Advancing Radiotherapy Outcomes: Ginkgo biloba Extract as a Potential Radiosensitizing Adjuvant in Breast Cancer

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Abstract: Despite the established efficacy of ionizing radiation in oncology, the clinical utility of chemical radiosensitizers is often limited by acute and chronic toxicities. Consequently, there is an urgent need to identify plant-derived phytochemicals that can selectively enhance tumor radiosensitivity while maintaining a favourable safety profile. This study investigates the radiosensitizing potential of *Ginkgo biloba* extract (GB) in breast cancer management. The in vitro radiosensitizing effects of GB were evaluated in MDA-MB-231 breast cancer cells. Cells were treated with varying concentrations of GB (10–350 µg/mL) prior to exposure to ionizing radiation. Cell viability was assessed using the MTT assay at 24, 48, and 72 hours post-irradiation. Preliminary findings indicate a dose-dependent response. While lower concentrations exhibited negligible effects, higher concentrations demonstrated a trend towards enhanced radiation-induced cytotoxicity. Cells pre-treated with higher concentrations of GB exhibited enhanced susceptibility to radiation-induced damage, resulting in further reductions in survival compared to either modality administered independently. In particular, high-dose GB pre-treatment markedly reduced cell viability across all evaluated time points, suggesting a pronounced radiosensitizing effect. These results indicate that GB extract may enhance the therapeutic efficacy of radiotherapy in breast cancer cells. Further research will assess selected high concentrations in combination with clinically relevant radiation doses and investigate associated molecular biomarkers of DNA damage and cellular response.

Methods

Cell Treatment and Irradiation

MDA MB 231 Cell lines were treated with different concentrations of GB (0, 10, 50, 100, 200, 300 and 350 µg/ml) either alone or in combination with clinically relevant dose (3 Gy) for radiotherapy by using 6 MV X-ray photons with dose rate 3 Gy/min and incubate for 24, 48 and 72 hours.

Cell Viability Assay (MTT)

The cytotoxic and radiosensitizing effects of GB were quantified using the MTT colorimetric assay. The absorbance was measured at 570 nm using a microplate reader for different time interval post irradiation with different GB concentrations. Cell viability was calculated as a percentage relative to the untreated control group.

Measurement of Intracellular Reactive Oxygen Species (ROS)

To evaluate the induction of oxidative stress, intracellular ROS levels were detected using the CellROX with selected effective GB concentrations from MTT results. The fluorescence intensity, reflecting the degree of ROS generation, was analyzed via flow cytometry (FACS) and expressed as mean fluorescence intensity (MFI).

Apoptosis Analysis by Flow Cytometry (FACS)

The percentage of apoptotic cell death was quantified using Annexin V-FITC and Propidium Iodide (PI) double staining. The distribution of viable (Annexin V−/PI−), early apoptotic (Annexin V+/PI−), late apoptotic (Annexin V+/PI+), and necrotic (Annexin V−/PI+) cells was analyzed using a flow cytometer (FACS).

Statistical Analysis

All experiments were performed in triplicate to ensure reproducibility. Data are presented as mean ± standard deviation (SD). Analysis of normal distribution of measured end-points and of variance (ANOVA) followed by LSD post-hoc test for multiple comparisons was carried out using Statistica software (Dell software, Round Rock, Texas, United States). Comparison between treatment conditions was performed using two tailed *t*-test. The results were considered significantly different at $p < 0.05$

