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Investigation of some tetraoxanes as potential antitumour agents

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Background: The search for new antitumor compounds is the imperative in modern oncology. The aim of this work was to investigate the antiproliferative activity and mode of cell death induced by several newly synthesized tetraoxanes against five human tumor cell lines in vitro.
Material and Methods: Stock solutions of investigated tetraoxanes were dissolved in DMSO at concentrations of 10 mM, and afterwards diluted by nutrient medium to various final concentrations. Target cells used were malignant human breast adenocarcinoma MDA-MB-361 and MDA-MB-453, cervix carcinoma HeLa, melanoma Fem-x and myelogenous leukemia K562 cells. Normal human peripheral blood mononuclear cells-PBMC (non-stimulated and stimulated with PHA) was used as healthy control cells. Antiproliferative activity of investigated compounds was assessed, measuring cell survival in standard, 72 h MTT test. In order to determine the mode of HeLa cell death induced by the investigated compounds, microscopic examination of morphological characteristics of acridine orange and ethidium bromide stained cells as well as DNA fragmentation assay were performed.
Results: Investigated tetraoxanes exerted a dose dependent antiproliferative action towards investigated cell lines with good selectivity in their action to tumor cells in comparison to normal immunocompetent cells. Concentrations inducing 50% decrease in cell survival (IC50) obtained from three independent experiments, and on mononuclear cells, were given on table. Microscopic examination of the mode of direct HeLa cell death induced by investigated tetroxanes, 24 h after continuous agents action in concentrations $2\times$IC50, showed morphological appearance of apoptosis (in the form of condensed and/or fragmented nuclei). This data was confirmed by detection of the ladder pattern on DNA electrophoresis, indicates DNA fragmentation in 180–200 bp characteristic of apoptotic state induced by all investigated compounds.
Conclusions: Results obtained showed that investigated compounds, could be promising agents for the treatment of human tumors, and are candidates for further analyses on experimental animals, in vivo.

IC50 values for the 72 h of action of investigated tetraoxanes determined by MTT test

	IC50 [μM]					
Cell lines	Do122	Do123	Do124	Do126	Do127	Do128
HeLa	5.32	4.87	6.89	6.13	6.67	6.92
Fem-x	4.27	4.49	8.82	5.00	7.02	6.69
MDA-MB-361	6.35	6.48	11.19	7.70	10.14	12.00
MDA-MB-453	4.64	4.77	9.26	6.59	8.45	8.55
K562	3.89	3.56	6.47	5.61	6.15	5.52
PBMC-PHA	11.85	9.22	134.95	18.98	19.33	84.8
PBMC+PHA	7.64	7.97	126.29	16.42	17.1	54.58

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Multiple therapeutic targets based novel herbal formulation withanti cancer and immunostimulatory activities

