INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD)
© 2026 IJNRD | Volume 11, Issue 7, July 2026 | ISSN: 2456-4184 | IJNRD.ORG

Radiosensitizing Effect of Inositol Hexaphosphate on Human Colon Cancer Cells: A Preliminary *In Vitro* Study

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Abstract

Background: Radiotherapy plays a key role in the management of colorectal cancer; however, the development of radioresistance limits its therapeutic efficacy. Identification of safe and effective radiosensitizing agents may improve treatment outcomes. Inositol hexaphosphate (IP6), a naturally occurring phytochemical, has demonstrated antiproliferative and pro-apoptotic properties in several cancer models. This study is intended to examine the preliminary radiosensitizing capacity of IP6 in SW480 human colon cancer cells.

Methods: SW480 cells were treated with increasing doses of IP6 to assess its effects on cell replication using BrdU labelling. Based on the proliferation data, an effective concentration of IP6 was selected for combination treatment with γ-radiation. We used the MTT assay to measure post-treatment cell survival, while apoptosis was quantified by Annexin V staining.

Results: IP6 progressively suppressed SW480 cell multiplication at higher concentrations, with 1.0 mM producing marked growth inhibition and therefore selected for subsequent experiments. Combining IP6 with γ-radiation killed significantly more cells than radiation alone, proving its ability to sensitise the cells. Furthermore, Annexin V analysis showed a higher proportion of apoptotic cells in the IP6 plus radiation group than in the radiation-only group, suggesting that IP6 potentiates radiation-induced apoptosis.

Conclusion: Radiotherapy outcomes in SW480 colon cancer cells improve when combined with IP6, which simultaneously blocks cell growth, limits viability, and accelerates apoptosis. Consequently, IP6 holds promise as a novel radiosensitizing tool for colorectal malignancies. Fully validating these initial observations will require deep mechanistic profiles alongside in vivo experimentation.

Keywords: Inositol hexaphosphate, IP6, colorectal cancer, SW480, radiosensitisation, γ-radiation, cell proliferation, apoptosis.

1. Introduction

Worldwide, colorectal cancer (CRC) stands out as a primary driver of cancer-related illness and mortality. Data compiled in the GLOBOCAN 2024 database places CRC among the top three most prevalent malignancies on Earth. Even with ongoing improvements in early screening protocols, imaging diagnostics, and clinical therapeutics, this disease continues to claim an exceptionally high number of lives every year. Although improvements in early detection and multimodal therapeutic strategies have enhanced patient survival, treatment resistance and disease recurrence continue to pose significant clinical challenges, particularly in advanced-stage disease (1,2).

Managing colorectal cancer today requires a combined approach that integrates surgery, chemotherapy, targeted drugs, immunotherapy, and radiation. While surgical resection remains the primary treatment for localised disease, radiotherapy is frequently employed in locally advanced colorectal tumors to improve local disease control and reduce recurrence. However, intrinsic and acquired resistance to ionising radiation considerably limits therapeutic efficacy, necessitating higher radiation doses that may increase toxicity to surrounding normal tissues (3,4).

Tumors resist radiation by accelerating DNA repair, activating survival pathways, altering cell cycle checkpoints, and blocking apoptosis. Among these, alterations in growth factor signalling pathways and suppression of programmed cell death contribute significantly to tumor cell survival following irradiation (5,6). Consequently, considerable efforts have been directed toward identifying radiosensitizing agents capable of enhancing tumor cell susceptibility to radiation while minimising damage to normal tissues.

Naturally occurring dietary phytochemicals have attracted increasing attention as potential adjuvants in cancer therapy because of their favourable safety profile and ability to modulate multiple signalling pathways simultaneously. Phytic acid, or inositol hexaphosphate (IP6), is a natural polyphosphorylated carbohydrate found in high amounts throughout grains, beans, nuts, and seeds. Broad experimental data reveal that IP6 slows growth, cuts oxidative stress, calms inflammation, and drives programmed cell death across several tumor models, notably colorectal cancer (7,8). In colon cancer models, IP6 has been shown to inhibit cellular proliferation, induce apoptosis, suppress Akt/mTOR signalling, and reduce tumor development in experimental animal models (9–11).

