AIN SHAMS UNIVERSITY
Women's College Arts and
Science and Education
Zoology Department

Metallic Nanoparticles as an Anti-proliferative Activity Against Human Hepatocellular Carcinoma

(In-Vitro study)

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ABSTRACT

We aim to evaluate cytotoxic effect of gold and silver metallic nanoparticles (AuNPs & AgNPs) on human cucasian hepatocellular carcinoma cell line model (HepG2) and their possible anti-proliferative activity**.** This new class of engineered nanoparticles with desired physicochemical properties can be applied as new therapeutic approaches against human liver cancer disease**.** HepG2 was used as a model of human liver cancer cells**.** Metallic nanoparticles were characterized using UV-visible spectra and transmission electron microscopy (TEM)**.** Cytotoxic effects of metallic nanoparticles on HepG2 cells were followed by colorimetric SRB and neutral red cell viability assays**.** Further investigation of cytotoxic effect of our nanomaterials were further investigated on a cellular and molecular level using cell cycle analysis, DNA fragmentation assay and some apoptotic genes expression on a level of mRNA for p53, Bak, Bax, Bcl2 and β actin was served as housekeeping gene**.** Treatment of HepG-2 with different concentrations of 35 nm diameter of AuNPs did not show alteration of cell morphology after 24 h of cell exposure**.** Such metallic nanoparticles did not reveal vigorous toxic effect at concentration up to 100 μM after 48 h of cell exposure**.** Cellular evaluation of AuNPs revealed progressive accumulation at G0/G1 and at G2/M phases of cell cycle**.** The expression of mRNA of P53, Bak, Bax, BCl2 without expression of mRNA of caspase 3 gene was observed in treated cells with AuNPs , suggesting involvement of intrinsic apoptotic caspase independent pathway**.** Treatment of HepG2 with different concentrations of 22 nm diameter of AgNPs did not show alteration of cell morphology after 24 h of

cell exposure**.** Also, cytotoxicity results revealed that; viability was 58% after cell treatment with 10 μM and decreased to 40.78% after treatment of cells with 1000 μM for 48 h**.** Cellular evaluation of AgNPs revealed progressive accumulation in the S phase of the cell cycle correlating with decreased number of cells in the G2/M phase followed by cellular DNA fragmentation**.** Extensive evaluation of cytotoxic effect of AgNPs showed mRNA apoptotic genes expression (P53, Bak, Bax, Bcl2) without expression of mRNA of caspase 3 gene which was expressed in untreated cells, same as the results were obtained by treating cells with AuNPs, suggesting intrinsic apoptotic casepase independent mechanism but may be induced by different molecules than that exerted by AuNPs**.** Our engineered gold nanoparticles (35 nm) and silver nanoparticles at size of 22nm showed genotoxic effect on human liver carcinoma cell line HepG-2 through intrinsic apoptotic caspase independent mechanisms**.** Further quantitative analysis and investigation for the impact of time on genotoxic effect are required before reaching a final conclusion and starting *in vivo* assays**.**

Key words: Metallic nanoparticles, anti-proliferative activity, HepG2, apoptotic genes expression

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ARABIC SUMMARY	1

LIST OF ABBREVIATIONS

11-MUA	11-mercaptoundecanoic acid
2(Bcl-w)	Bcl-2-like protein 2
A2780 cells	Human ovarian cancer cells
A549	Human carcinoma lung cell line
Ag (+)	silver ions
Ag^{+}	Silver ion
AgNPs	Silver nanoparticles
AIF	Apoptosis-inducing factor
Apaf-1	Apoptotic protease activating factor 1
ASM	Human airway smooth muscle cells
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
Au(0) nanoparticle	Gold nanoparticles
Au1+	Aurous ion
Au3+	Auric ion
Au-CNDs	Gold composite nanodevices
AuNP	Gold nanoparticles
BAD	BCL2-Associated Agonist of Cell Death
BAK	Bcl-2 homologous antagonist/killer
Balb/3T3	Mouse fibroblasts cells
BAX	Bcl2-associated X protein
Bcl-2	B-cell lymphoma 2
BCL-XL	B-cell lymphoma-extra large
BEAS-2B cell	Normal human lung cell line
BECs	Human bronchial epithelial cells
BH domains	Bcl-2 homology (BH1–4) domains
BH1–4	Bcl-2 homology (1–4) domains
BHK21	Baby hamster kidney
BID	BH3-interacting domain death agonist
BOK	Bcl-2 related ovarian killer
bp	Base pair
BPEI-AgNPs	Branched polyethyleneimine coated AgNPs
C17.2	Neural progenitor cells
Caco2	Human colorectal adenocarcinoma cell line
Caspase	Cysteine-aspartic proteases
CCDs	Charge-coupled devices camera
CD95	Cluster of differentiation 95 (FasR)
Cdks	Cyclin-dependent kinases
cDNA	Complementary DNA
Ch	Chromatin
CHK-1	Checkpoint kinase 1
CHK-2	Checkpoint kinase 2
Cit-AuNPs	Citrate capped gold nanoparticles
CM	Cell membrane
CMs	Cell membranes

