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Evaluation an *in vitro* anticancer and cytotoxic potential of local herb *achillea aleppica*

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Abstract:

Cancer is a major public health in the world .Its two main characteristics are uncontrolled cell growth and metastasis. Natural products represent a rich source of compounds that have found many applications in various fields of medicine and therapy including cancer therapy. The objective of this study was to analyze the anticancer property of the aerial parts of local herb achillea aleppica .The ethanolic extract was prepared by using standard protocol .The antiprolifirative effect of the ethanolic extract was evaluated in vitro by employing MTT assay. The potency of extract concentration was calculated in term of inhibition percentage in viable AMGM and REF cell lines as compare to control value. The extract showed time dependent antitumor activity ,since 42.5% of cell were inhibited at 200µg/ml after 24h exposure, while 67.3% of cell inhibited at the same concentration after 72h exposure, in other hand , normal cell REF was slightly affected with increased concentration.

Keywords: *achillea aleppica , AMGM , REF, MTT assay*

Introduction:

Cancer is a leading cause of death worldwide and a diverse group of diseases (1) . According to the World Health Organization (WHO), it was estimated 12.7 million people globally were diagnosed with cancer and about 7.6 million people died in 2008. As estimated in this report, more than 21 million new cancer cases and 13 million deaths are expected by 2030. Although cancer accounts for around 13% of all deaths in the world, more than 30% of cancer deaths can be prevented by modifying or avoiding key risk factors (2) .

Due to the societal and economical implications of this pathology, tremendous efforts have been made over the past decades to improve the available therapeutic options. Although a large number of potent chemotherapeutic anticancer agents have been identified and successfully used in clinical practice, while minimizing their toxic side effects. Indeed, most anticancer agents display a narrow therapeutic window due to their lack of selectivity against cancer cells. Besides, the ability of the anticancer compounds to actually reach their target is often impaired by a number of physiological barriers (3), as well as the chemoresistance, is considered to be the responsible for treatment failure in over 90% of patients with metastatic cancer (4).

Herbal plants and plant-derived medicines have been widely used as natural alternatives to produce new potential therapeutic compounds for treatment combating diseases (5). The health promoting effects of plant constituents and extracts are being increasingly studied and their consumption is on the rise (6). Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their tumoricidal actions against various cancers(7).

Investigations in the traditional ethnobotany and ethnomedicine presented that Achillea species has been used to treat haemorrhoids, cancer, vertigos, anemia, anorexia, dyspepsia, gastralgia, haemorrhage, dysmenorrhoea; very efficacious in female complaints(8). Flowers are used for treatment of gastritis, tannins occurring in this species render it is used, for treatment of haemorrhage, dysmenorrhoea and diarrhoea; it is also used to treat haemorrhoids and as a tonic and cicatrizant (9).

The anti-tumor activity of the derived flavonoid Casticin of Achillea millefolium by G2/M arresting(10) . Also, the antihepatoma activity of Achillea millefolium on five human liver-cancer cell lines, that is, HepG2/C3A, SK-HEP-1,

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HA22T/VGH, Hep3B and PLC/PRF/5 have been investigated (11) antioxidant, anti-inflammatory and antiproliferative properties of Achillea millefolium (12). In another research, it was found that A. millefolium was effective against mouse leukemia cells as anticancer agent(13) .

Phytochemical investigations of Achillea species have revealed that many components from this genus are highly bioactive. The essential oils obtained by water distillation from aerial parts of Achillea schischkinii Sosn. and Achillea aleppica DC. subsp. aleppica were analyzed by gas chromatography and gas chromatography/mass spectrometry. 1,8-Cineole (32.5 and 26.1%, respectively) was the main component in both oils. The oil of A. aleppica subsp. aleppica was also found to be rich in bisabolol and its derivates. When tested for their antimicrobial, antiinflammatory, and antinociceptive activities, the oil of A. aleppica subsp. aleppica showed significant antiinflammatory, antinociceptive, and moderate antimicrobial activities(14,15).

In current research, investigations were performed for studying the anticancer activity of local herb achillea aleppica extract against two Iraqi cell lines ; AMGM as a brain cancerous cell line, and REF as a normal murine cell line.

Methods:

Plant material

The plant material was collected in June 2013 from arbil governorate a north area of Iraq and the taxonomic identity of the plant was confirmed by the Iraqi national herbarium.

Extraction

The aerial parts of plant were collected and dried under shade. The dried samples were powdered and used for solvent extraction. For extract preparation, 250 g of dried sample was macerated with absolute ethanol, for 48 h with shaking and the procedure was repeated once. The extract was filtrated through Whatman No. 1 and combined, then concentrated using a rotary evaporator under reduced pressure at 40°C. The dry extract obtained was kept in refrigerator at 4C.

