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Cytotoxic Effect of Aqueous Extract of Anastatica hierochuntica L. on AMN-3 Cell Line in vitro

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Abstract
Anastatica hierochuntica L. is distributed throughout Arabain Peninsula, and elsewhere it is locally called "Kuffe Maryam" .All parts of the plant are used in folk medicine. This study amid to investigate the effect of aqueous extract of anastatica hierochunctica *L*. on the cancer cell lines AMN-3. Anti cancer activity of aqueous extract of anastatica hierochunctica *L*. showed anticancer activity against AMN-3 cell line for twelve concentrations (0.04, 0.09, 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100) mg/mL in comparison with negative control.
Keywords: Anastatica hierochuntica L., Cytotoxic activity.

Introduction

There is a global increase in the use of medicinal plants for health reasons. In developing countries herbal drugs and traditional remedies are relatively more popular because of cultural acceptability and belief that being natural, they are safe and non-toxic. However, toxicity of such drugs is yet not well understood [1-3]. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoid and phenolic compounds [4].

A. hierochuntica L. (Family: Brassicaceae) locally called 'Kaff-e-Maryam, is a well known desert zone medicinal plant. Novel melanogenisis inhibitor flavonoids with antioxidant potential were isolated from it [5,6]. Kaff-e-Maryam is a monocarpic annual plant species characterized by topochory/ ombrohydrochory type of seed dispersal [7-8] (Figure 1).

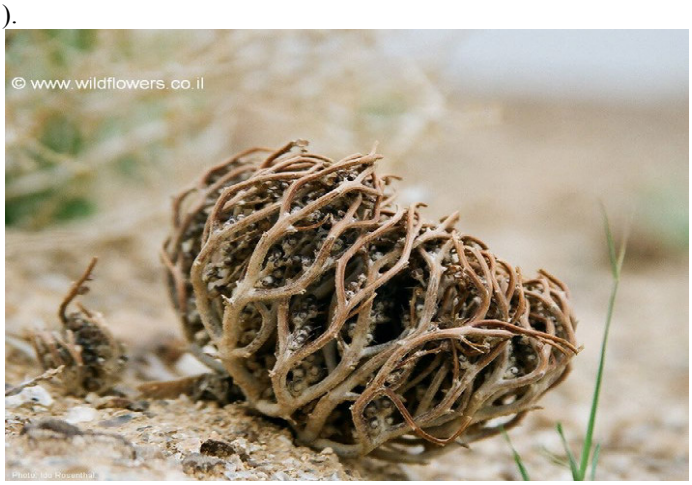


Figure 1: Kaffe Maryam [9]

All parts of *A. hierochuntica* are famous and used in folk medicine to provide cure against various disease states [10,11]. Based on its frequent medicinal use and popularity among the people of central Asia, Africa, Arabian Peninsula, and else-where, Kaff-e-Maryam was selected for present study. The plant is available on sale throughout Saudi Arabia in mini vegetable markets, road side, and on the shops of medicinal plant sellers [12] .

A decoction of Kaff-e-Maryam (*A. hierchuntica*) has been in use against stomach upset, as antispasmodic, against fatigue, uterine haemorrage, menstrual cramps, depression. It is believed to be a cure for arthritis inflammations, infections, diabetes, asthma, viral and autoimmune diseases. It is also used for pain relief after surgery [8,13-15]. Its freshly prepared decoction is commonly used as local disinfectant, to ease childbirth, as liver tonic, to stop vomiting, to treat mouth ulcers, and provide cure against stomach cancer [16,17]. All parts of the plant were reported to possess antimicrobial activity [18].

Breast cancer is a type of cancer originating from breast tissues, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk[19].The size, stage, rate of growth and other characteristic determine the kind of treatment [20]. Breast cancer may be invasive or noninvasive. Invasive means it has spread from the milk duct or lobule to other tissues in the breast. Noninvasive means it has not yet

invaded other breast tissue[21].

Aim of study

The aim of this study was to assess cytotoxic effects of Aqueous Extract of Anastatica hierochuntica L. on AMN-3 Cell Line in vitro.