Although the anticancer properties of IP6 have been extensively investigated, relatively few studies have examined its ability to enhance the therapeutic response to ionising radiation in colorectal cancer. Since radiation-induced apoptosis represents a major mechanism of tumor cell elimination, agents capable of augmenting apoptotic signalling may improve radiosensitivity and therapeutic outcomes. Therefore, identifying naturally derived compounds that act as radiosensitizers offers an excellent strategy to boost radiation impact while sparing healthy tissues to improve the efficacy of radiotherapy while reducing treatment-associated toxicity (12).

We designed this study to test whether IP6 can make human SW480 colon cancer cells more vulnerable to radiation. The effects of IP6 pretreatment on cellular proliferation, radiation-induced cytotoxicity, and apoptosis were investigated using BrdU incorporation, MTT viability, and Annexin V apoptosis assays.

2. Materials and Methods

2.1 Cell Line and Culture Conditions

Human SW480 colorectal adenocarcinoma cells were propagated in Leibovitz's L-15 medium (Gibco, USA) containing 10% fetal bovine serum (Invitrogen, USA). We enriched this mixture with 1.6 mM L-glutamine and a standard penicillin-streptomycin cocktail (both from Gibco, USA). Incubation settings were stabilised strictly at 37°C under a 100% atmospheric air environment. For all subsequent experimental protocols, we exclusively harvested cells during their logarithmic growth phase.

2.2 IP6 Formulation

Stock solutions of inositol hexaphosphate (IP6) were generated using sterile saline as the solvent. From this stock, working doses (0.1, 0.25, 0.5, 1.0, and 2.0 mM) were mixed fresh before each assay by diluting the compound directly into serum-free Leibovitz's L-15 medium.

2.3 Experimental Design

SW480 cells (5×10^4 cells/well) were left to adhere overnight post-seeding. We then applied varying concentrations of IP6 for a 24-hour window to pinpoint the optimal dosage for growth suppression. Based on the proliferation studies, 1.0 mM IP6 was selected for subsequent radiation experiments.

For radiosensitisation studies, cells were pretreated with 1.0 mM IP6 for 24 hours and subsequently exposed to 2 Gy γ-radiation. Following irradiation, we kept the plates in a standard incubator and analysed using cell viability and apoptosis assays.

2.4 MTT Cell Viability Assay

We used an MTT assay to evaluate cell survival after radiation exposure. In short, we treated the cultures with IP6 and irradiated them according to the steps detailed above. After the incubation window, we swapped the old medium for serum-free media containing the MTT reagent, letting it react for 3 hours at 37°C. We then dissolved the blue formazan crystals with an appropriate MTT solvent and read the absorbance values at 590 nm with a microplate reader. Survival rates were quantified as a percentage of the untreated control baseline.

2.5 BrdU Cell Proliferation Assay

We monitored cell replication using a commercial BrdU assay kit, following the provided protocol. In short, we exposed the SW480 lines to varying doses of IP6 for a 24-hour period. Bromodeoxyuridine (BrdU) labelling solution was added during the final incubation period to allow incorporation into newly synthesised DNA. Following fixation, incorporated BrdU was detected using an anti-BrdU antibody, and we used a microplate reader to read the absorbance, calculating cell replication as a percentage of the untreated control group.

2.6 Annexin V Apoptosis Assay

We tracked apoptotic cell death by running an Annexin V staining protocol. Following our combined IP6 and radiation workflows, we collected the cells, rinsed them twice with phosphate-buffered saline (PBS), and mixed them into Annexin V binding buffer. Next, we applied the Annexin V and propidium iodide (PI) stains per the supplier's manual, leaving the tubes to react in the dark for 15 minutes at room temperature. We then used fluorescence microscopy to count the apoptotic fractions, pulling data across several independent experimental runs to complete our quantitative analysis.

2.7 Radiation Exposure

Cells were irradiated using a γ-radiation source following IP6 pretreatment. Based on optimisation studies, a radiation dose of 2 Gy was selected for subsequent experiments. After irradiation, we placed the plates back into the incubator under standard culture conditions until the respective experimental endpoints.

2.8 Statistical Analysis

All assays were performed in triplicate unless noted otherwise, with data expressed as mean ± standard error (SE). Inter-group comparisons were evaluated via one-way analysis of variance (ANOVA) alongside an appropriate post hoc test, defining statistical significance at $p < 0.05$.

