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Effect of phenolic compound extract of *Artemisia herba alba* on the Ultra-structural and BCl2 expression

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Abstract

The results of the IHC showed there was positive expression for bcl2 in the control group with high score (++) high and this expression will decrease with the increase of the dose of the PC extract in the treated group .And the results of the ultrastructural examination by using TEM showed there was increase in the N/C ratio, well developed ER, increase number of variably sized mitochondria in the control group while the treated group revealed there was many vacuoles in the cytoplasm , plasma membrane blebbing and formation the apoptotic bodies. From our study we concluded that there was clear effect on the ultrastructural after using PC as anti- tumor.

Introduction

The genus *Artemisia* L. (family Asteraceae, tribe Anthemideae), comprises a variable number of species (from 200 to over 400, depending on the authors The genus *Artemisia* is known to contain many bioactive compounds; artemisinin exerts not only antimalarial activity but also profound cytotoxicity against tumor cells (1). Heredity and environmental changes affect the susceptibility to cancer. More than 30% of cancer could be prevented by modifying or avoiding key risk factors, including: tobacco use, being overweight or obese, low fruit and vegetable intake, physical inactivity . ((Saklani & Kutty, 2008) .Side effects in current cancer therapies lead for need to search for new treatments like regimens . Herbal medicine is a growing area of health care that demands attention, It is also an important branch of alternative medicine (2).

Material and Method

The phenolic compound extracted according to the (3). By taking 100 g of dry weight from the erial part of the plant then adding 400ml from acetic acide (0.2%) in flask with 1000ml capacity and extracted it by reflex condenser for 8 h then we take the extract and adding equal quantities of n-propanol and NaCl until saturated then we will saw the separation two layers the upper one is the phenolic compound. we took it and concentrated by the rotary evaporator and then dried by in Petridis in the oven at(45-50C) to be powder and keep it until use . Transplantation of tumor cells :

The source of tumour cells (Mammary adenocarcinoma- AM3) (4) was supplied from ICCMGR were gotten from a tumour- bearing mouse and used to obtain tumour-cell suspension, that transplanted into other mice. At the first week after tumour transplantation, the volume of

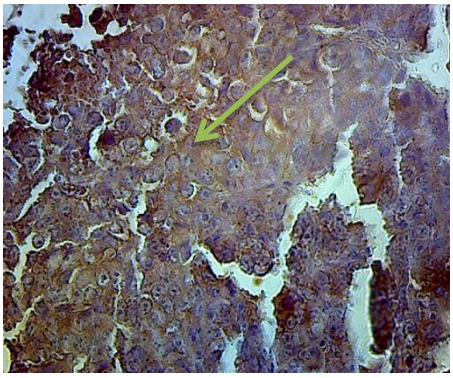
tumour growth was estimated by measuring with two perpendiculars (width and length) by using Vanier calibre . The animal were divided into 4 groups treated with different doses of the phenolic extract of *Artemisia herba alba* (0.45,0.9 ,1.8)g/kg for 4 weeks and the control group was treated by acetic acid only . after 4 weeks the animal were sacrificed and the sample was taken from the tumor mass (0.2 -0.3)mm and fixed in paraffin (10% con) for Immunohistochemical reaction and other sample is fixed using qluteraldyhyd for transmission electron microscopy.

The Result

The results of this study showed inhibitory action of the phenolic compound extract on the mammary tumor cell growth when compared with the untreated groups **.**

The immunhistochemical assessment (Scoring system).

BCL2 expression were considered as positive in cell with cytoplasmic staining . Specimen that contained less than 10 % positive tumor cell were scored as negative(fig 2) ,those with more than10% positive tumor cell were scored positive(fig 1). To minimize the risk of false positivity , acotoff value of 10% was used to define Bcl2 positivity on tumor basis . Staining for Bcl2 was subjectivly estimated as negative or no staining low than(10%) (scattered positve tumor cell), intermediate (10to50%) ,and more than (50%) scored as high (5). Lymphoid infiltarated in at tumor cell serves as internal control (positive for bcl2 expression and negative for p53 expression(fig 4).Fig 3) showes the completely hiden of bcl2 expression after treatment with (1.8g/kg)og the phenolic compound which represent the negative scoring and presence the necrotic tissue.