Materials and Methods

Samples of whole dried A. hierochuntica were brought from Iraqi market in Baghdad, then aerial parts of the plant were isolated and kept in airtight glass containers till the time of the experiment. Then the dried plant was ground to fine powder. 25 gm of the powder, mixed with 250 mL of distilled water and were incubated for 3hrs at (60) C°. Extracts were filtered and concentrated using a rotary evaporator at low temperature and pressure. The crude extracts were weighed and stored at -20°C until use.

Method of Cytotoxicity Assay

Single cell suspension was prepared by treating 25 cm2 flask of tissue culture at passage 13 with 2 ml 25% trypsin solution incubated for 2 min at 37°C in an incubator supplemented with (5%) CO2 after detachment of the cells from the flask surface. Single cell suspension gently taping of the flask. This was followed by the addition of 20 mL of growth medium supplemented with 10% fetal calf serum. Then, the viability test of the cells was made by using trypan blue dye which stains the dead cells. Cell suspension was well mixed followed by transferring 200µ/well of the 96 well flat bottom micro titer plate using automatic micropipette containing (1x105 cell/ well). Plate were incubated at 37°C for 24 hrs in an incubator supplemented with (5%) CO2 until 60-70% confluence of the internal surface area of the well for AMN-3 cell line. The cells were then exposed to different concentration of new compouned (0.04, 0.09, 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100) mg/mL respectively, each compouned was added to the cells in triplicate from of with culture media represented the negative control, then the 96 well cell culture plate incubated at 37°C in an incubated supplemented with (5%) CO2 for 24 hrs. After elapsing the incubation period, 50 µl/well of neutral red dye freshly prepared were added to each well and incubated again for 2 hrs, viable cells will uptake the dye and the dead cells will not. The plates washed by PBS to remove the excess dye, then 100µl/well of eluent soilution were added each well to draw out the dye from the viable cells. Optical density of each well was measured by using ELISA reader at a transmitting wave length on 492 nm.

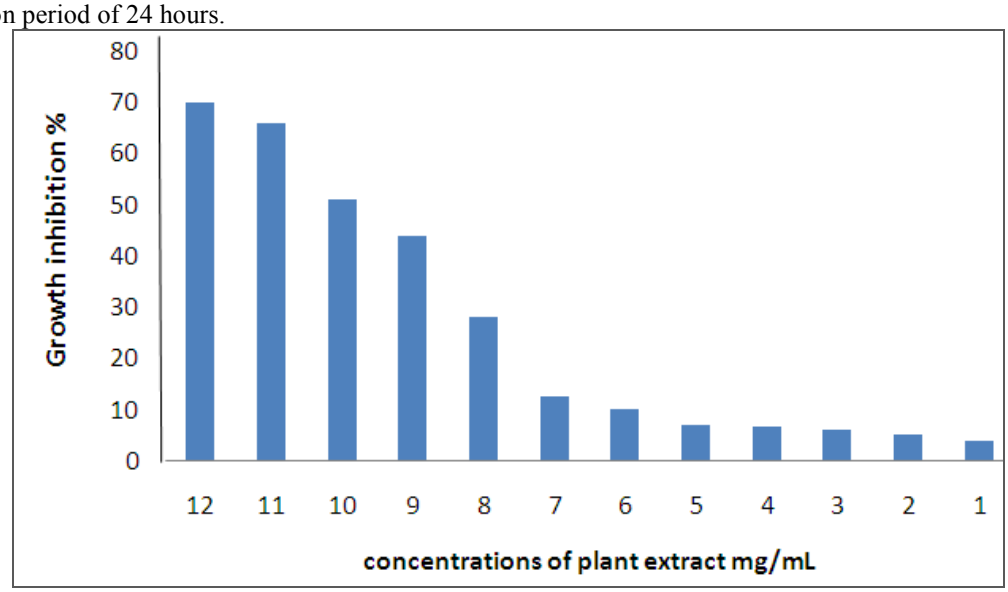
$$\text{Inhibition rate \%} = \frac{\text{Absorbance of negative control} - \text{Absorbance of test}}{\text{Absorbance of negative control}} \times 100$$

