

## **Abstract**

Four multifloral honey types from different Indian origin were analyzed for rheology and thermal properties. Controlled shear rate viscometry indicated the Newtonian behavior in the honey samples. Differential Scanning Calorimetry (DSC) analysis revealed the inflection transition temperature ( $T_g$ ) of the honey samples ranged between  $-51.40\text{ }^{\circ}\text{C}$  to  $-30.64\text{ }^{\circ}\text{C}$ . Further honey samples were analyzed for their phenolic constituents using high performance liquid chromatography (HPLC). HPLC and Mass Spectroscopy analysis revealed that dihydroxy benzoic acid, caffeic acid, ferulic acid and cinnamic acid were the major phenolic constituents of the tested honey. The total anti-oxidant potential and free radical scavenging ability varied among the honey samples and exhibited a significant correlation with their phenolic content. Thiazolyl blue tetrazolium bromide (MTT) assay revealed that the honey samples containing higher phenolic content showed significant antiproliferative effect against colon cancer cells. From the above-said experiments, Sample C among the four was found to be better based on its physical properties, phenolic, antioxidant and antiproliferative effects. Hence, Sample C was chosen to decipher the molecular mechanism behind the honey-induced apoptosis. Honey induced the apoptotic signal via initial depletion of intracellular non protein thiols, consequently reducing the mitochondrial membrane potential (MMP) and increasing the reactive oxygen species (ROS) generation. p53 up-regulation and modulation of pro and anti-apoptotic proteins were observed in the honey induced apoptosis. We had screened the antiproliferative effect of some reported phenolic constituents of honey namely p-coumaric acid, gallic acid and eugenol in colon cancer cells. We had selected eugenol based on their better antiproliferative nature. Reduction of intracellular non-protein thiols, increase in ROS generation and modulation of apoptotic proteins were observed in eugenol-induced apoptosis. Finally we had studied the anticancer activity of honey samples (Samples B and C) and eugenol against Ehrlich ascites carcinoma. We found that Sample C remarkably inhibited the tumor growth by 40% compared to Sample B (12.17%) when given intraperitoneally (25% v/v; i/p). Eugenol with a dose of 100 mg/kg showed nearly 29% tumor inhibition.

**Keywords:** Honey, Phenolic content, Antioxidant, Apoptosis, ROS, Eugenol, Ehrlich