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Mangiferin induces cell death against rhabdomyosarcoma through sustained oxidative stress

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ABSTRACT

Background: Embryonic rhabdomyosarcoma (RD) is the most prevalent type of cancer among children. The present study aimed to investigate cell death induced by mangiferin in RD cells.

Methods: The Inhibitory concentration (IC_{50}) value of mangiferin was determined by an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. Cell death induced by mangiferin against RD cells was determined through lactate dehydrogenase and nitric oxide release, intracellular calcium levels, reactive oxygen species generation, antioxidant status, mitochondrial calcium level, and mitochondrial membrane potential. Furthermore, acridine orange/ethidium bromide staining was performed to determine early/late apoptotic event.

Results: Mangiferin induced cell death in RD cells with an IC_{50} value of 70 μM. The cytotoxic effect was reflected in a dose-dependent increase in lactate dehydrogenase leakage and nitric oxide release during mangiferin treatment. Mangiferin caused dose dependent increase in reactive oxygen species generation, intracellular calcium levels with subsequent decrease in antioxidant status (catalase, superoxide dismutase, glutathione-S-transferase, and glutathione) and loss of mitochondrial membrane potential in RD cells. Further data from fluorescence microscopy suggest that mangiferin caused cell shrinkage and nuclear condensation along with the occurrence of a late event of apoptosis.

Conclusion: Results of the present study shows that mangiferin can act as a promising chemopreventive agent against RD by inducing sustained oxidative stress.

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1. Introduction

Cancer continues to represent the largest cause of mortality in the world.[1] Rhabdomyosarcoma (RD) is the most common type of cancer in children under the age of 15 years, and embryonal RD is its most common subtype that develops in the head and neck region and the genitourinary tract. Treatment of this cancer involves a combination of chemotherapy and radiation along with surgery. Chemotherapeutic agents currently available for treating RD have been found to possess several toxic effects such as hepatotoxicity and cardiotoxicity.[2,3] An extremely promising strategy employed for cancer prevention today involves the use of natural compounds. Several studies showed the protective or curative effect of phytochemicals against various diseases including cancer, and in-depth studies are being carried out to understand their mechanism of action. Among them, polyphenols, which are naturally present in plants, are of great interest as chemopreventive agents.[4]

Mangiferin (2-β-D-glucopyranosyl 1-1,3,6,7-tetrahydroxy xanthone), a xanthone C-glucoside from *Mangifera indica* L. (Anacardiaceae), is consumed worldwide as a fruit, and as a culinary and flavoring agent. Fruits, bark, and leaves of *M. indica* have been reported to possess diverse medicinal properties and are widely used in several medicinal preparations. Mangiferin has been reported to contain antioxidant, antitumor, antiviral, antibacterial, antihyperglycemic, analgesic, anti-inflammatory, antidiarrheal, anti-HIV, immunostimulant, and immunomodulatory properties.[5–11]

The antioxidant activity of mangiferin is attributed to its being a polyphenol.[12] Anticarcinogenic potential of mangiferin in bowel carcinogenesis[9] has been reported earlier. Mangiferin inhibited proliferation and induced apoptosis in K562 leukemia cells[13] and HL-60[14] in a dose- and time-dependent manner. Previous reports show that studies on the anticancer activities of polyphenols against RD are sparsely reported. Since mangiferin is a well-established pharmacophore, the present study was aimed at investigating the possible anticancer activity of mangiferin against RD cells.

2. Methods

2.1. Chemicals

Mangiferin, 2,7-dichlorodihydrofluorescein diacetate (DCF-DA), Fura 2-AM, Rhod 2-AM, and propidium iodide were purchased from Sigma Aldrich Chemicals Private Limited, Bangalore, India. Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (CCCP) and 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) were procured from Calbiochem, La Jolla, CA, USA. Dulbecco's modified eagles medium, fetal bovine serum, trypsin, antibiotics (penicillin, streptomycin, and gentamycin), and other fine chemicals were purchased from the Himedia Laboratories Private Limited, Mumbai, India.

2.2. Cell culture and solubility

RD cells were procured from the National Centre for Cell Science, Pune, India. The cells were maintained in DMEM + 10% fetal bovine serum supplemented with antibiotics (100 units/mL penicillin, 30 μg/mL streptomycin, and 20 μg/mL gentamycin). The cells were grown in a CO_2 incubator (5% CO_2, 37 °C). Cells at 80% confluency were trypsinized and used for assays. Mangiferin was dissolved in dimethyl sulfoxide; the final solvent concentration used in culture did not exceed 0.01%.

2.3. Cell viability

Cell viability was determined by an MTT assay.[15] Cells were seeded at a density of 10^4 cells/well and allowed to attach for 1 hour in a CO_2 incubator. Next, the cells were treated with mangiferin at various concentrations (10 μM, 30 μM, 50 μM, 70 μM, 90 μM, and 110 μM) for 24 hours. After the treatment schedule, MTT was added (5 mg/mL) and the cells were incubated for 5 hours. The formed purple formazon crystals were solubilized using dimethyl sulfoxide, and absorbance was measured at 570 nm in a spectrophotometer (Bio-Tek Instruments, Winooski, VT).

2.4. Treatment schedule

The treatment groups were as follows: Group I was the control group; Group II consisted of cells treated with mangiferin at a concentration of 50 μM, Group III consisted of cells treated with mangiferin at 70 μM, and Group IV consisted of cells treated with mangiferin at 90 μM. Cell count in each group was 5×10^6. After attachment, the cells were treated with different concentrations of mangiferin (as mentioned in different treatment groups) and incubated for 24 hours.

After treatment, the supernatant was used for estimating the release of lactate dehydrogenase (LDH) and nitric oxide (NO). The cells were trypsinized and suspended in Tris–EDTA phenyl methyl sulfonyl fluoride buffer used for estimating the levels of DNA, RNA, protein, lipid peroxidation, and nonenzymic antioxidant [glutathione (GSH)], and activities of enzymic antioxidants such as super oxide dismutase, catalase, glutathione-S-transferase.

2.5. Cytostatic effect

Cytostatic effect of mangiferin on RD cells was determined by estimating the levels of DNA, RNA, and protein. The cell suspension was treated with 5% Trichloro acetic acid (TCA) to precipitate nucleic acids and proteins. The precipitate was washed with 10% TCA (ice cold) and 95% ethanol to remove lipids. To the resulting precipitate 5% TCA was added and the mixture was incubated at 70 °C for 15 minutes. After centrifugation (10,000 g for 10 minutes), the supernatant was used for DNA and RNA estimation.

2.6. Estimation of DNA

To the nucleic acid extract, 1N perchloric acid and diphenylamine reagent were added and the mixture was incubated at 95 °C for 10 minutes. A blank and the standard (calf thymus DNA) samples were also tested concurrently. Absorbance was read at 640 nm, and the values were expressed as $\mu g/5 \times 10^6$ cells.[16]