Results and Discussion

Cytotoxic effect of aqueous extract of anastatica hirochuntica L. on AMN-3 cell line after an incubation period of 24 hour:

The percentage of plant extract growth inhibition values represented in figure (1) appeared that after 24 hours incubation growth inhibition of AMN-3 cell line was increased with the increased of plant extract of A. hierochuntica L. concentration when compared with the negative control. Aqueous extract of plant has significant differences of cytotoxic effect on AMN-3 cell line ($P<0.05$), 70%, 66%, 51%, 44%, 28%, 12.5%, 10%, 7%, 6.4%, 6%, 4.9%, 3.7% these percentage of growth inhibition rate were showed at concentration 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.09 and 0.04 mg/mL respectively.

Figure (1): Cytotoxic effect of aqueous extract of anastatica hirochuntica L. on AMN-3 cell line after an incubation period of 24 hours.



Due to the importance of some of the plants from a medical point has varied uses and characteristics abounded popular that pharmacological effectiveness and speed of its impact when used in therapeutic herbs or powders complete preparations derivative in the treatment of many human diseases without relying on synthetic compounds industrially[22].

Attributed various plants ability to inhibit different cancer cell lines to the presence of oily chemical compounds in these plants. The plants and medicinal herbs have many of the chemical compounds that have different events such as toxic inhibitory effectiveness of cancer cells within the body of an organism or outside. These compounds have several mechanisms that lead to the inhibition of tumor cell's ability Inhibition of tumor cell capacity and reduce the growth and spread and kill it [23].

The results obtained in this study supported including reached by many researchers. About owning plant extracts of the effectiveness of anti-cancer cells, and this activity mainly depends on the focus the user and the duration of exposure also depends on the type extract effective compounds in which and the extent of the cancer cells sensitive to all that, and the results obtained have shown the effect of aqueous extract of the plant A. hierochuntca and its ability to inhibition higher growth of cancer cells AMN-3 which was characterized by an increase of the percentage of inhibition increased concentration and duration of exposure. Many researchers referred to in their studies use of extract of plant of the effectiveness of anti-cancer cells like Achillea Fragrantisime, which tested its ability to inhibit cell lines cancer vero, REF, AMN-3, AMG3 as the use of aqueous extracts of four different concentrations of the plant and three times been 24, 48 and 27 hours and were generally results characterized by an increase of the percentage of inhibition increased concentration and duration of exposure with variations[24].

The observed protective effect of A. hierochuntica may be attributed to the antioxidant properties of this interesting desert plant . In earlier studies , compounds such as qurecetin, luteolin, and kaempferol derivatives, and anastatica was found to be anti-ulcerogenic compound with significant protective activities [25,27]. Hence, the observed cytoprotective and antioxidative property of A. hierochuntica are ascribed to the presence flavonoids and other antioxidants present in the plant extract under study [28,30]. Besides several biological activities, various flavonoids have been reported to be useful in the treatment of certain gastrointestinal disorders and as inhibitors of free radical generation [31,33]. Nevertheless, quercetin was earlier confirmed to exercise its gastroprotective effect by its antiperoxidative, antioxidant, and antihistaminic nature[34,35]. Flavonoids contain hydroxyl functional groups, are responsiple for the antioxidant effect in the plants. The high antioxidant activities can be attributed to their phenolic and β-carotene contents and a wide range of β- carotene (ranging from 1 to 190 µg/g d. w.) [36]. These results were disagreement with other investigations (A.A. AL-Mussawi) [37], which showed anticancer activity of flavonid extract of A. hierochuntica showed no anticancer activity against Hep2nd, AMN-3 cell lines for four concentration (3.125, 6.25, 12.5, 25 mg/L) in comparison with negative control.

Conclussion

Through the current study, we conclude that the aqueous extract of the plant anastatica hierochuntica is a medicinal plant for its ability inhibitory effective in inhibiting the growth of cancer cells as well as its

importance in the treatment of other diseases.

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