Effect of germanium complexes on hepatocellular carcinoma cell line

A thesis submitted for the degree of Master of Science as a partial fulfillment for requirements of the Master of Science

By

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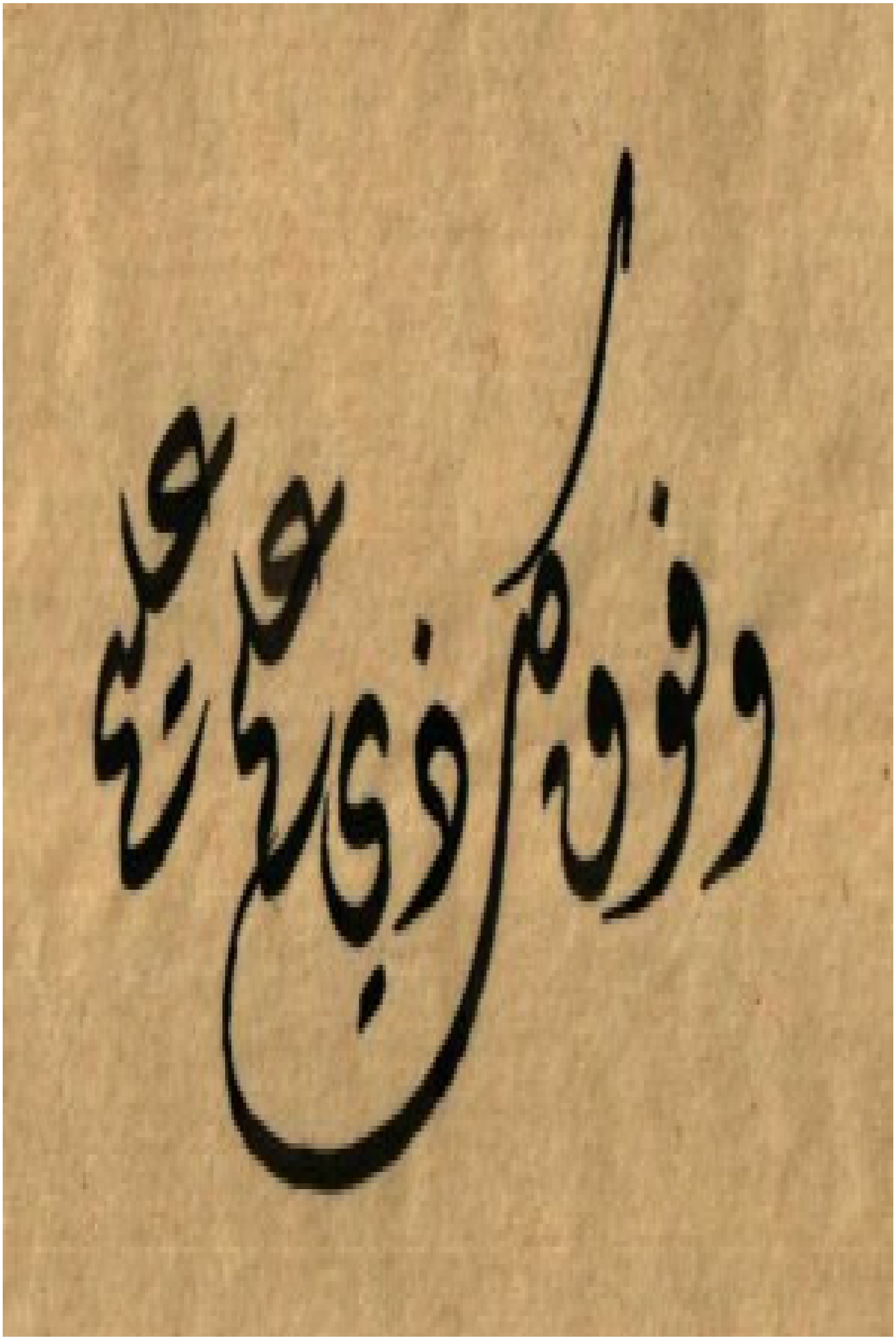
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List of abbreviation