3. Results

3.1 Pretreatment with IP6 Enhances Radiation-Induced Loss of Cell Viability

To evaluate whether IP6 functions as a radiosensitizer, SW480 cells were primed with 1.0 mM of the compound before exposure to escalating levels of γ-radiation, followed by metabolic tracking via an MTT assay (Figure 2A). While standalone radiation restricted cellular viability in a standard dose-response pattern, priming the cultures with IP6 drove an even sharper drop in survival. This heightened vulnerability was observed across every single radiation threshold tested when compared to the corresponding radiation-only cohorts. These findings indicate that IP6 potentiates the cytotoxic effect of ionising radiation, thereby enhancing the radiosensitivity of SW480 colon cancer cells.

3.2 Dose-Response Suppression of SW480 Cell Proliferation by IP6

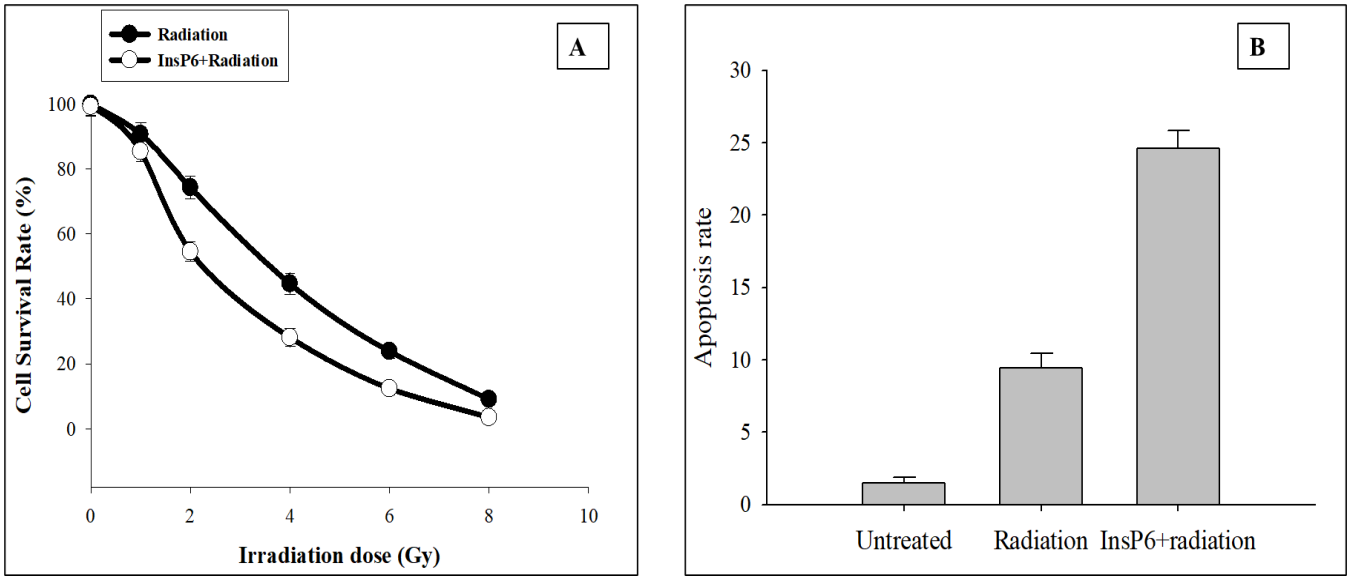
Optimal IP6 dosing for later irradiation assays was identified by screening SW480 cultures against incremental compound concentrations (0.1–2.0 mM) via a BrdU assay (Figure 1). A stepwise suppression of cell proliferation occurred in tandem with rising doses, a pattern clearly reflected by the diminishing BrdU signal at elevated compound exposures. A significant reduction in proliferative activity was observed at higher concentrations of IP6, with 1.0 mM producing a pronounced inhibitory effect. Based on these findings, 1.0 mM IP6 was selected for all subsequent radiation experiments

3.3 IP6 Potentiates Radiation-Induced Apoptosis in SW480 Cells

Annexin V staining assessed whether the observed viability decline correlated with active apoptosis across the different treatment conditions (Figure 2B). Apoptotic percentages were recorded in the control, radiation-alone, and combination arms. Exposure to radiation by itself shifted cells toward apoptosis compared to unexposed controls. Notably, introducing IP6 before irradiation drove a distinct secondary increase in the Annexin V-positive population, proving that the combination actively accelerates apoptosis.