(Fig 1)) the cytoplasmic expression of bcl2 protein appear with (more than 50%) scoring of the tumor ( ) cell. In the control group(400x)

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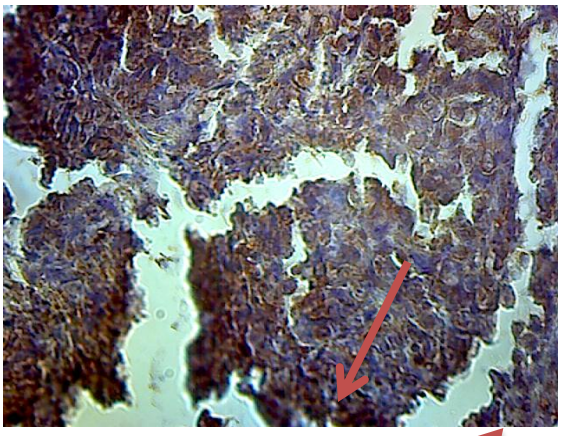
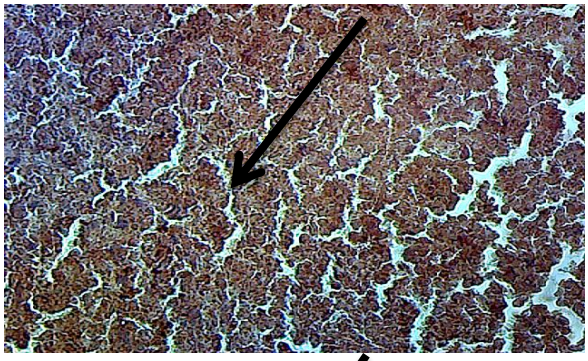
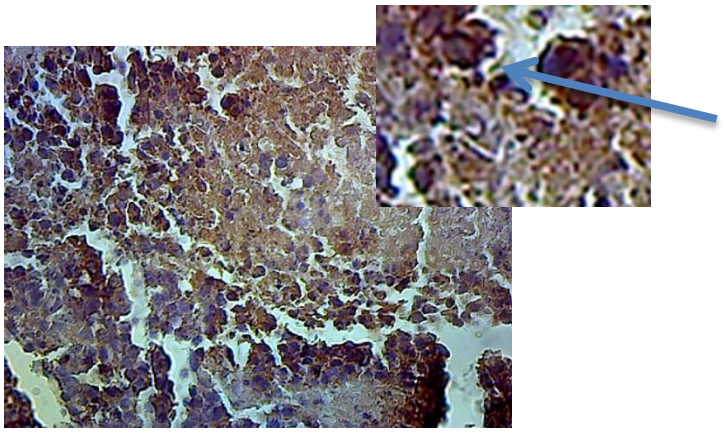


(Fig 2). the protein expression of the bcl2 protein is conceder negative ( ) according to the scoring less than (10%) 0f the tumor cell after reated with(0.9g/ kg) of the phenolic compound(400x)



(Fig 3)shows the completely hiden of the bcl2 expression (after treatment with(1.8g/kg) of the phenolic compound which represent the negative scoring and presence the necrotic tissue (400x).



(Fig 4)shows the lymphocyt which conceder as internal control for the bcl2 protein expression in the control

( ) group(400x).

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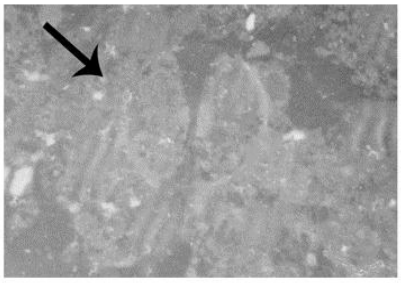
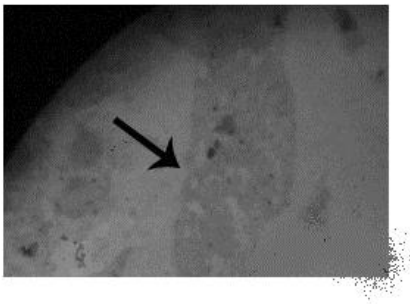
The ultrastructural study

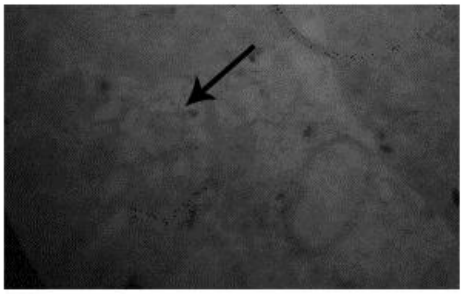
Ultrastructural examination of the tumor cells in different studying groups that done by using TEM revealed the characteristic featurs of the tumor cells involving well- developed endoplasmic reticulum (fig 1) ,increase the number of mitochondrai with size varaibility(fig 2), increas the N/C ratio prominent nucleolus as well as presence of cytoplasmic vacuoles(fig 3), The blebbing of the plasma membrane (fig 4) and formation of the apoptotic bodies(fig 5) were the most prominent features of treated tumor cells particularly in treated group with highest dose of PC .