Abbreviation	Description
ANOVA	Analysis of variance
ATCC	American tissue culture center
Asc	Ascorbic acid
BCC	Basal cell carcinoma
Bcl2	B-cell lymphoma 2
BCLC	Barcelona clinic liver cancer classification
DMSO	Dimethyl Sulfoxide
EAC	Ehrlich ascites carcinoma
EBV	Epstein–Barr virus
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal Bovine Serum Albumin
Ge	Organogermanium
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HepG2	Human liver carcinoma cell line
HEPG2	Human liver carcinoma cell line as per some American references
HPV	Human papilloma viruse
IARC	International agency for research on cancer
IC 50	50 % inhibitory concentration
ICC	intrahepatic cholangiocarcinoma
IL2	Interleukin-2
IL6	Interleukin-6
Ki67	cellular marker for proliferation
LD50	Lethal dose 50%
LGDNs	Low grade dysplastic nodules
MTX	Methotrexate
NCI	National cancer institute
NHL	Non Hodgkin lymphoma
NK	Natural killer
PBS	Phosphate buffer saline

RFA	Radiofrequency
RPMI	Roswell Park Memorial Institute (culture medium)
SCC	skin cancer
SRB	Sulphorhodamine-B
TACE	Transarterial chemoembolization
TARE	Transarterial radioembolization
TCA	Trichloroacetic acid
UV	ultraviolet

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Abstract

Background and aim: Organogermanium (Ge) complexes are used as dietary supplement. Studies revealed that Ge complexes exhibited analgesic, anti-inflammatory, antioxidant, immune-modulating, antiviral, and anticancer activity. Ascorbic acid is an essential vitamin for human body it is well known with its antioxidant activity. High doses of ascorbic acid showed anticancer activity in different cancer cells. The aim was to determine the antitumor effect of organogermanium complexes, in vitro and in vivo.

Methods: The effect of 12 Ge complexes was tested on HEPG2 cell line, the inhibitory concentration 50 (IC50%) was calculated for each of the complexes. The best 4 complexes, with least IC_{50}, were used for further studies. For the in vivo experiments, 2 complexes were chosen and tested on mice bearing Ehrlich ascites carcinoma. Their effect on EAC and the immune response interleukin 2 (IL-2) and interleukin 6 (IL-6) were compared to that of doxorubicin.

Results: The results were compared to doxorubicin as positive control. The four complexes were tested alone or combined with high dose of ascorbic acid. The results showed a strong antitumor activity of the Ge-complexes. IC_{50} values were less than that of doxorubicin, indicating a stronger inhibition of tumor growth. Furthermore, a significant decrease was observed in the proliferation marker Ki67, and the anti-apoptotic marker bcl-2, together with an increase in the apoptotic marker caspase3. The combination with ascorbic acid

increased the activity of some complexes acting in synergetic way. The in vivo results revealed an increase in the survival rate of mice treated with the complexes than the untreated group. When compared to doxorubicin; the positive control, the complexes showed a comparable effect on mice survival, ascites volume, and number of dead cells; whether alone or combined with vitamin C. Furthermore, a decrease in IL-2 and IL-6 levels was observed, indicating a decrease in cancer progression.

Conclusion: The present results showed the potential anticancer activity of germanium complexes, however, their effect on the whole body compared to the well-known doxorubicin needs further investigations.

1-Review

1.1.Cancer

1.1.1.Definition

Cancer is a general term for a group of diseases that affect any part of the body. It is the uncontrolled growth and spread of abnormal cells with the ability to invade or spread to other parts of the body. This process is known as metastasizing. Metastases are a major cause of death from cancer***(WHO, 2018).***

The basic abnormality resulting in the development of cancer is the continual unregulated proliferation of cancer cells and the lack of responding appropriately to the signals that control normal cell behavior. Cancer cells grow and divide in an uncontrolled manner, invading normal tissues and organs and eventually spreading throughout the body. The loss of growth control by cancer cells is the result of abnormalities accumulating in multiple cells, which affect the cell behavior, an important aspect that distinguish cancer cells from their normal counterparts ***(Cooper & Hausman, 2016)***.

1.1.2. Classification

Cancers are classified according to the type of cell from which they arise, there are more than 100 distinct types of cancer that