بسم الله الرحمن الرحيم

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The efficiency of nanoparticles conjugated with the snake venom against the hepatocellular carcinoma cell line HEPG2.

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RESEARCH ARTICLE

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The efficiency of crude viper venom *Cerastes cerastes*-conjugated chitosan nanoparticles against the hepatocellular carcinoma cell line HEPG2

ABSTRACT:

Snake venoms (SnVs) are mixture of numerous proteins and peptides, and several studies have demonstrated the therapeutic values of some bioactive compounds extracted from SnVs on various cancer cell lines, as well as in some models *in vivo* as cytotoxic, anti-tumour and apoptosis-inducing agents. In the current study, we evaluated the anticancer potential of crude *Cerastes cerastes* snake venom conjugated with chitosan nanoparticles (CSNPs) on hepatocellular carcinoma cell line (HEPG2) at different concentrations for 24-hrs incubation through performing MTT assay and using Transmission Electron Microscope (TEM) for investigations. Also, some measurements were carried out on CSNPs to examine their stability, surface charge and detect the appropriate size for using as drug carrier through zetasizer instrument which gave some fundamental information about Nanoparticles (NPs); as average zetapotential, average size, and polydispersity index (PDI), also NPs were examined morphologically by using TEM. The cells were incubated for 24-hrs with venom-conjugated NPs for MTT cell viability assay and results revealed significant cytotoxic potency of venom-conjugated NPs. The determined IC_{50} of venom-conjugated NPs was 0.709 µg/ml and ultrastructural investigations of HEPG2 cells treated with venom-conjugated NPs at three different doses 1/2 IC_{50}, IC_{50} and $2IC_{50}$ for 24-hrs showed some degrees of necrosis and various cellular alterations. The venom-conjugated NPs proved high potency against HEPG2 cells after 24-hrs treatment and in a dose-dependent manner. On the contrary, there had been non-significant cytotoxic effect of free low molecular weight CSNPs (LMWT-CSNPs) on HEPG2 cells.

KEY WORDS:

Snake venoms, *Cerastes Cerastes*, HEPG2, Chitosan, Nanoparticles, Anticancer.

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INTRODUCTION:

Cancer is a multi-genic and multi-cellular disease; unfortunately, it can arise from all kinds of cells and organs with multi-factorial etiology (Baskar *et al.*, 2012). Certainly, the development of cancer occurs through multi-step carcinogenesis process depending on many survival mechanisms to progress as self-sufficiency in growth signalling, unresponsive to inhibitory growth signalling, avoidance of apoptosis, endless proliferative potential, sustained angiogenesis, tumour invasion and metastasis (Hanahan and Weinberg, 2011). The hepatocellular carcinoma (HCC) is the third leading cause of cancer related death


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CONTENTS

LIST OF ABBREVIATIONS

AnVs	Animal Venoms
Bcl-2	**B-Cell Lymphoma 2**
BmK-CREB	***Buthus martensi Karsch*- cAMP Response Element Binding protein**
BAX	**BCL2-associated X protein**
CCs	**Cancerous Cells**
CTX-111	**Cardiotoxin-111**
CC	***Cerastes cerastes***
CCV	***Cerastes cerastes* Venom**
C.C-PLA$_2$-1	***Cerastes cerastes* Phospolipase A$_2$-1**
C.C-PLA$_2$-2	***Cerastes cerastes* Phospolipase A$_2$-2**
C.c.gasperetti	***Cerastes cerastes gasperetti***
C.vipera	***Cerastes vipera***
CSNPs	**Chitosan Nanoparticles**
CS	**Chitosan**
CrTX	**Crototoxin**
CP-LAAO	***Cryptelytrops purpureomaculatus* l-Amino Acid Oxidase**
CTL	**C-Type Lectin**
DDSs	**Drug Delivery Systems**
DD	**Degree of Deacetylation**
D.r.russelii	***Daboia russelii russelii***
DIS	**Disintegrins**
EAC	**Ehrlich Ascites Carcinoma**

