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Extract from Clematis erecta Inhibits Breast Cancer Cell Migration, Invasion, and Apoptosis

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ABSTRACT

One of the main characteristics of breast cancer is the migration and invasion of cancer cells to other regions of the body. The illness gets more difficult to control and cure when cancer cells invade more areas of the body. There are medications that kill cancer cells but also induce general cytotoxicity; no medication has been found to stop cancer cells from spreading. It has already been shown that the natural ingredients have invasive and anticancer potential. Syphilitic, malignant, and other nasty ulcers are historically treated with an infusion of the leaves of Clematis erecta L. (Ranunculaceae). Furthermore, there was notable analgesic and anti-inflammatory effect in the ethyl acetate fraction and methanolic extract. The scientific literature continues to provide no evidence that C. erecta has anticancer properties. Investigating the anticancer effects of C. erecta aerial parts on breast cancer cells was thus planned. The findings indicate that C. erecta may have anti-invasive properties against MDA-MB-231, a kind of triple-negative human breast cancer cell. The effects of three distinct extracts (water, methanol, and chloroform) from the aerial portions of C. erecta on the migration and proliferation of MDA-MB-231 human breast cancer cells were assessed. It's interesting to note that aqueous extract reduces cell growth by over 50% and reduces invasion and migration by 40% and 50%, respectively. Further evidence that C. erecta has the ability to destroy cancer cells came from the fragmentation of DNA in extract-treated cells.

Introduction

As the most common cause of mortality and one of the most complex medical conditions, cancer is a deadly illness.[1] The condition is still difficult to cure even with cutting-edge scientific methods, early diagnosis, therapy, and preventative measures.[2-4] Normal cells may become malignant due to the unchecked proliferation of cancer cells caused by genetic instability and other cell changes.[4] Worldwide, women get the most breast cancer diagnoses out of all cancer types.[5] The capacity of breast cancer cells to penetrate and spread is a hurdle to the disease's identification and therapy.[6,7] Mutations in the genome give cancer cells the capacity to break out from the main tumor site, break down the extracellular matrix (ECM), and infiltrate stromal tissues.These cells then intravasate, travel via vascular or lymphatic pathways, extravasate in distant tissues, and start to self-home in the new location [8, 9].[10–12] Inhibiting metastasis has the ability to lower the disease's fatality since it is a complicated process involving the whole cell apparatus. In order to prevent metastasis by obstructing the components essential to the adhesion, migration, and invasion of cancer cells, scientists are now investigating a wide range of medications and substances.[6,13–15]

It is becoming more well acknowledged that the process of invasion and metastasis offers a wealth of potential targets for the creation of more advanced medications that might function as inhibitors by limiting invasion and metastasis.[16] Studies have shown the chemopreventive properties of phytochemicals found in the human diet, and they have also raised the possibility of bioactive natural substances having anticancer properties.[17–19] Several medications containing anticancer ingredients have been created from natural sources.[20, 21] Scientific research has recently concentrated on the bioactive elements of these substances and their processes for causing cell death.[22–24] Consequently, it seems that natural phytochemicals may be used to stop, slow down, or even cure cancer.

The genus Clematis has garnered significant interest recently due to its anticancer properties.[25–28] Therefore, we chose Clematis erecta for our present research. L. C. erecta is a member of the Ranunculaceae family and is often referred to as upright virgin's bower. Syphilitic, malignant, and other nasty ulcers are historically treated with an

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infusion of C. erecta leaves.[29, 30] Significant analgesic and anti-inflammatory activity was shown by the methanol and ethyl acetate fractions, while the plant's aqueous extract had fungicidal and bactericidal properties.[30, 31]
There aren't many studies in the literature on C. erecta's capacity to fight cancer. Therefore, it was intended to look into the aerial sections of C. erecta's potential anticancer properties. The anticancer potential of the plant extracts was found in the activation of apoptosis and the inhibition of migratory and invasive capacity. Various plant extracts were examined for in-vitro anticancer studies on chosen cancer cell line.

