1. **Plant extract P2Et from Caesalpinia spinosainhibits in vitro generation of Cancer Associated Fibroblasts**

**Global Use of Natural Products in Cancer Patient Management**

**Background**: Several paracrine signals (cytokines, growth factors, oxidative stress) are generated in the tumor microenvironment. These signals increase the generation and activation of stromal fibroblasts to Cancer-Associated Fibroblasts (CAF) that proved strong support in carcinogenesis and tumor progression. Alternative therapies based on plant extracts or natural compounds targeting TME are a new topic in cancer research; in that sense, the antioxidant activity of natural products might affect tumor oxidative stress and signaling involved in CAF generation during carcinogenesis.

**Purpose**: Evaluate the role of P2Et extract obtained of *Caesalpinia spinosa* plant on TME interactions using an *in vitro* CAF-like and tumor cells model of breast cancer.

Study Design and Methods: CAF-like model induced by exogenous TGF-β addition was developed from 3T3 murine fibroblasts cell line. Conditioned media of CAF-like were used to evaluate stromal paracrine interaction on 4T1 murine breast cancer cell line. Non-lethal concentrations of P2Et were used to determine its effects on CAF generation and activation, and indirect interaction with the tumor compartment.

**Results**: Phenotypic and metabolic transformation of 3T3 fibroblasts after TGF-β addition was compatible with features reported for CAF. We observed a decrease in Caveolin-1 expression associated with mitochondrial mass loss and glucose consumption increase. Together with ROS increase, these events represent mitophagy induction and increased glycolytic metabolism. In the other hand, conditioned medium of TGF-β treated fibroblast promoted 4T1 colonies formation in the clonogenic assay and tumor cells migration in wound healing assay, functionally confirming the CAF phenotype. P2Et extracted showed activity on TME. It counter resting CAF conversion from TGF-β treated 3T3 fibroblasts, restoring caveolin-1 expression, attenuating metabolic (catabolic) transformation, and oxidative stress induction and functionally decreasing cancer cells growth and migration induced by CAF-conditioned medium.

**Conclusion**: Results suggest that antioxidant activity of phenolic compounds could inhibit CAF generation driven by tumor-stromal metabolic coupling and oxidative stress, showing future directions for the development of natural therapies directed to TME.

**Keywords:** Tumor microenvironment, Cancer Associated fibroblast, Plant extracts, natural products

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