EPR	**Enhanced Permeability and Retention**
ECM	**Extracellular Matrix**
FDA	**Food and Drug Administration**
FADD	**Fas-Associated protein with Death Domain**
FA	**Formula A**
FB	**Formula B**
HCC	**Hepatocellular Carcinoma**
IL-6	**Interleukin 6**
IL-1β	**Interleukin 1 beta**
KDa	**kilo Dalton**
LMWt-CSNPs	**low Molecular Weight Chitosan Nanoparticels**
MMP	**Matrix Metalloproteinase**
MDR	**Multi Drug Resistance**
NPs	**Nanoparticles**
N.n.atra	***Naja naja atra***
N.n.oxiana	***Naja naja oxiane***
NF-kB	**Nuclear Factor Kappa light chain enhancer of activated B cells**
NDDSs	**Novel Drug Delivery Systems**
NDs	**Novel Drugs**
OH-LAAO	***Ophiophagus Hannah* L-Amin Acid Oxidase**
PLA_2	**Phospholipase A_2**
p50	**Protein 50 (Transcription factor)**
p53	**Protein 53(Tumor suppressor)**
p65	**Protein 65(Transcription factor)**
PTEN	**Phosphatase and Tensin homolog**

RGD	**Tripeptide Arg-Gly- Asp**
STAT3	**Signal Transducer and Activator of Transcription 3**
SnVs	**Snake Venoms**
SnV	**Snake Venom**
SnV-MMP	**Snake Venom MatrixMetalloProteinase**
SnV-LAAO	**Snake Venom L-AminoAcid Oxidase**
SRNCs	**Stimuli-Responsive Nanocarriers**
SPs	**Serine Proteases**
TRAIL	**Tumor necrosis factor-Related Apoptosis-Inducing ligand**
TPP	**Tri-PolyPhosphate**
VnS	**Venomus Species**
V. l. turnica	***Vipera lebtina turnica***

LIST OF FIGURES

ABSTRACT

Snake venoms (SnVs) are mixture of numerous proteins and peptides,and several studies have demonstrated the therapeutic values of some bioactive compounds extracted from SnVs on various cancer cell lines as well as in some models *in vivo;* as cytotoxic,anti-tumor and apoptosis-inducing agents. In the current study, we evaluated the anticancer potential of crude *Cerastes cerastes* (*C.cerastes*) snake venom (SnV) alone and when conjugated with low molecular weight chitosan nanoparticels (LMWt-CSNPs) on hepatocellular carcinoma cell line HEPG2 at different concentrations and time intervals through performing MTT assay.Also,we used inverted light microscope for morphological studies, Transmission Electron Microscope (TEM) for ultrastructural investigations.Also,some measurements were carried out on chitosan nanoparticles (CSNPs) to examine their stability, surface charge and detect the appropriate size for using as a drug carrier through zetasizer instrument. We got some fundamental information about nanparticles (NPs); such as average zetapotential, average size, and polydispersity index (PDI).Nanoparticles were also examined morhologically by using TEM.Spectrophotometer analysis was carried out on SnV for qualitative and quantitative measurements.Also, it was necessary to determine the surface charge of *C.cerastes* venom by zetasizer

before conjugation process with chitosan.The cytotoxicity of HEPG2 cells treated with free crude venom and venom-conjugated NPs of formula B (FB) was measured by MTT assay.The MTT results revealed significant cytotoxic potency of both of them, wherase there wasn't significant cytotoxic potencyof free LMWt-CSNPs.Besides,the determined IC_{50} values of venom and venom-conjugated NPs were highly promising. The IC_{50} was 3.67 µg / ml for *C.cerastes* venom and 0.709 µg / ml for venom-conjugated NPs.So, the cytotoxicity of cells treated with venom-conjugated NPs appeared to be more potency than those treated with venom alone.Morphological studies and ultrastructural investigations of HEPG2 cells treated with free venom and venom-conjugated NPs at three different doses 1 ⁄ 2 IC_{50}, IC_{50} and $2IC_{50}$ for 1, 3, 6 and 24 hrs time intervals showed various forms of cytotoxic effect of venom on HEPG2 cells.They were in the form of: decrease in the number of filopodia,blebbing and rupture of plasma membrane,lytic necrosis,coagulative necrosis,swelling of mitochondria with cristolysis,appearance of apoptotic bodies,pyknosis,karyorrhexis and karyolysis.The data of zetapotential,size,and PDI of CSNPs and venom-conjugated NPs showed reasonable values,reflecting the stability and uniformaity of NPs.Besides,surface charge of *C.cerastes* venom was in negative value.The results of spectrophotometer analysis of