Figure 1. Radiosensitizing effect of IP6 in SW480 cells.

(A) MTT assay showing decreased cell viability following γ-radiation and enhanced cytotoxicity after pretreatment with 1.0 mM IP6. (B) Quantitative analysis of Annexin V-positive cells demonstrating increased apoptosis in the IP6 plus radiation group compared with untreated and radiation-alone groups



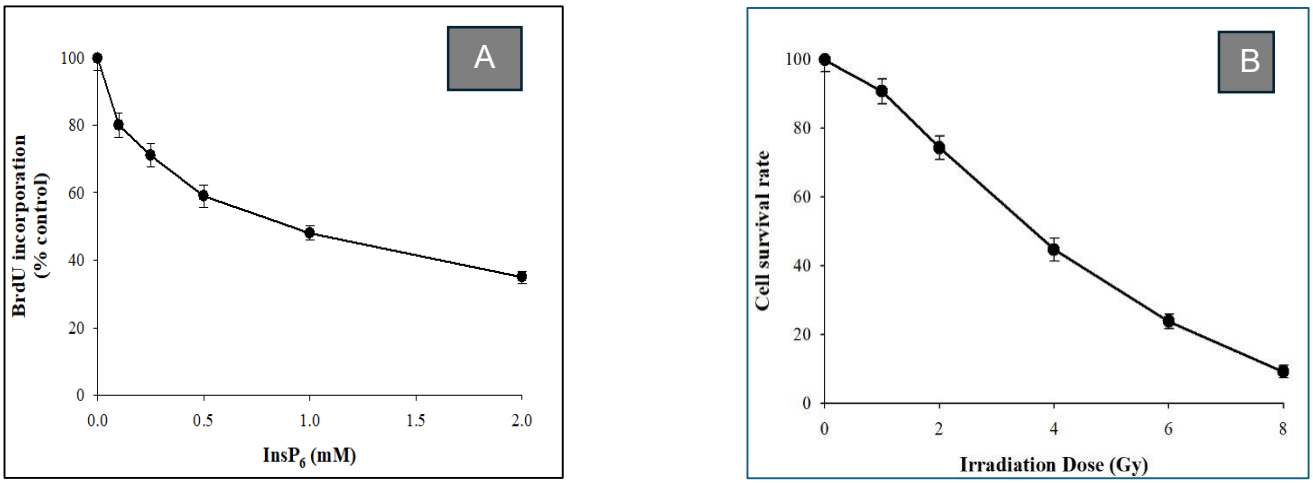


Figure 2. (A)Dose-response impact of IP6 on SW480 cell multiplication using BrdU tracking. Cells underwent a 24 h incubation with escalating IP6 doses ranging from 0.1 to 2.0 mM. The compound induced a stepwise reduction in active DNA synthesis, guiding the selection of a 1.0 mM concentration for upcoming combination radiotherapy testing.

(B) Effect of ionising radiation on cell survival rate. Cell survival and proliferation were assessed using a BrdU incorporation assay following exposure to increasing doses of radiation (0, 2, 4, 6, and 8 Gy). The cell survival rate decreases in a dose-dependent manner. Data points represent the mean ± standard error of the mean (SEM) [or standard deviation (SD)] from three independent experiments (n = 3).