LINAC Accelerator with self designed phantom for cells irradiation

Results

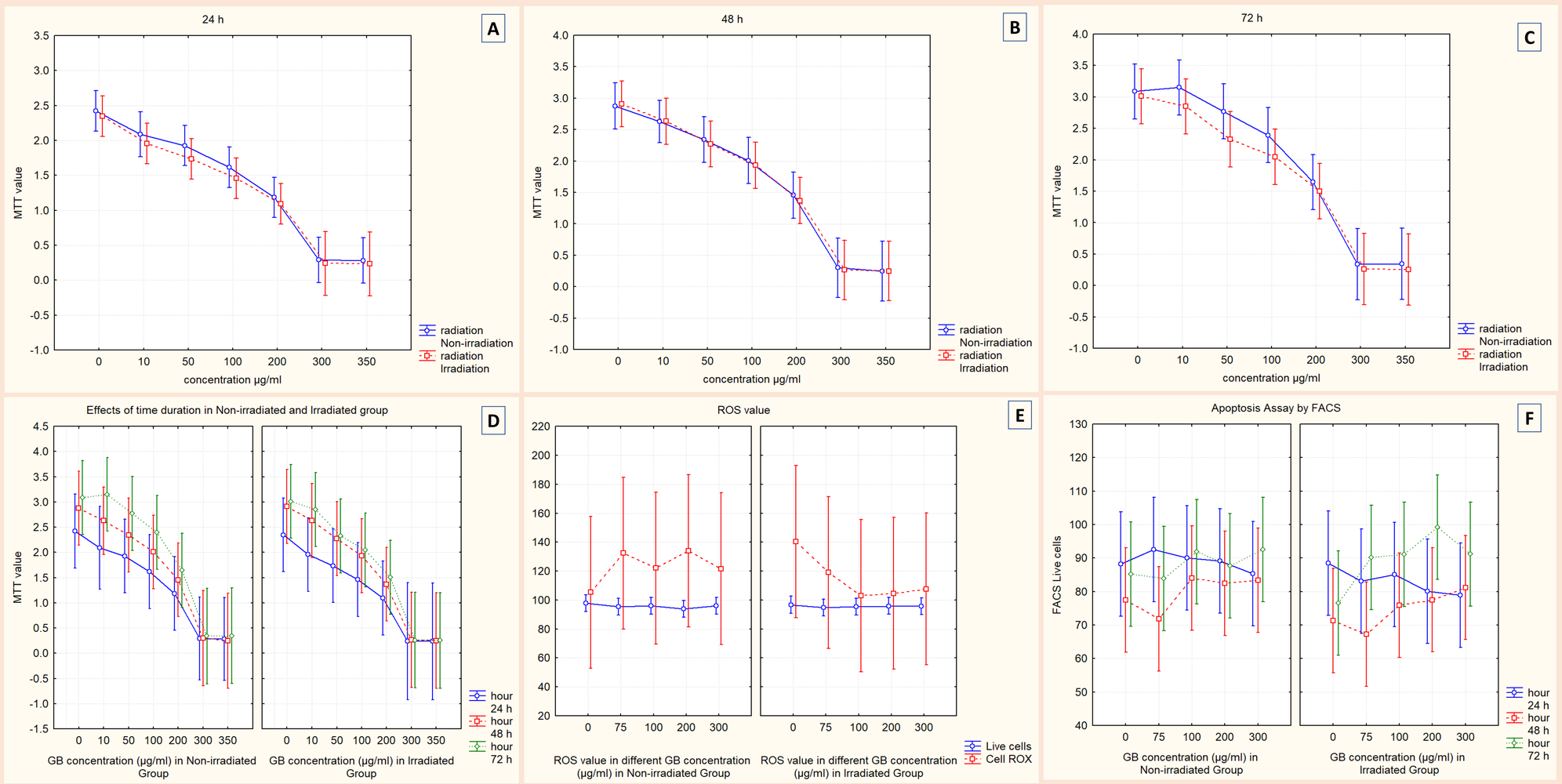

Figure: Cell viability with different concentrations of GB post irradiation A); 24 hrs. post irradiation B) 48 hrs. post irradiation; C) 72 hrs. post irradiation; D) combined effects of GB concentration with time duration post irradiation; E) ROS value with selected higher GB concentration post irradiation; F) Live cells through FACS after different time duration post irradiation for selected higher GB concentrations.

Conclusion: In this study, we evaluated the concentration-dependent effects of Ginkgo biloba extract (EGB-761) in combination with 3 Gy ionizing radiation in MDA-MB-231 breast cancer cells. Although higher concentrations (200–300 µg/mL) showed a trend toward enhanced radiosensitivity and increased apoptosis, these effects did not reach statistical significance under the tested conditions. The observed modulation of ROS levels and changes in live-cell populations suggest a potential interaction between GB treatment and radiation-induced cellular stress pathways. Further studies with optimized dosing and larger sample sizes are required to clarify the radiosensitizing potential of GB extract.

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