Ain Shams University
Faculty of Science
Department of Biochemistry

Biochemical Study on the Effect of Metallo-surfactant as Anticancer Drug

Thesis

Submitted in the Partial Fulfillment for the Requirement

of

M.Sc. Biochemistry

By

Ahmed Mohsen Mohammed

(B.Sc. Biochemistry / Chemistry June ٢٠٠٣)

Faculty of Science - Ain Shams University

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Under Supervision

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بسم الله الرحمن الرحيم

"رب أوزعني أن أشكر نعمتك التي أنعمت علي و على والدي و أن أعمل صالحا ترضاه و أدخلني برحمتك في عبادك الصالحين"

صدق الله العظيم

"سورة النمل.....أية رقم ١٩"

For

My Father,

My Mother,

My Brother,

and

My Sister

جامعة عين شمس

كلية العلوم

قسم الكيمياء الحيوية

أسم الطالب : أحمد محسن محمد موسى

الدرجة العلمية : ماجيستير في العلوم

القسم التابع له : قسم الكيمياء الحيوية

إسم الكلية : كلية العلوم

الجامعة : جامعة عين شمس

سنة التخرج : يونيو ٢٠٠٣

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*First and foremost all praise to **Allah** the most gracious and most merciful, **who** guides us to the straight path, for all **his** gifts thought life and for helping to complete this work*

And the peace and greeting are upon the prophet Mohamed as well as his family and his companions.

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Abstract

Ahmed Mohsen Mohamed." Biochemical Study on the Effect of Metallo-surfactant as Anticancer Drug"

M.Sc. Thesis, Ain Shams University, Faculty of Science, Department of Biochemistry.

The study aimed to synthesize and evaluate the antitumor and antibaterial effect of copper cetyl trimethyl ammonium bromide and Cu-CTAB loaded cyclodextrin nano-analogue (CD-CTAB). The nano-analog was synthesized by physical loading using grinding with ball mill. The ratio between Cu-CTAB and cyclodextrin oligosaccharide was 1 Cu-CTAB: 3 cyclodextrin. The particle size of the nano-analogue was determined using the transmitted electron microscope (TEM). The structure was evident using FTIR, NMR and inductive coupled plasma, the surface properties of Cu-CTAB were evaluated and used to explain the penetration power inside the cancer and bacterial cells. The *in vitro* anticancer activity of the compounds on Ehrlich ascites carcinoma (EAC), colon cancer cells (HCT-119), liver cancer cells (Hepg-2), breast cancer cells (MCF-7), and cervix cancer cells (Hela) were investigated using MTT assay. The *in vivo* cardiotoxic effect was estimated by measuring lactate dehydrogenase and creatine phosphokinase enzymes in rats treated with the compounds under investigation in comparison with rats treated with Doxorubicin. The Cu-CTAB showed a little effect against the cardiac muscle compared to the doxorubicin. The *in vivo* antitumor activity, liver function, hematological and antioxidant status of the compounds under investigation were evaluated using Ehrlich ascites carcinoma (EAC) bearing female mice. After 24 h of

tumor inoculation, a tenth of the LD_{50} of Cu-CTAB was inoculated day after day for one week. After administration of the last dose, mice were sacrificed to investigate for alterations in the hematological profile, liver biochemical parameters, antioxidant status and histopathological changes. The results showed Cu-CTAB decrease in tumor volume, body weight and increased the mean survival time, thereby increasing life span of EAC tumor bearing mice. Hematological profile reverted to more or less normal levels in the target compounds except for the doxorubicin group which showed a severe neotropenia. Treatment with Cu-CTAB and its nano-analogue slightly increased the levels of superoxide dismutase which was more in case of doxorubicin group. The histopathological examinations of the liver specimens showed acute inflammation in doxorubicin group compared with a mild effect in Cu-CTAB and its nano-analogue treated mice. The Cu-CTAB and its nano-analogue posses a promising antitumor effect and a less cardiovascular side effects as compared with the doxorubicin. The antibacterial effect of Cu-CTAB was also screened against *Desulfonamonas pigra*, *Escherichia coli* and *Staphylococcus aureus* by measuring the inhibition zone, also the antifungal effect was screened against *Candida albicans* by the same manner. The antibacterial and antifungal activity of the Cu-CTAB showed a potential activity comparable to the parent compound cetyl trimethyl ammonium bromide (CTAB).

