# SYNTHESIS OF BIOCOMPATIBLE FOLIC ACID-CARBON DOT NANOCONJUGATES TARGETING BAX/BCL-2 PATHWAY IN BREAST CANCER CELLS.

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**Keywords:**
Carbon dots, Biosynthesis, Folic Acid, Anti-cancer, Therapeutics


**Introduction:**

Carbon dots (CDs), first reported in 2004, are <10 nm carbon-based nanoparticles with $sp^2$ domains, known for tunable optical/electronic properties, strong luminescence, low toxicity, and high stability, making them suitable for biomedical applications [1]. *Annona reticulata* (Ramphal), widely used in ayurveda, possesses antimicrobial, antioxidant, antidiabetic, anticancer and anti-inflammatory properties and serves as an economical green precursor for CD synthesis. In this study, Folic acid–conjugated CDs (FA-CDs) were synthesized via a hydrothermal method to enable receptor-mediated targeting of breast cancer cells and investigate apoptosis and angiogenesis pathways. Cancer involves uncontrolled cell proliferation and spread, where apoptosis plays a key role; the BAX/BCL2 ratio regulates this process [2]. The pro-apoptotic protein BAX induces cytochrome c release, forming the apoptosome with Apaf-1 and activating caspase-3 via caspase-9 [3]. This work evaluates folate receptor expression and compares FA-CD uptake with CDs using BAX/BCL2 RT-PCR, aiming to demonstrate selective targeting and improved therapeutic efficacy with reduced toxicity.

**Objectives:**

1. Develop an eco-friendly and cost-effective method for synthesizing carbon dots using bioactive compounds from Ramphal fruit.
2. Functionalize CDs with folic acid via a one-pot method to enhance targeted delivery to cancer cells.
3. Characterize synthesized CDs using UV-Vis, FTIR, TEM, SEM and PL analysis.

4. Evaluate anticancer activity in MCF-7 breast cancer cells by determining $IC_{50}$, cell migration and clonogenic potential.
5. Analyze apoptosis through AO/EtBr staining and gene expression of BAX and BCL-2 using RT-PCR.
6. Assess anti-inflammatory activity using a carrageenan-induced rat paw edema model.

**Methodology:**

A calculated amount of Ramphal fruit pulp was extracted and blended with 200 ml distilled water. To 55 ml of fruit extract, 5 ml of folic acid was mixed for one-pot synthesis of carbon dots. The mixture was subjected to hydrothermal treatment at 180°C for 5 hrs, followed by filtration, centrifugation and lyophilization. The resulting folic acid–carbon dot nanoconjugates were prepared efficiently through this green synthesis approach. Characterization was carried out using UV-Vis, FTIR, TEM, SEM and PL analysis to evaluate their properties. The cytotoxicity of the synthesized conjugated CDs was evaluated in MCF-7 cells using an MTT cell viability assay. Cells were seeded in 96-well plates and treated with varying concentrations of synthesized conjugated CDs and doxorubicin. After incubation, MTT solution was added to assess cell viability. The optical density was measured at 492 nm using Magellan software. Additionally, cell migration assay was performed to evaluate the ability of cells to migrate by measuring a closure of a scratch. In Vitro Anticancer Activity will also be evaluated through clonogenic assay. The mechanism of apoptosis and apoptotic stages will be evaluated by AO/EtBr staining and the mechanism of BAX/BCL-2 expression will be analyzed by the means of RT-PCR. Anti-inflammatory activity will be evaluated by using a carrageenan-induced rat paw edema model.