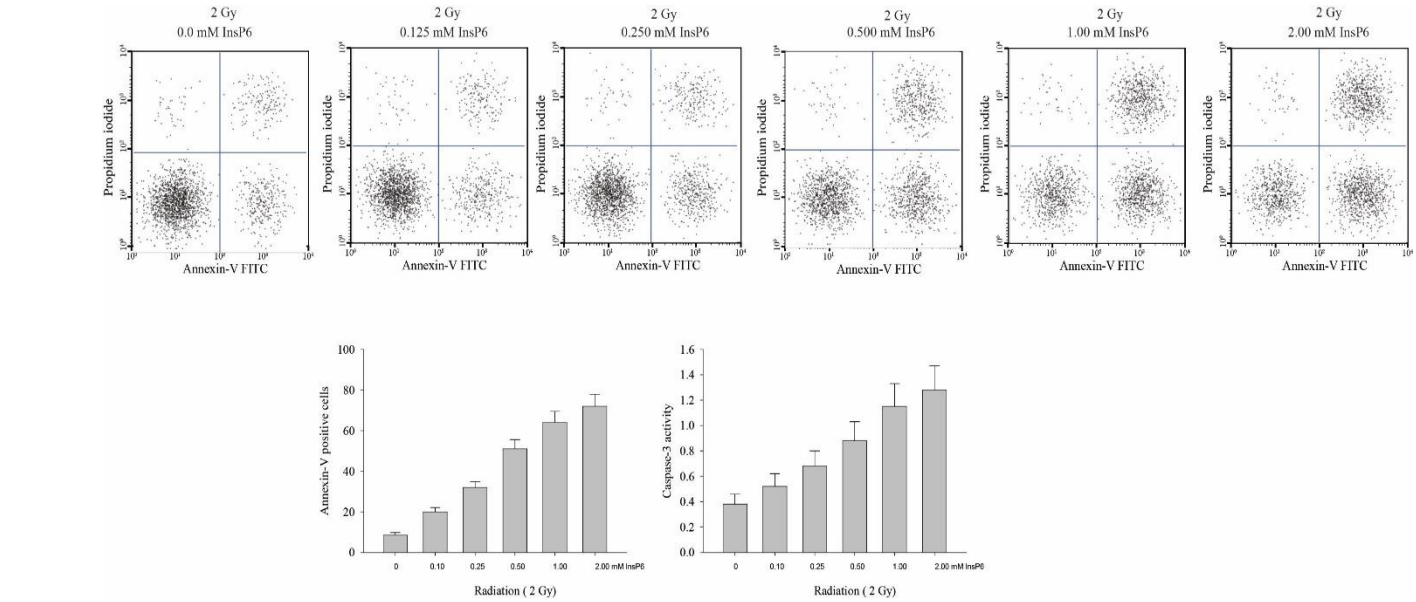


Figure 3. Stimulation of Apoptosis by combined IP6 and γ-radiation treatment in SW480 cell lines.

(A) Representative flow cytometry dot plots capturing Annexin V-FITC and propidium iodide (PI) shifts after exposing cells to a fixed 2 Gy dose of radiation paired with incremental concentrations of IP6 (0.0–2.00 mM). (B) Quantitative evaluation of the total Annexin-V positive cell fraction across the indicated exposure groups. (C) Spectrophotometric measurement of relative Caspase-3 enzyme activation following the parallel combined treatments.

4. Discussion

The therapeutic efficacy of ionising radiation against SW480 colon cancer cells is amplified by IP6 through a multi-pronged mechanism involving the arrest of cell division, a drop in survival rates, and the induction of programmed cell death. These findings support previous reports describing the antiproliferative and pro-apoptotic activities of IP6 and further suggest its potential utility as a radiosensitizing agent in colorectal cancer.

Our finding of dose-dependent proliferation arrest is supported by prior research linking IP6 to the widespread disruption of survival signalling cascades in colorectal cancer cells. As documented by Kapral et al., cell division in colon cancer lines is shut down by IP6 via the inactivation of the Akt/mTOR pathway. This inhibitory profile is further driven by the concomitant stimulation of apoptotic networks and cell-cycle regulators (9). Similar antiproliferative effects have also been observed in other colorectal cancer models, supporting the broad anticancer activity of this naturally occurring phytochemical (10,11).

An important finding of the present study was the enhanced reduction in cell viability following combined IP6 and radiation treatment compared with radiation alone. Ionising radiation induces DNA damage that ultimately results in cell-cycle arrest and apoptosis; however, activation of cellular survival pathways frequently limits its therapeutic effectiveness (5,6). The additional reduction in viability observed following IP6 pretreatment suggests that IP6 increases the susceptibility of colon cancer cells to radiation-induced cytotoxicity. Similar observations have been reported for several naturally derived phytochemicals that enhance radiation responses by suppressing tumor cell survival mechanisms while preserving normal tissue integrity (12,13).