Material and Methods

Collection and Identification of Plant Material

In September 2018, the aerial parts of C. erecta were acquired from KR Indo German, Kurukshetra. By comparing the plant's macroscopic and microscopic characteristics to an authentic sample of C. erecta that was previously identified from the National Institute of Science Communication and Information Resources, New Delhi, India (Ref. No. NISCAIR/RHMD/ Consult/-2008-09/1192/224, dated 09/04/2009), the plant's identity has been confirmed in the Pharmacognosy laboratory of DPSDR, Punjabi University Patiala.
Various crude extracts of C. erecta leaves were prepared using LR grade solvents, namely methanol (S.D. Fine Chemicals, Mumbai, India), n-hexane, and chloroform (E Merck, Delhi, India).
Getting Ready for Extracts
Aerial sections of C. erecta were ground in a grinder after being sun-dried. On a water bath kept at 80°C, dried powdered plant material (1.4 kg) was extracted one at a time by refluxation method using solvents (3 X 4 L each) in increasing order of polarity, n-hexane, chloroform, and methanol. Using a distillation assembly, solvents from crude extracts were recovered to provide n-hexane extract (HE), chloroform extract (CE), and methanol extract (ME). The remaining marc was then removed using a hot plate decoction method and 4L of distilled water. A hot air oven set at 70 to 80°C was used to dry the water/aqueous extract (WE). It was discovered that the yields of HE, CE, ME, and WE were, respectively, 4.50, 2.32, 4.07, and 2.14% w/w.[32]
Cultured Cells and Cell Lines
The National Centre for Cell Science, located in Pune, India, provided the breast cancer cell lines (MDA-MB-231). Cell lines were cultivated at 37°C in a humidified environment with 5% CO2 in DMEM containing 10% FBS (GIBCO), 100 IU of penicillin G/mL, and 100 µg of streptomycin/mL.

Results

Cell Viability Assay

Using the cell viability test, anticancer activity was measured (MTT test). A tetrazolium salt is MTT 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide. Chloroform, methanol, and the water extract of C. erecta were shown to have antiproliferative effects on the development of human breast cancer metastatic cell line (MDA-MB-231). After exposing the cells to doses ranging from 20 to 200 µg/mL of methanol, chloroform, and C. erecta aqueous extract, the cells were incubated for a full day. Figure 1 clearly illustrates each extract's capacity for cytotoxicity. As the concentration of the extract was raised, the percentage of viable cells after treatment exhibited a decreasing trend.

The assay's findings point to the extract's potential for cytotoxicity.

Assay for Invasion and Migration

We used the transwell assay to evaluate the characteristics of the 200 µg aqueous extract of C. erecta in order to investigate its impact on MDA-MB-231 cell migration and invasion. 200 µg of the extract were applied to the cells.

The findings showed that, during a 24-hour treatment period, the MDA-MB-231 cell line's capacity for migration and invasion was considerably inhibited by the aqueous extract of C. erecta. MDA-MB-231 cell movement was 40% decreased by the extract (Figs. 2A & 2B). A same suppression pattern was also seen for the cells' ability to invade,

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with the amount of invaded cells being almost half decreased by the aqueous extract (Figs. 3A & 3B). Our findings suggested that the aqueous extract could prevent the MDA-MB-231 cell lines from migrating and invading.

Assay for DNA Fragmentation

We also looked at the part that C. erecta's aqueous extract played in the MDA-MB-231 cells' programmed cell death. The test for DNA fragmentation was used to investigate the impact of C. erecta aqueous extract on apoptosis. One of the distinguishing characteristics of cells going through apoptosis is DNA fragmentation. The MDA-MB-231 cells were treated with several amounts of the C. erecta aqueous extract in order to look for any alterations in the cells' DNA. Six well culture plates were used to plate the cells, and following

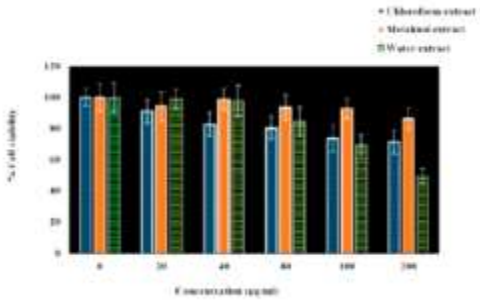

Fig. 1: Percent cell viability of MDA-MB-231 cell lines in different extracts of *C. erecta*

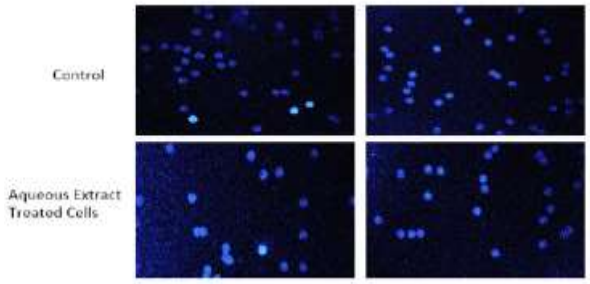

Fig. 2 A: Representative photomicrographs showing the inhibitory effect of aqueous extract of *C. erecta* on the migration of MDAMB- 231 cancer cells