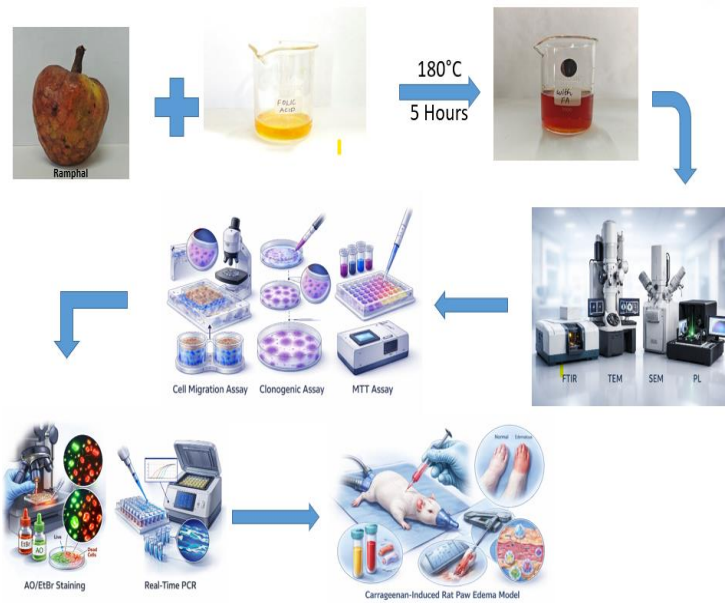


Figure 1: workflow of the methodology

**Result and Discussion:**

The UV–Vis spectrum shows peaks at ~224 nm and ~282 nm with a visible tail, confirming carbon dot formation and folic acid functionalization. The FTIR spectrum shows typical functional groups in bare carbon dots, while enhanced peaks, shifts, and stronger bands (especially at 3200–3500 $cm^{-1}$ and ~1650 $cm^{-1}$) in folic acid-functionalized CDs, confirming successful surface modification and conjugation. TEM provided inference on internal structure at the nanometre scale ranging from 5-200 nm. MTT assay with varying FA-CDs concentrations showed reduced cell viability when compared to control, enabling $IC_{50}$ determination. The scratch assay results (0–48 hrs) showed substantial wound closure in control, moderate closure in cells treated with FA-CDs and minimal closure in doxorubicin-treated cells, indicating reduced migration. AO/EtBr staining revealed viable cells in control, early apoptosis in FA-CD treated cells and late apoptotic/necrotic cells in doxorubicin-treated samples, confirming apoptosis.

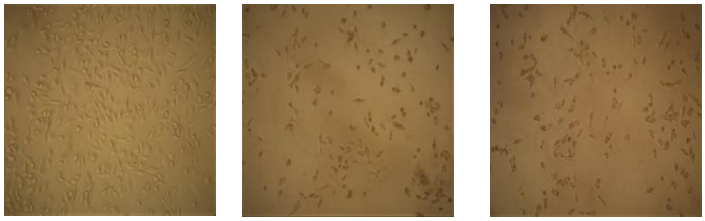

Figure 2: Control, Treatment with FA-CDs and Doxorubicin: MTT Assay

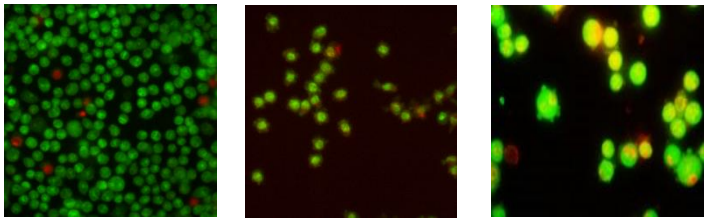

Figure 3: Control, Treatment with FA-CDs and Doxorubicin: AO/EtBr Staining

a) 0 Hrs

b) 48 hrs

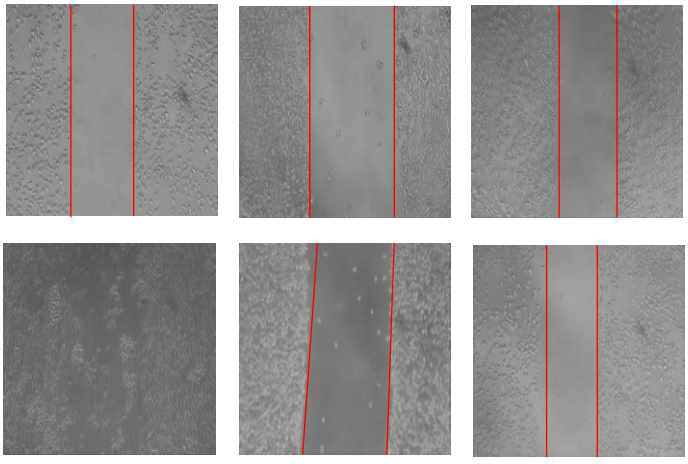

Figure 4: Control, Treatment with FA-CDs and Doxorubicin: Cell Migration Assay

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**Future Scope:**

Synthesis from Ramphal supports green nanotechnology, making them cost-effective, biocompatible, and sustainable for biomedical use. This research lays a solid groundwork for future investigations in the following promising areas.

1. Targeted Cancer Therapy- FA-CDs can be used for selective drug delivery to folate receptor–overexpressing cancer cells (e.g., breast, ovarian), improving therapeutic efficiency and minimizing toxicity to normal cells.
2. Bioimaging Applications- Due to their strong fluorescence, FA-CDs can serve as bioimaging probes for early cancer detection and real-time cellular tracking.
3. Personalized Medicine- FA-CDs can be tailored for patient-specific treatments, especially in cancers with high folate receptor expression.
4. Clinical Translation Potential- With further research, FA-CDs could be advanced into clinical trials for targeted cancer diagnostics and therapy.