significantly elevated after 24 h incubation. Incubation with Manilkara zapota leaf water extract for 72 h triggered the transcriptional elevation of the adenomatous polyposis coli (APC), glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), AXIN1, and casein kinase 1 (CK1). The  $\beta$ -catenin mRNA levels were reduced accordingly when the concentration of the Manilkara zapota leaf water extract was increased. Our results suggested that Manilkara zapota leaf water extract offer great potential against colorectal cancer through modulation of Wnt/ $\beta$ -catenin signaling pathway, caspase-dependent pathway, and antioxidant enzyme.

**Keyword:** Apoptosis; Beta-catenin; Caspase-dependent pathway; Cytotoxicity; Manilkara zapota