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Effect of Cynanchum paniculatum (Bge.) Kitag. on various diseases: an overview of current research progress

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ABSTRACT

Traditional medicine and compounds identified from the medicinal plant extract and nutraceuticals were known to show significant beneficial effects on human health. Cynanchum paniculatum is a medicinal herb successful in treatment of many microbial infectious and metabolic disorders such as cancer. Management of tumors are still a big challenge for oncologists because there are several complex mechanisms in the cancerous cells, that influence the host system to support the tumor microenvironment. One of the well-established properties of cancerous cell is its metastasizing ability, which makes tumor development in other organs and higher recurrence rate. Cancer cells attains the metastatic property by inducing a specific pathway called epithelial to mesenchymal transition (EMT). It was observed that the inhibition of cell migration was effective in both CP extract and conditioned medium supplemented cells compared to their respective controls. We found that, in MCF-7 cells, conditioned medium showed better activity than direct CP extract treatment. This shows that CP extract interferes with cell migration and pertaining intracellular signals. We presume that the major reason behind this would be the effect of CP on modulation in exosomal pathway and their communication through secreted biomolecules. In conclusion, EMT signaling plays a crucial role in tumor metastasis and CP extract played a significant role in inhibition of cell migration through intra/extracellular signaling of cancer cells. This can be an important finding for future studies on the role of CP constituents against cancer metastasis and recurrence.

Keywords: Cynanchum paniculatum, Epithelial to mesenchymal transition, Cell migration, Breast cancer, Triple-negative cells.

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1. INTRODUCTION

Folklore medicine has been very successful centuries together in several countries and their cultures (Kala et al., 2006; Efferth and Kuete 2010; Yuan et al, 2016). They have provided many invaluable plants that possess significant medicinal values and were successful in the management of several ailments and even provide a complete cure for certain complex disorders that are challenging to medical experts (Nasri et al., 2014, Chen et al., 2020). It was obvious that even the global population believes and seeks herbal medicines more than the allopathic approach because it is safer and available in abundance (Pawar et al., 2016). Traditional medicines consist of several herbs and Cynanchum paniculatum (Bge.) Kitag. (CP) is one of such celebrated and well-explored plant species that was used in traditional Chinese medicine for more than a thousand years and it is known as Xuchangqing in Chinese. It was known for its ability

to treat inflammation and the oldest record of CP was from Han Dynasty where it was used to treat mental disorders, malaria, and some viral infections (Chen et al., 2020). The plant parts are composed of several bioactive metabolites and are mostly phenolic derivatives. Paeonol is a phenolic compound that accounts for about 1% of the dry weight of CP and other components include, cynapanosides, phenanthroindolizidine, cynanversicosides, 3-hydroxy-4-methoxy-acetophenone and, etc. Recently, around 21 steroidal glycosides such as paniculatumoside, neocynapanogenin were identified and are being explored for its biological properties (Wang et al., 2002; Kim et al., 2013; Fu et al., 2015; Xiong et al., 2018).

The extracts from CP have been reported to avoid vital organs toxicities and several metabolic diseases involving those organs. Most of the activities rendered by CP were mainly executed by the phenolic compounds and it was reported that the compounds obtained from CP have been interfering with several pathological signaling processes that help in disease progression (Chen et al., 2020). Inflammation, oxidative stress, apoptosis, are some common pathways influenced by CP and its compounds. These pathways targeted by CP play a vital role in cancer progression and though the effect of CP on treating malignant conditions was not seriously explored in detail, the anti-inflammatory action of this plant can provide a connection to anti-cancer ability. Apart from cancer, several other diseases conditions such as alcohol induced liver injury, renal injury & fibrosis, cerebral ischemia, depression and etc., were successfully managed by CP-based treatment (Chen et al., 2020; Zhou et al., 2020).