Cell Growth

human cerebral glioblastoma multiform (AMGM) and fibroblastic and epithelial cells with normal chromosomal picture (REF) a normal murine cell lines were kindly provided from Iraqi center of cancer and medical genetic researches , were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, Streptomycin (100 U/ml), penicillin (100 U/ml), and incubated in 5% CO2 at 37 ºC for 24 h. Cell counts determined using 0.2 ml of trypan blue solution and 1.6 ml PBS , then subculture when monolayer's cells were confluent(11). Afterwards, 200 µl of cells in growth medium were added to each well of a sterile 96-well microtiter plate. The plates were sealed with a self-adhesive film, lid placed on and incubated in 5% CO2 at 37°C. When the cells are in exponential growth, i.e. after lag phase, the medium was removed and serial dilutions of ethanolic extract in SFM (200µg/ml,100µg/ml,50µg/ml,25µg/ml,12.5µg/ml and 6.25 µg/ml) were added to the wells. four replicates were used for each concentration of ethanolic extract. The middle two columns as control (cells treated with SFM only). Afterwards, the plates re-incubated under the same condition for the selected exposure times (24, 72 hrs).

Cytotoxicity assay

200µl of cell suspension (Confluent monolayer's) of both AMGM and REF were seeded into wells of a 96-well plate. After 24 hrs of incubation 200 µl of crude extract of two fold serial dilutions were added. Four replicates were used for each concentration of extract. Afterwards, the plates were re-incubated at 37°C for the selected exposure times (24, 72 hrs). The cytotoxicity test was determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (12) . In brief, 50 µl of MTT was added to the wells, the cells were cultured for additional 4 hrs at 37ºC. Then 100 µl of DMSO was added to the wells. The solubilized formazan was measured at 570 nm using microplate spectrophotometer (Multiskan, Finland).

The % Inhibition were calculated with the following formulae :

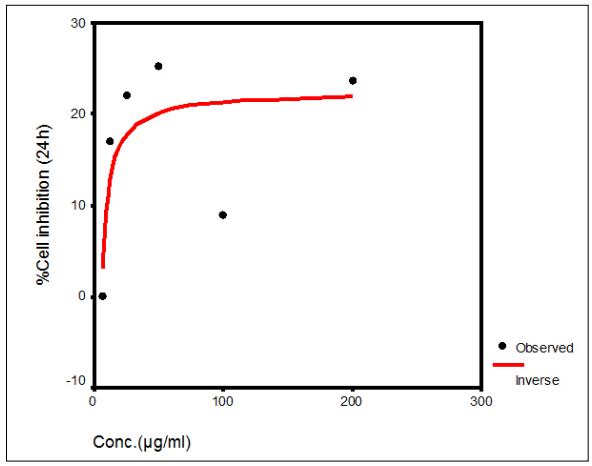
Inhibition % = 1-(OD of sample / OD of control) x 100

Results:

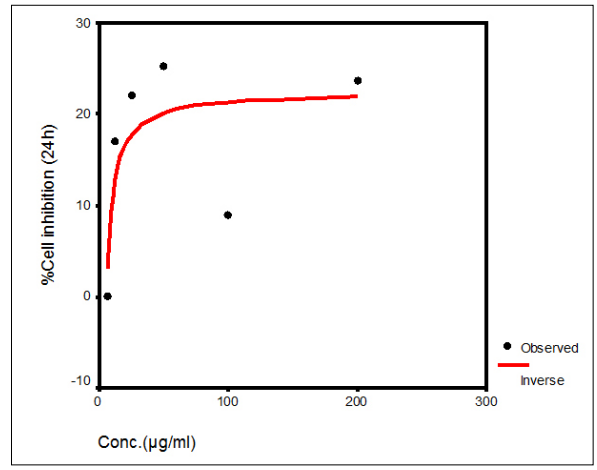
The result of the cytotoxic activity of ethanolic extract tested against AMGM brain cancer cell line determined by MTT assay and percentage of inhibition calculated by microplate reader at 570nm , were listed in table (1).

Table 1: cytotoxicity on AMGM by ethanolic extract of achillea aleppica after 24h ,72h exposure.

No.	Conc. (µg/ml)	%Cell inhibition (24h)	% Cell inhibition (72h)	C.S
1	6.25	-	-	Wilcoxon Signed Ranks Test Z = - 1.214 P=0.225 NS
2	12.5	16.8	-	
3	25	19.1	52.8	
4	50	35	38.8	
5	100	36.1	46	
6	200	42.5	67.3	
7	Cell control	-	-	



Figure(3):cytotoxicity on REF by ethanolic extract of achillea aleppica after 24h exposure.