COOH	Carboxylic acid
CP70	Human ovarian cancer cells
CT	Computerized tomography scan
CTAB	Cetyltrimethylammonium bromide
Cyt-c	Cytochrome c
Cyto	Cytoplasme
d-ATP	Deoxyadenosine triphosphate
dATP/ATP	deoxyadenosine triphosphate/adenosine triphosphate
DD	Death Domains
DFF	DNA fragmentation factor
DISC	Death-inducing signal complex
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide
dsDNA	Double stranded DNA
DSV	Digital Streaming Video
DU145	Human prostate cancer cells
EC50	Half maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
ENDOG	Endonuclease G
EPR	Enhanced permeability and retention
ER	Endoplasmic Reticulum
FADD	Fas associated death domain
FasL	Fas ligand
FDA	Food and Drug Administration
G1	Gap 1 phase of cell cycle
G2	Gap 2 phase of cell cycle
GF	Growth factor
Glu-GNPs	Glucose-capped GNPs
GNP	Gold nanoparticles
GNPs	Gold nanoparticles
GNRs	Gold nano rods
GNS	Gold nanostar
GSH	Glutathione
H2O2	Hydrogen peroxide
HaCaT	Immortal Human keratinocyte line
HAT	Histone acetyltransferase
HAuCl4	Chloroauric acid
HAuCl4	Tetra chloroauric (III) acid
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCT116	Colon cancer cell line
HCV	Hepatitis C virus
HDF	Human dermal fibroblast normal cells
HeLa cell	Human epithelial malignant cells, derived from a cervical carcinoma
HepG2	Human hepatoma cell line
hMSC	Human mesenchymal stem cells
HRTEM	High-resolution transmission electron microscope
HT29	Human colon adenocarcinoma cell lines

i.v.	Intravenous
IARC	International Agency for Research on Cancer
IC50	The half maximal inhibitory concentration
IL1-beta	Interleukin-1 beta
IL-6	Interleukin 6
IMR-90	Normal human lung fibroblast cells
IONPs	Iron oxide (Fe_3O_4) nanoparticles
IP	Intraperitoneal
IR	Infrared
IV	Intravenously
K562	Human chronic myelocytic leukaemia
KCl	Potassium chloride
KCs	Kupffer cells
kd	Kilo Dalton
kDa	Kilo Dalton
kV	Kilovolt
L132	L132 lung epithelial cells
LSPR	Localized surface plasmon resonance
M	Mitotic phase of cell cycle
M	Mean
MCF-7	Breast cancer cell line
MDA	Malondialdehyde
MDA-MB-231	Breast cancer cells
MDM2	Muring double minute 2
MOMP	Mitochondrial outer membrane permeabilization
MPS	Mononuclear phagocytic system
MRC-5 cells	Human fetal lung fibroblast cells
mRNA	Messenger ribonucleic acid
MTs	Microtubules
MTT	3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide
N	Nucleus
NCCD	Nomenclature Committee on Cell Death
NCRP	National Cancer Registry Program
NLS	Nuclear localization signal
NM	Nuclear membrane
Noxa	Latin for damage, pro-apoptotic member of the Bcl-2 protein family
NPs	Nanoparticles
NR	Neutral red
NRC	National Research Center
NRs	Nanorods
OD	Optical density
OM	Outer mitochondrial membrane
OMM	Outer mitochondrial membrane
P21	Cyclin-dependent kinase inhibitor
p21CIP1	P21(Cdk-interacting protein)
p21WAF1	P21 (wild-type p53-activated fragment 1)
P53	Tumor suppressor p53

PAMAM	Polyamidoamine dendrimers
PARP-1	Poly (ADP-ribose) polymerase-1
PARP-1	Poly- ADP-ribose polymerase 1
PAT	Parenteral anti-schistosomal therapy
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PC12	Rat pheochromocytoma cell
PCD	Programmed cell death
PCR	Polymerase chain reaction
pdi	Polydispersity index
PEG	Poly ethylene glycol
PI	Propidium iodide
PMN	Phagocytic mononuclear cell
PTP	Permeability transition pore
PUMA	P53 upregulated modulator of apoptosis
PVP	Polyvinylpyrrolidone
RAW264.7	Mouse macrophage-like cell line
RBC	Red blood corpuscle
RER	Rough endoplasmic reticulum
RES	Reticuloendothelial system
RME	Receptor mediated endocytosis
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute
RT –PCR	Reverse transcription polymerase chain reaction
S	Synthesis phase of cell cycle
SD	Standard Deviation
SEER	US Surveillance, Epidemiology, and End Results
SGC 7901	Human gastric cancer cells line
SiO(2) NPs	Silica nanoparticles
smARF	Small ADP ribosylation factor
SOD	Superoxide dismutase
SRB	Sulforhodamine B
TAE	Tris-Acetate buffer
tBID	Truncated BID
T-cells	T lymphocytes
TEM	Transmission electron microscopy
TGF-b	Transforming growth factor b
TGN	Trans-Golgi network
THP-1	Derived human macrophages cell line
TJs	Tight junctions
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNFα	Tumor necrosis factor alpha
TRADD	Tumor necrosis factor receptor type 1-associated DEATH domain protein
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand

U251	human glioblastoma cells
UVB	Ultraviolet B
WBC	White blood cells
WHO	World Health Organization
ZFL	Zebrafish liver cells
ZS	Zetasizer
ΔΨm	Mitochondrial transmembrane potential