FACULTY OF SCIENCE
ENTOMOLOGY DEPARTMENT

ANTIPROLIFERATION EFFECT OF CANTHARIDIN EXTRACTED FROM BLISTER BEETLES ON HEPATOCELLULAR CARCINOMA CELL LINE

Thesis Submitted To

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Sarah S. Hegazy

Abstract

Hepatocellular carcinoma is one of the most common cancers worldwide, with high prevalence and very low survival rates because of its resistance to conventional anti-cancer therapies. Cantharidin extracted from blister beetles was considered a promising candidate regarding this problem because of its potent medicinal value. Nuclear factor-κB has been linked to a variety of human diseases, particularly cancers. It is constitutively activated in many types of cancer and can exert pro-tumorigenic functions. **This study aimed** to evaluate the anti-cancer effect of cantharidin extracted from the oil blister beetle *in vitro* on human hepatocellular carcinoma and normal human embryonic kidney cell lines (HepG2 and HEK-293). **The materials and methods** involved extracting cantharidin, testing its purity by FT-IR, and testing its cytotoxicity against HepG2, as a hepatocellular carcinoma model, and HEK-293, as normal control. Afterwards, the NF-κB activity was measured in HepG2 cells after 24 and 48 hours. **The results** of the cytotoxicity assay on HepG2 and HEK-293 showed that cantharidin exhibited a high inhibitory effect on HepG2 in a time- and dose-dependent manners, this was evidenced by reduction in viable cells count and a statistically significant reduction in NF-κB activity ($P<0.01$) when compared with the untreated control. The results were confirmed by microscopical observations of cell morphology and NF-κB fold changes. The direct cytotoxicity of cantharidin was much lower against HEK-293 compared with HepG2, indicating a possible selective behavior. **It was concluded** that cantharidin could be considered a potential anticancer agent against hepatocellular carcinoma.

Keywords: Cantharidin, NF-κB, HepG2, HEK-293, Hepatocellular carcinoma.

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LIST OF ABBREVIATIONS

Abbreviation	Full Term
°C	Degree Celsius
%	Percent
×*g*	Times Gravity
µL	Microliter
µm	Micrometer
CA	Cantharidin
cDNA	Complementary Deoxyribonucleic Acid
cm	Centimeter
CO_2	Carbon Dioxide
CRC	Cantharidin-Related Compound
C_t	Cycle Threshold
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetate
EGF	Epidermal Growth Factor
FT-IR	Fourier-Transform Infrared
g	Gram

h	Hour
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
IC_{50}	Median Inhibitory Concentration
IκBα	Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-cells Inhibitor, Alpha
km	Kilometer
L	Liter
M	Mean
MDR	Multi-Drug Resistance
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
mm	Millimeter
mRNA	Messenger Ribonucleic Acid
NF-κB	Nuclear Factor-KappaB
nm	Nanometer
OD	Optical Density
PBS	Phosphate-Buffered Saline

PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
qRT-PCR	Quantitative RT-PCR
RHD	Rel Homology Domain
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RT-PCR	Real Time-PCR
SD	Standard Deviation
sec	Second
sqRT-PCR	Semi-quantitative RT-PCR
TAE	Tris-Acetate-EDTA
TNFs	Tumor Necrosis Factors
U	Unit
USA	United States of America
V	Volts

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