(fig 2) mitochondrai with size varaibilty)



(fig 1) well developed E.R( )



(fig 3) the cytoplsmic vacules (

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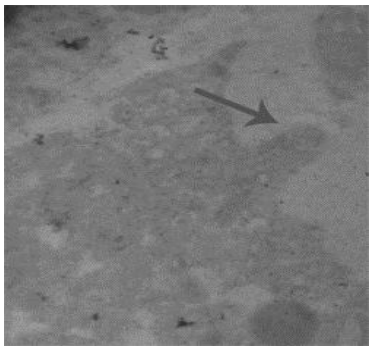
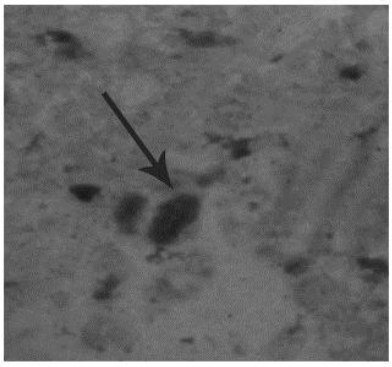


(f ig 4) the cytpoplasmic blebbling(



(fig 5)the formation the apoptotic bodeis

Disscution

The result of this study revealed that there was clear expression of the Bcl2 protein with high score which mean more than 50% (fig 1) and the expression is decreased with the increasing concentration of phenolic compound extract (fig 2) .

In the(1.8g/kg) the score is be negative and the tumor cell transform to necrotic tissue (fig 3). and the presence of the lymphocyte as internal control for bcl2 as in(fig4).

The present result were consistent with the findings of(6) that Bcl-2 protein has a role in the inhibition of apoptosis and its overexpression is found in human follicular lymphoma and disagree with the results of (7) that suggest Bcl-2 expression was negative in all rat samples.

Our suggestion is that the extraction of PC effect as anticancer drug that might be contain active hydroxyl group which can participate in different reactions by making cross linked with other protein and thus will causes formation conformational change in the Bcl2 protein which found in the membrane of the mitochondria then release the cytochrome c from the internal mitochondria membrane which then activate the caspase 8,9 and activation the caspase 3 and formation the apoptic bodies . The current study revealed there was difference in the ultrastructural occurs as a result of treatment with different doses of phenolic compound. The control group showed well-developed endoplasmic reticulum (fig 5) with increase it concentraition the number of mitochondrai with size varaibility (fig6) More over incres in the N/C ratio prominent nucleolus as well as presence of cytoplasmic vacuoles (fig7) The blebbing of the plasma membrane(fig 8) and formation of the apoptotic bodies (fig 9) .

These findings are in agreement with the study of (8) who mentioned that there was ultra structural effect of parental MCF-7 cells are more sensitive to Dox. which causes more expressed cytotoxic alterations than DDP does. In the majority of cells dystrophic changes were detected that were expressed in significant vacuolization of cell cytoplasm and decrease of the number of cytoplasmic organelles. Some cells were in a state of necrobiosis, with significant nuclear alterations, in particular, with loosen euchromatin, while cytoplasm contained a big number of vacuoles of various sizes . The usual ultrastructural examination of the tumor cell which indicat that many changes in the cytoplasm organelles,occurred which are in consistent with the results of (9) who mentioned that the ultra- structural analysis revealed that T-47D cells treated with the *Epipremnum pinnatum* chloroform extract demonstrated different stages of apoptotic and non-apoptotic type of cell death. There was clumping of nuclear chromatin margination of chromatin against the nuclear membrane ,a poptotic bodies cell blebbing and vacuolated type of morphology identified as Type II non-apoptotic programmed cell death.

The role of phenolic compound as drug for mammary gland tumor treatment shows inhibition of cell proliferation , apoptotic bodies formation and necrosis. The interpretation for such alteration is that apoptosis process is initiated by both physical-biological and pathological stimuli and this study revealed that Bcl2 might exist in a hypo phosphorylated condition to maintain its anti- apoptic .Apparently a microtubule –damaging drug ,through the activation of kinase or cascade of kinase ,can cause Bcl2 hyper- phosphorylation and thus lead to its loss of function by dissociation of its dimeric partner Bax .

Conclusions . there was an effect for the phenolic coumpond extract on the bcl2 expression and the ultrastructure changes,that was obvious in cell programmed death like vaculation of the cytoplasm , blebbing the plasma membrane , and formation the apoptotic bodies

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