2. BREAST CANCER AND THEIR TYPES

Breast cancer remains the 5th most common form of cancer and the genotypic variation results in different types of breast cancer phenotypes with variations in histopathological appearance, molecular changes like presence and absence of progesterone receptor (PR), estrogen receptor (ER), Human Epidermal Growth Factor Receptor 2 (HER-2) and presentation of clinical features (Table 1) (Kast et al., 2015; Dai et al., 2017; Jeibouei et al., 2019).

Breast cancers are divided into five subgroups based on its phenotypic expression of certain receptors and differences in their cellular characteristics (Table 1). Normal breast cells like cancers possess expression patterns of noncancerous breast tissue cells. Luminal A & B breast cancer cells are ER-positive cells with epithelial markers. Cells that show elevated expression of epidermal growth factors and HER2 are termed HER2 positive breast cancer cells. Basal-like breast cancer cells are a very challenging type of cells to treat and they possess characteristics of mammary epithelial cells with basal cytokeratins. Based on the expression levels of claudin protein basal-like breast cancer cells were characterized as claudin-low basal-like breast cancer cells. These cells are also called triple-negative breast cancer cells (TNBC) as they show reduced expression of ER, PR, and HER2 proteins compared to other types of breast cancer cells. They actively undergo Epithelial to Mesenchymal Transition (EMT) and metastasis with typical features like immune evasion and the ability to gain stem cell characteristics. They show the highest incidence and recurrence rate (Dai et al., 2017; Fedele et al., 2017).

Metastatic potential and chemoresistance, differ according to different types of breast cancer cells and hence treatment strategies also change accordingly. Generally, survival rates of patients with ER and PR positive (either or anyone) breast cancers are high because the treatment strategies are targeted towards estrogen and progesterone receptors modulations at later stages, while these tumors respond to radiotherapy at early stages (Ethier et al., 1993; Malhotra et al., 2010). On the other hand, the ER/PR negative but HER2 positive cells comprised of 10-15% of reported breast cancer cases and their prognosis very hard and show metastatic potential with high recurrence rate. Inhibition of receptor tyrosine kinases was found to be a better therapeutic strategy for HER2 positive and ER/PR negative breast cancers (Ogba et al., 2014; Zubor et al., 2015).

Table 1. Types of breast cancer cells and their genomic characteristics (Dai et al., 2017)

Types of Breast cancer cells	Genomic characteristics	Cell lines
Luminal A	ER (+); PR (+); HER2 (-)	MCF-7; T47D
Luminal B	ER (+); PR (+); HER2 (+)	BT-474; BSMZ
Triple-negative/claudin-low	ER (-); PR (-); HER2 (-)	MDA-MB-231; SUM1315
Triple-negative/basal-like	ER (-); PR (-); HER2 (-)	MDA-MB-468
HER2 positive	ER (-); PR (-); HER2 (+)	SK-Br-3

The major challenge for oncologists is the management of triple-negative cells that have no expression of ER, PR, and HER2 and their type of breast cancer progression. They are classified as basal-like breast cancer cells and 5–10% of all breast cancers are of this subtype. The major feature of this subtype is that the cells show characteristics of stemness, immune evasion and have features for EMT. The prognosis of triple-negative breast cancers is difficult and patients must rely only on chemotherapy as the no possibility of targeted therapy (Ferraiuolo and Wagner, 2019).

