



**Anticancer and radiosensitization efficacy of nanocomposite
Withania somnifera extract on mice bearing tumour cells**

A thesis

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I declare that this thesis has been composed by myself and that the work of which it is a record has been done by myself. It has not been submitted for a degree at this or any other university.

Hebat Allah Ibrahim Mohamed

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Abstract

The objective of this study was to evaluate the anticancer and radio-sensitizer efficacy of *Withania somnifera* extract /Gadolinium III oxide nanocomposite (WSGNC) in mice bearing tumour cells.

Ehrlich Solid Carcinoma was induced in female Swiss albino mice by the intramuscular inoculation of 0.2 ml of Ehrlich Ascites Carcinoma Cells (EAC), containing 2.5×10^6 viable cells, in the right thigh of the lower limb of each mouse. Mice with a palpable solid tumour diameter of 10mm^3 were used. WSGNC (227 mg/kg body weight) was given to Solid Ehrlich Carcinoma bearing mice via i.p. injection 3 times/week for 3 weeks. Irradiation was performed by whole body fractionated exposure to 6 Gy fractionated, applied in 3 doses each of 2 Gy/week during 3 weeks.

Cell viability was assayed *in vitro* and *in vivo* studies, tumour size and weight was also determined. DNA fragmentation, caspase-3 activity, mitochondrial enzymes activities, NADH-ubiquinone oxidoreductase (complex I), NADH-cytochrome c oxidoreductase (Complex II) and succinate-cytochrome c oxidoreductase (Complex III) were determined in cancer tissues. In addition oxidative stress was assayed by measuring the content of malondialdehyde (MDA) as a marker of lipid peroxidation.

Reduced glutathione (GSH) as well as superoxide dismutase (SOD) and catalase activities were determined as markers of the antioxidant status. Treatment of Solid Ehrlich Carcinoma bearing mice with WSGNC combined with γ -radiation led to a significant decrease in the tumour size and weight, mitochondrial enzyme activities and SOD activity, on the other hand, a significant increase was observed in DNA fragmentation, caspase-3 activity while a non-significant change was recorded in GSH content, MDA concentration and CAT activity in cancer tissues.

So, it is concluded that WSGNC possesses a radio-sensitizer and anticancer modulator suggesting its possible role in reducing the radiation exposure dose during radiotherapy.

List of Abbreviations

| | |
|-----------------------------|--|
| ADP | Adenosine diphosphate |
| AIF | Apoptosis inducing factor |
| Akt | a serine / threonine-specific protein kinase |
| ALT | Alanine transaminase |
| AST | Aspartate transaminase |
| At | Absorbance of test sample |
| ATP | Adenosine triphosphate |
| Bcl-2 | anti-apoptotic protein |
| BSA | Bovine serum albumin |
| b.w | body weight |
| CAT | Catalase |
| CdC2 | Cyclin- dependent kinase |
| DDT | dichlorodiphenyltrichloroethane |
| D.F | degrees of freedom |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| DTNB | (5,5-dithio-bis-(2-nitrobenzoic acid)) |
| DTPA | Diethylene triamine penta acetic acid |
| D.W | Dry weight |
| EAC | Erlich ascites carcinoma |
| EC | Erlich carcinoma |
| EDTA | Ethylene diamine tetra-acetic acid |
| (EDTA)Na₂ | Disodium hydrogen Ethylene diamine tetra-acetic acid |
| EGTA | Ethylene glycol tetraacetic acid |
| EPR | Enhanced Permeability and Retention |
| ETF-Q | Electron transfer flavin protein-Q oxidoreductase |
| FAD | Flavin Adenine Dinucleotide |
| 2Fe-2S | Iron- sulfur cluster |

| | |
|--------------------------------------|--|
| FMN | Flavin mono nucleotide |
| FMN H2 | Flavin mono nucleotide- reduced form |
| FTIR | Fourier transform infrared spectroscopy |
| Gd | Gadolinium |
| Gd₂O₃ | Gadolinium III oxide |
| GdBNs | Gadolinium-based nanoparticles |
| Gd-NGO | Functionalized nanographene oxide |
| GSH | Reduced glutathione |
| GSSG | Oxidized glutathione |
| H[•] | Hydroxyl radical |
| H₂O₂ | Hydrogen peroxide |
| H.S | highly significant |
| IAPS | Inhibitor apoptosis proteins |
| IC₅₀ | Half maximal inhibitory concentration |
| IR | Ionizing radiation |
| KCN | Potassium cyanide |
| KH₂PO₄ | Potassium dihydrogen phosphate |
| LD₅₀ | Half lethal dose 50 |
| LDH | Lactate dehydrogenase |
| LPO | Lipid peroxidation |
| MAPKs | Mitogen-activated protein kinases |
| MDA | Malondialdehyde |
| MDH | Malate dehydrogenase |
| MnSOD | Manganese – superoxide dismutase |
| MOMP | Mitochondrial outer membrane permeability |
| MRI | Magnetic resonance imaging. |
| MTT | (3-[4,5-dimethylthiazole-2,5-diphenyltetrazolium bromide |
| Na₂HPO₄ | Disodium hydrogen phosphate |
| NADH | Reduced nictotinamide adenine dinucleotide |
| NBT | Nitroblue tetrazolium |
| NCT | Neutron capture therapy |

| | |
|-----------------------|---|
| NF-kB | Nuclear factor kappa |
| NPs | Nano particles |
| N.S | Non significant |
| OH· | Hydroxyl radical |
| PARP | poly ADP ribose polymerase enzyme |
| p-ERK | Extracellular-signal regulated kinases |
| p-JNK | C - Jun - N - terminal kinases phosphorylation |
| Py-Gd | Pyrene gadolinium |
| Q | ubiquinone |
| QH₂ | Reduced ubiquinol |
| r1 | Relaxivity value |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| RPMI- 1640 | Roswell Park Memorial Institute medium |
| RT | Radiotherapy |
| S | Significant |
| S.E | Standard Error |
| S.D | Standard deviation |
| SDS | Sodium dodecyl sulfate |
| SOD | Super oxide dismutase |
| T1 | Longitudinal relaxation time |
| TBA | Thiobarbituric acid |
| TCA | Trichloroacetic acid |
| TGF-β | Tumour growth factor β |
| TNF | Tumour necrosis factor |
| TNFR | Tumour necrosis factor receptor |
| TRAILR | TNF-related apoptosis-inducing ligand receptor |
| WS | <i>Withania somnifera</i> |
| WSGNC | <i>Withania somnifera</i> extract/Gadolinium III oxide nanocomposite |

| | |
|------------------|-------------------|
| γ - ray | Gamma irradiation |
| ZrO ₂ | Zirconium dioxide |