Keywords: Antitumor agents, human cancer cell lines, *in vitro* anticancer activity, *in vivo* anticancer activity, nano-analog, surfactant complex.

Contents

List of Abbreviations

Abb.	*Full Name*
ADP	*Adenine diphosphate*
AIDS	*Aquired immune deficiency virus*
ALK	*Alkaline phosphatase enzyme*
ALT	*Alanine transaminase enzyme*
A_{min}	*Minium surface area*
CD	*Cyclodextrin*
CMC	*Critical micelle concentration*
CPC	*Cetyl pyrdinium chloride*
CPK	*Creatine phosphokinase*
CT	*Computarized Tamography*
CTAB	*Cetyl trimethyl ammonium bromide*
$CuCl_2$	*Copper dichloride*
CD-CTAB	*cetyl trimethyl ammonium bromide copper complex loaded cyclodextrine nano-analogue*
Cu-CTAB	*cetyl trimethyl ammonium bromide copper complex*
Cu-SOD	*Copper Superoxide dismutase enzyme*
Cu-Zn SOD	*Copper zinc superoxide dismutase enzyme*
DMSO	*Dimethyl sulfoxide*
DNPH	*Dinitro phenyl hydrazine*
DNPH-one	*Dinitro phenyl hydrazone*
DOX	*Doxorubicin*
EAC	*Ehrlich ascites carcinoma*
EDTA	*Ethylene diaminetetraacetic acid*
ELISA	*Enzyme linked immunosorbant assay*
FeS	*Ferrous sulfide*

FTIR	***Fourier transformed infrared***
GUPCO	***Gulf petroleum company***
H^{+}NMR	***Proton Nuclear Magnetic Resonance***
HBV	***Hepatitis B virus***
HCT-116	***Humane colon cancer cell line***
HELA	***Humane cervix cancer cell line***
HEPG-2	***Humane liver cancer cell line***
HIV	***Human Deficiency virus***
HLB	***Hydrophilic lipophilic balance***
HPV	***Humane papilloma virus***
IC_{50}	***Inhibition concentarion 50***
ICP	***Inductive coupled plasma***
LCT_S	***Long chain triglycrides***
LD_{50}	***Lethal dose 50***
LDH	***Lactate dehydrogenase enzyme***
M.S.T	***Mean survival time***
MCF-7	***Humane breast cancer cell line***
MCT_S	***Medium chain triglycrides***
MIC	***Microbial increased corrosion***
mNm	***Newton meter***
MTT	***(3-(4,5-dimethyl thiazol-2-yl) 2,5 diphenyl tetrazolium bromide***
N	***Avogadro's number***
NAD	***Nicotinamide adenine dinucleotide***
$NADH^{+}$	***Nicotinamide adenine dinucleotide Reduced***
NADPH	***Nicotinamide adenine dinucleotide phosphateReduced***
NCI	***National Cancer Institute***
PC_{20}	***Efficiency***
QAC_S	***Quaternary ammonium compounds***

R	***General gas constant***
RF	***Radio frequancy***
ROS	***Reactive oxygen species***
RPMI-1640	***Growth media***
SGOT	***Serum glutamate oxaloacetate transaminase enzyme***
SGPT	***Serum glutamate aspartate transaminase enzyme***
SOD	***Superoxide dismutase enzyme***
SRB	***Sulfate reducing bacteria***
TEM	***Transmission electron microscope***
VEGF	***Vascular epidermal growth factor***
v-onc	***Viral oncogene***
V_s	***Volume of sample***
V_t	***Volume of test***
Zn-SOD	***Zinc Superoxide dismutase***
γ	***Surface tension***
ΔG^0_{ads}	***Free energy adsorbed***
ΔG^0_{mic}	***Free energy micellized***
ε	***Molar absorbitivity***
μkat/L	***Micro katal per liter***
Π_{cmc}	***Effectiveness***
Γ_{max}	***Maximum surface excess***