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Cancer is the one of the major cause of the death in the world and to find out safe and effective therapy for its control is the major challenge of the day. The medical therapies (surgery, radiation, chemotherapy) are aggressive in inhibiting cancer growth, far more so than any of the natural therapies. Radio therapies are drugs against cancer have major problem of adverse effects related to their potential of inhibition of cancer cell growth and cause immune suppressive conditions. Keeping this view, we attempt to make a natural healing formulation (WS), from Withania somnifera a traditional herb used by people, for years as a nutritional supplement. WS, composition of different chemical constituents shows strong anticancer activity in vitro against panel of cell lines as well as in vivo mice tumor models with simultaneous immune stimulating activities.
Materials and Methods: Cell proliferation – MTT reduction asay was used for cell proliferation assay. FACS – DCFHDA and JC-1 were used for ROS generation and Mitochondrial Potential loss, FITC and PE labelled antibodies were used for immune studies. ELISA – Sandwich ELISA was performed for Cytokine and Immunoglobulin isotype estimation. Immunoblotting – Western Blot analysis was done for different pro and antiapoptotic protein expression studies. Tumor models – Ehrlich and Sarcoma 180 Mice Tumor models.
Results: WS shows strong anti prolifeartive activity against panel of different cell lines In vitro, IC_{50} ranging from 10−30 μg/ml. Induction of apoptosis was observed by annexin V/ PI staining in HL-60 cells using flow cytometry. WS induced ROS generation (60% DCF +ve cells) and loss of mitochondrial potential change (72%) in HL-60 cells stained with DCFHDA and JC1 respectively after 6h tratment. Cytochrome c release and Bax translocation in WS treated cells was observed by Immunoblotting. WS induce the expression of Caspase3 and 9 in HL60 cells. WS was aslo found to be strong inhibitor of NF-κB in HL60 cells performed by EMSA and also stop its nuclear translocation. Mice with Ehrlich's solid tumors were given oral dose of WS (100–300 g/kg) for nine days and it was found there was 60% tumor regression and the same time treated mice were found healthy and no mortality occurred during trteatment period. Further there was a significant increase in $CD3^+$, $CD4^+$, $CD8^+$ and NK cells population in WS treated, tumor bearing mice.
To investigate the imuno stimulatory properties of WS in normal BALB/c mice, it was found that WS dose dependently stimulated Th1 (IFN-g, IL-2) immunity and macrophage function (IL12, TNFa and NO). Flow cytometric analysis of T cells ($CD3^+$, $CD4^+$, and $CD8^+$) and B cells ($CD19^+$) indicated enhanced proliferation and differentiation these cells. WS dose dependently modulate the expression of co stimulatory molecules CD80, CD86, CD11a, CD11b in mice splenocytes.
Conclusion: The studies showed that WS bearing immunostimulating and anti cancer activity With no toxicological consequences might find useful therapeutic applications against cancer.

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Differential response of melanoma B16 cell lines in the control of the mitotic arrest induced by proteasome inhibitors

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The ubiquitin-proteasome pathway plays a central role in cell cycle control and cancer. Proteasome inhibitors block cell cycle and promote apoptosis of normal and cancer cells. Bortezomib, a proteasome inhibitor is now in clinical use, for multiple myeloma treatment. Numerous studies have showed that bortezomib inhibits cell growth and induces apoptosis in cancer cells mainly through the Nuclear Factor-kappaB pathway.
The proteasome is implicated in chromosome segregation since the separation of sister chromatids depends on the degradation of securins and cyclin B. The proteasome is also involved in chromosome condensation because it regulates degradation of Aurora A y B kinases. These kinases phosphorylate Histone H3 (Ser10) which is related to chromosome condensation. Very few studies have been carried out to address the relation between the effect of proteasome inhibitors and chromosome segregation.
Using mouse melanoma B16 cells (both the poorly – B16F0 – and the highly metastatic – B16F10 – sublines) we have previously demonstrated that the proteasome inhibitor MG132: arrests cell cycle in G2/M, increases proportion of mitosis and anaphases, induces alteration in chromosome segregation pattern and enhances the level of Histone H3 (Ser10). In this work we aimed to establish if the effect of MG132 in B16 was: Cell specific, specific for high grade metastatic cell lines, species specific and if can be induced by other proteasome inhibitors. To this end, we have analyzed the effect of MG132 in other mouse tissues cell lines: fibrosarcoma (L929), normal fibroblasts (G7); in two cell lines with different metastatic potential: B16F0 and B16F10; in other melanoma cell line: human melanoma (A375) and in different species: human cervix carcinoma (HeLa) and rat adrenal pheochromocytoma (PC12). To determine if other proteasome inhibitors have an effect comparable to MG132, we have analyzed the effect of Bortezomib, Lactacystin and Epoxomycin in B16F10 cells. We have found that Lactacystin and Epoxomycin, but not Bortezomib, showed similar effects that those observed with MG132 in B16. The effect of proteosome inhibitors on chromosome segregation was not observed in other cell lines tested, indicating that B16 cell lines behave in a cell-specific manner in response to proteasome inhibitors, the possible mechanisms responsible of these differential responses are being currently under study.
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