Figure(4): cytotoxicity on REF by ethanolic extract of achillea aleppica after 72h exposure.

In 24h exposure the lower concentration 6.25µg/ml showed no cytotoxicity , while slightly inhibition rate 5% at the same concentration after 72h exposure time.
Inhibition percentage was slowly increased with increasing the concentration(17%,22%,25.2%) (12.5,25,50,50µg/ml) and long exposure time (12.5,25,200µg/ml)(28.4%,28.2%, 20.9%) respectively, despite of no significant difference at (P= 0.075).

Discussion:

The local herb achillea aleppica is used in Iraqi folk medicine. Very little researches known about its scientific medicinal value. In this study the aerial parts extract was found to exhibit anticancer activity .The anticancer mechanism of action was found to be induce apoptosis.

However the GC-MS analysis of ethanolic extract for these herb was done by the authors in previous work revealed the presence of more pharmacologically active compounds such as citral , carveol , α-bisabolol , Andrographolide , other flavonoids and unsaturated fatty acids(16) . also reported the activity of some phyto-components with compound with nature of flavonoids; unsaturated acid and linolenic as antioxidant ,cancer preventive. The same compounds which exist in our herb could have contributed to the observed anticancer activity of the herb extract. Similarly Andrographolide treatment inhibited the in vitro proliferation of different tumor cell lines, representing various types of cancers. The compound exerts direct anticancer activity on cancer cells by cell-cycle arrest at G0/G1 phase through induction of cell-cycle inhibitory protein p27 and decreased expression of cyclin-dependent kinase 4 (CDK4)(17). Furthermore, this compound has strong anticancer activity against human colorectal carcinoma LoVo cells by inhibiting cell cycle progression(18).It is also Lim , demonstrated that the anticancer mechanisms for andrographolide include the inhibition of Janus tyrosine kinases-signal transducers and activators of transcription, phosphatidylinositol 3-kinase and NF-κB signalling pathways, suppression of heat shock protein 90, cyclins and cyclin-dependent kinases, metalloproteinases and growth factors, and the induction of tumor suppressor proteins p53 and p21, leading to the inhibition of cancer cell proliferation, survival, metastasis, and angiogenesis(19). The anti-proliferative activities of carveol have been shown in a number of cancer cell lines including human metastatic breast cancer (MDA-MB-231 and MCF-7). However, its effect on prostate cancer has heretofore not been known, much less its effect on the human prostate carcinoma cells, LNCaP and PC-3(20). The anti-metastatic potential of α-bisabolol and its possible mechanism of action were investigated. Its induced a decrease in cell proliferation and viability in pancreatic cancer cell lines (KLM1,KP4,Panc1,MIA Paca2), but not in pancreatic epithelial cells. α-bisabolol treatment induced apoptosis and suppressed AKt activation in pancreatic cell lines(21) .
Furthermore, α-bisabolol might induce dose- and time-dependent apoptosis in HepG2 cells. Western blot data also showed a cascade activation of caspases-8,-9,-3 and promoted expression of Fas, implying caspase-8 might function as an upstream regulator, and the Fas-related pathway might be involved in this process(22) . In conclusion the antiprolipherative effect of achillea aleppica due to its rich source of biological active compounds like andrographolide and flavonoids.

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تقييم الفعالية المضادة للسرطان والقاتلة للخلايا للنبات المحلي الاخليليا خارج الجسم الحي

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الخلاصة:

يعتبر السرطان من اكبر المشاكل التي تواجه صحة المجتمع في العالم , وهو ذات خاصيتين ، نمو الخلايا غير المسيطر عليه وانتشار المرض في أعضاء الجسم. . تعتبر النواتج الطبيعية مصدر غني لمركبات كثيرة ذات خواص تطبيقية في مجالات مختلفة من الطب ومنها علاج السرطان. في هذه الدراسة تم تحديد الخاصية المضادة للسرطان التي تتمتع بها الأجزاء الهوائية للنبات المحلي الاخليليا . إن عملية الاستخلاص تمت وفق معايير قياسية وان التأثير المثبط لتكاثر الخلايا تم تقييمه باستخدام اختبار MTT فضلا عن ذلك فان قوة تأثير المستخلص حسبت على أساس نسبة التثبيط للخلايا الحية AMGM و REF وان المستخلص اظهر فعالية تثبيطية تعتمد على فترة التعريض .