Immunomodulatory potential of Clinacanthus nutans extracts in the co-culture of triple-negative breast cancer cells, MDA-MB-231, and THP-1 macrophages

ABSTRACT

Triple-negative breast cancer is the main type of breast carcinoma that causes mortality among women because of the limited treatment options and high recurrence. Chronic inflammation has been linked with the tumor microenvironment (TME) in breast cancer progression. Clinacanthus nutans (CN) has gained much attention because of its anticancer properties, but its mechanism remains unclear. We aimed to study the qualitative phytochemical content and elucidate the cytotoxicity effects of CN on human triple-negative breast cancer (TNBC), MDA-MB-231 and human macrophage-like cells such as THP-1 by using sulforhodamine B (SRB) assay. As highly metastatic cells, MDA-MB-231 cells can migrate to the distal position, the effect of CN on migration were also elucidated using the scratch assay. The CN effects on ameliorating chronic inflammation in TME were studied following the co-culture of MDA-MB-231/THP-1 macrophages. The cytokine expression levels of IL-6, IL-1β and tumor necrosis factor-alpha (TNF-α) were determined using ELISA assays. The results showed that both ethanolic and aqueous CN extracts contained alkaloid, phenol and tannin, flavonoid, terpenoid, glycoside and steroid. However, saponin was only found in the aqueous extract of CN. CN was not cytotoxic to both MDA-MB-231 and THP-1 cells. The ability of MDA-MB-231 to migrate was also not halted by CN treatment. However, CN ethanol extract decreased IL-6 at 25 μg/mL ($p = 0.02$) and 100 μg/mL ($p = 0.03$) but CN aqueous extract increased IL-6 expression at 50 μg/mL ($p = 0.08$) and 100 μg/mL ($p = 0.02$). IL-1β showed decreased expression after treated with CN ethanol and CN aqueous both at 25 μg/mL ($p = 0.03$). TNF-α were significantly decreased after CN ethanol treatment at concentration 25- ($p = 0.001$), 50- ($p = 0.000$) and 100 μg/mL ($p = 0.000$). CN aqueous extract slightly inhibited TNF-α at all 25-50- and 100 μg/mL ($p = 0.001$, $p = 0.000$, $p = 0.000$, respectively). Overall, CN acts by ameliorating the pro-inflammatory condition in the TME and may be a potential strategy for its anticancer mechanism on highly metastatic breast cancer condition. The major pathways that link both cancer and inflammation were NF-κB and STATs thus further study on the upstream and downstream pathways is needed to fully understand the mechanism of CN extracts in cooling the inflamed TME in breast cancer.