3. EPITHELIAL TO MESENCHYMAL TRANSITION (EMT)

EMT is a combination of intracellular and extracellular signalling processes where epithelial cells acquiring mesenchymal stem cell features. When EMT occurs during embryogenesis, it is termed as type 1 EMT, while occurrence during tissue regeneration is termed as type 2 EMT. The pathological basis of EMT event results in tumor malignancy (Fig. 1), metastasis, and invasion of other cells and at this state, it is termed as type 3 EMT (Mani et al., 2008; Felipe Lima et al., 2016; Ye et al., 2017). As discussed earlier, EMT plays a central role in the metastasis of cancer cells. Under normal physiological conditions, epithelial cells adhere to the basement membrane with the help of integrins and possess the apical-basal polarity. During tumor development, the cancerous cells subset loses apical-basal polarity which leads to loss of its characteristic cell-cell junctions and acquires mesenchymal cells' properties. This enables migration of cancerous cells and increases the circulating tumor cells to help to invade distant organs (Lamouille et al., 2014; Mittal, 2018). EMT leads to phenotypic changes in the cells organized by transcription factors like TWIST1, SNAIL1, etc leading to downregulation of E-cadherin which is the prominent epithelial marker. As a result, the cells acquire stem cells like properties and possess greater chemoresistance with possible epigenetic mechanisms, upregulation of drug efflux pumps, and DNA repair machinery (Lamouille et al., 2014; Dai et al., 2017).

3.1 Role of TGF-B and Other Signaling Pathways In EMT and Breast Cancer Progression

There are several signaling pathways involved in the initiation of EMT, where in TGF-β plays the primary role and is considered as the major player in tumorigenesis in most breast cancer cases (Massagué, 2008; Lamouille et al., 2014). TGF-β regulates a series of intracellular signaling molecules that makes epithelial cells lose their polarity, junctional stability, and cytoskeletal remodelling, which induces motility, focal adherence, interactions with adherent junctions (Miettinen et al., 1994). TGF-β is also activated in autocrine mode through receptor tyrosine kinases (RTKs), Wnt/ β-catenin pathway, Notch signaling, and Hedgehog pathway that help the cancer cells to acquire the stem cells like characteristics (Fedele et al., 2017). Urokinase plasminogen activator system (uPA) is a well-established paracrine mechanism that induces EMT and breast cancer progression through conversion of plasminogen to plasmin that upregulates ECM components and secretion of growth factors that stimulate migration and metastasis of tumor cells (Deryugina and Quigley, 2012; Duffy et al., 2014). The autocrine and paracrine mechanisms of EMT induction involve several proteins secreted by the cancerous cells collectively called secretome (Fedele et al., 2017). Among several factors secreted by cancer cells and other types of cells from tumor environment, exosomes have gained a special interest recently because of their biochemical profile and their role in tumor metastasis and progression (Giordano et al., 2020).

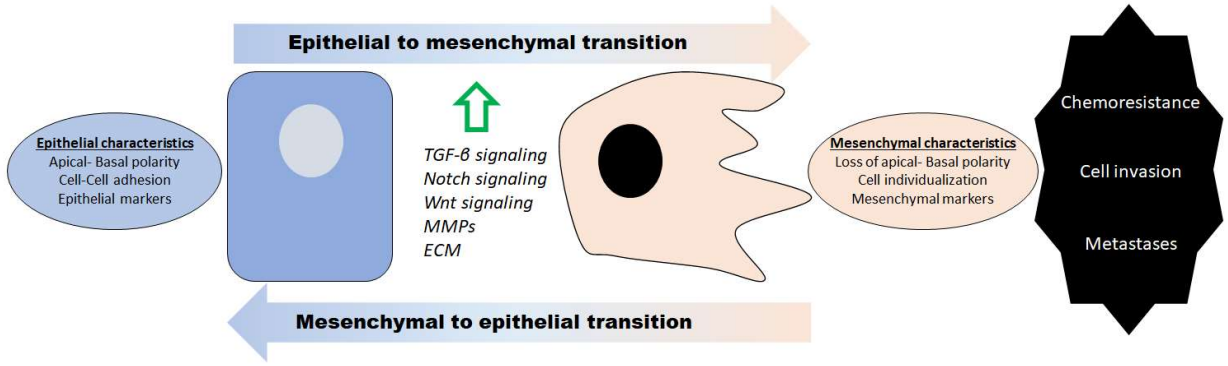

Fig. 1. Mechanism of epithelial to mesenchymal transition. The figure depicts the group of signaling molecules and pathways that triggers and accelerates epithelial to mesenchymal transition. The epithelial cells possess certain characteristics and they are lost when they acquire mesenchymal characteristics which is a major step in breast cancer progression. The mesenchymal characteristics help the cancer cells to metastasize, invade the neighbouring cells and evade the chemotherapeutic action. TGF-β – Transforming growth factor β; Wnt – Wingless-related integration site; MMPs – Matrix metalloproteinases; ECM – Extracellular matrix