Apoptosis represents one of the principal mechanisms responsible for radiation-mediated tumor cell elimination. In the present study, Annexin V analysis demonstrated that combined treatment with IP6 and radiation produced a greater proportion of apoptotic cells than radiation treatment alone. These findings are

in agreement with previous reports showing that IP6 activates apoptotic pathways through modulation of caspase activity and inhibition of prosurvival signalling pathways in colorectal cancer cells (9,10). Enhanced apoptosis following combined treatment suggests that IP6 may improve radiation efficacy by facilitating programmed cell death rather than solely inhibiting cellular proliferation.

The present investigation represents a preliminary evaluation of the radiosensitizing effects of IP6 *in vitro*. Although the findings demonstrate enhanced cytotoxic and pro-apoptotic responses following combination treatment, the molecular mechanisms responsible for these effects were not investigated in the present study. Earlier research indicates that the therapeutic traits of IP6 stem from its ability to alter growth factor signalling, manage oxidative stress, and trigger apoptosis. To fully chart how IP6 primes cells for radiotherapy, upcoming studies must blend deep molecular pathway mapping with validation in animal models.

Overall, the present findings indicate that IP6 enhances radiation-induced apoptosis and growth inhibition in SW480 colon cancer cells. Considering its natural origin, low toxicity profile, and reported anticancer properties, IP6 may represent a promising adjuvant for improving radiotherapeutic efficacy in colorectal cancer. Further preclinical studies are warranted to establish its therapeutic potential and optimise treatment strategies.

Study Limitations

This work serves as an initial *in vitro* assessment of the radiosensitizing effects of IP6 on SW480 cells. This work focused solely on functional assays—specifically cell division tracking, metabolic survival, and apoptosis profiles—to chart the biological impact of combining IP6 with radiation. The specific molecular pathways driving this observed radiosensitisation were not explored in this preliminary study. In addition, the findings are limited to a single colon cancer cell line and require validation in additional cell models. Since these experiments were performed under *in vitro* conditions, the clinical applicability of the findings remains to be established through animal studies and translational investigations.

Future Perspectives

Despite these limitations, the present findings provide preliminary evidence supporting the potential of IP6 as a radiosensitizing agent in colorectal cancer. Future studies should investigate the molecular mechanisms responsible for the enhanced radiation response, including alterations in EGFR/ErbB signalling, DNA damage repair pathways, oxidative stress, and apoptosis-associated proteins. Extending this research to a wider range of colorectal cell lines, 3D culture networks, and live tumor models remains an essential next step to solidify these baseline conclusions. Furthermore, evaluating IP6 in combination with clinically relevant radiotherapy regimens may facilitate the development of novel adjuvant strategies aimed at improving radiotherapeutic efficacy while minimising radiation-associated toxicity.

Conclusion

Ultimately, the vulnerability of SW480 colorectal adenocarcinoma cells to radiotherapy was markedly enhanced by prior IP6 exposure, driven by concurrent proliferation suppression, diminished survival, and accelerated apoptosis. These findings suggest that IP6 has the potential to improve the response of colon cancer cells to ionising radiation. Although the present work is preliminary, it provides a foundation for further mechanistic and preclinical investigations to establish the therapeutic value of IP6 as an adjuvant to radiotherapy in colorectal cancer.

Funding

This work was supported by the Department of Atomic Energy (DAE), Board of Research in Nuclear Sciences (BRNS), Government of India, under Grant No. 37(1)/14/10/2017-BRNS/37207.

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Acknowledgements

The authors gratefully acknowledge the Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre (BARC), Mumbai, for financial support under the Board of Research in Nuclear Sciences (BRNS) project and for providing the necessary facilities to carry out this research. The authors also acknowledge the Department of Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, for providing laboratory infrastructure, institutional support, and technical assistance during the course of this study.

Declarations

Ethics Approval and Consent to Participate

Not applicable. This study was performed using established human colorectal cancer cell lines (SW480) and did not involve human participants, animal experiments, or patient-derived clinical samples.

Consent for Publication

Not applicable.

Availability of Data and Materials

The data generated and analyzed during the current study are included in this published article. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Department of Atomic Energy (DAE), Board of Research in Nuclear Sciences (BRNS), Government of India, under Grant No. 37(1)/14/10/2017-BRNS/37207.