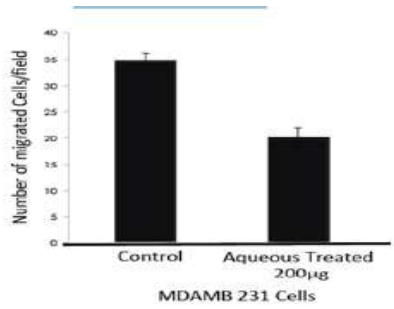

Fig. 2 B: Inhibitory effect of aqueous extract of *C. erecta* on the migration of MDA-MB-231 cancer cells

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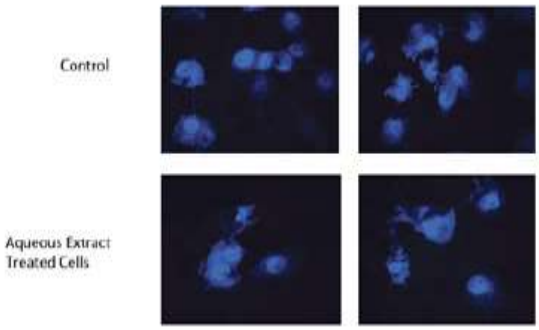

Fig. 3 A: Representative photomicrographs showing the inhibitory effect of aqueous extract of *C. erecta* on the invasion of cancer cells

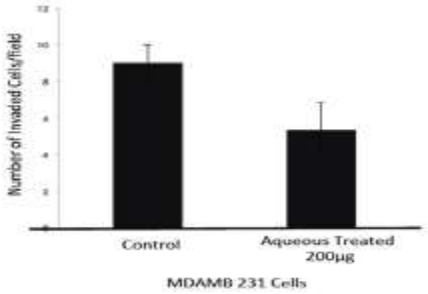

Fig. 3 B: Inhibitory effect of aqueous extract of *C. erecta* on the invasion of cancer cells

Fig. 4: DNA fragmentation in MDAMB 231 cells after exposure to an aqueous extract of C. erecta A DMSO-treated control is shown, and a DNA ladder is seen in the last lane.

The aqueous extract was applied to the cells at the stated doses for a duration of 24 hours (Fig. 4). For the experiment, the cells that were given DMSO treatment acted as the control. After that, the cells were treated by being incubated for a whole day. Following the manufacturer's instructions, the DNA of the treated cells was extracted using a Thermo Fisher DNA extraction kit. Next, the concentration was determined using nanodrop quantification of DNA.

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A comparable quantity of DNA was deposited onto the 1.8% agarose gel that contained ethidium bromide. When the cells were treated with aqueous extract of C. erecta at higher doses (100 and 200 µg), DNA fragmentation was observed. DNA fragmentation is shown in smear form in Fig. 4. These findings point to the potential function of C. erecta extract in inducing MDA-MB-231 cell death.

Discussion

Globally, breast cancer is the primary cause of mortality for women.[5] Metastasis of breast cancer continues to be a cause of death. Various chemotherapeutic treatments are available in clinics [36, 37], but they can have harmful side effects.38 As a result, a different strategy that uses less harmful chemicals, such medicinal herbs and their preparations, may be very valuable and cause less harm to normal cells. Among the key bioactive components of therapeutic plants are phytochemicals.[17, 18] The traditional medical system has made use of a wide range of plants and plant extracts.[20] From ancient times till the present, people have used these traditional treatments. Many chemotherapy medications, including etoposide, paclitaxel, and others, were first made from plants.[39] Numerous plant species include bioactive components with potential for use in medicine and chemoprevention. It is essential that we comprehend the process behind their actions.

Research on breast cancer has shown the efficacy of plant-based chemicals as anticancer agents.[24, 27, 40] In this work, we demonstrated the anticancer activities of C. erecta extract. According to the current research, C. erecta extract has strong cytotoxic activity and may kill breast cancer cells when applied topically. The ability for C. erecta extract to kill MDA-MB-231 cells has been shown by its treatment of these cells. These findings are in line with several earlier investigations that have shown the cytotoxic effects of different plant extracts on cancer cells.[21, 39, 41] It has previously been shown that Clematis species are antibacterial, anti-inflammatory, and useful against cancer and syphilis ulcers.[29] It was also intriguing to see that the C. erecta extract inhibits the cancer cells' capacity to migrate and invade other areas.

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