3.2 Role af Exosomes in EMT and Breast Cancer Progression

Exosomes are small vesicles secreted by the exocytosis process and they contain a variety of macromolecules that has a potential role in several intracellular and intercellular communications in the tumor microenvironment aiding in metastasis (Luga et al., 2012; Vella, 2014). Biomolecules secreted along with exosomes by different cell types have been reported to regulate several processes like angiogenesis, proliferation, etc including cancer cell plasticity by regulating EMT (Vella 2014). Exosomes derived from cancer-associated fibroblasts induce metastasis, while exosomes of mesenchymal stem cell and macrophage origin induce Wnt pathway-mediated migration and/or invasion of breast cancer cell lines (Cho et al., 2012; Menck et al., 2013). Hence, regulation of exosome secretion or modulation of their biochemical profile will have a serious effect on cancer metastasis and related mechanisms.

4. HERBAL MEDICINES FOR BREAST CANCER TREATMENT

Traditional medicines based on herbs are sought out more because compounds from these medicinal plants are safer to use and induce fewer side effects (Yuan et al., 2016). Compounds like curcumin, quercetin, kaempferol, and other polyphenolic compounds were identified from herbs and were reported for several health benefits. Several of the phytocompounds or plant parts are being successfully marketed (Abu Bakar et al., 2021). Among the reported biological properties of herbal compounds, anticancer effects were largely explored and those studies reported that mechanisms like oxidative stress, inflammation, apoptosis induction are predominantly targeted by the herbal extract and their constituent. Consumption of herbal formulations is successful in prevention and treatment of breast cancers also through regulating certain pathways like cell cycle, apoptosis, EMT, cell adhesion, etc (Laskar et al., 2020). More than 100 anti-cancer drugs were identified and marketed for breast cancer treatment in the past 35 years and around 80% of them are herbal compounds or their derivatives. Serious efforts are required for plant-based medicines to be translated to the clinical arena (Zyad et al., 2018; Amaral et al., 2019).

Most breast cancers are curable mainly because 40-60% of the reported cases were luminal breast cancers and inhibition of estrogen receptors has shown a significant success rate (Orlando et al., 2010). Plant-based phytoestrogens and their ability to reduce breast cancer incidence were observed in the population where soy was consumed in a regular diet. Phytoestrogens act as an analog for human estrogen and was able to inhibit estrogen receptors mediated signaling (Lamartiniere, 2000; Samavat and Kurzer, 2015). Herbal medicines and nutraceuticals (fruits and vegetables) had gained significant success and plant-based estrogen-like compounds or phytoestrogens were developed mainly in this regard. Naturally occurring flavonoids, diterpenes, anthocyanins, quinones and, etc, have also shown significant inhibition in tumor development through mechanisms like anti-inflammation, apoptosis, etc. were also reported to reduced breast cancer progression (Laskar et al., 2020).

5. MEDICINAL VALUE OF CP AND ITS ROLE IN VARIOUS DISEASE MANAGEMENT

CP has been successful in interfering with many signaling pathways that help in the progression of diseases and this makes this plant material as an extract or the phytoconstituents effective against many challenging diseases that target single or many organs at the same time (Table 2). Literature on the phytoconstituents from CP that provide many successful biological properties, narrows down to paeonol, which seems to be the active principle that carries out the activities against cancer, diabetes, and many other metabolic complications (Chen et al., 2020).

5.1 Respiratory and Cardiovascular Diseases

Traditionally, CP was prescribed for primary respiratory discomforts like cough and breathlessness (Li 2006), while currently several reports are being generated regarding constituents of CP and their effect against, asthma, inflammation in the respiratory tract, fibrosis and, etc (Zhou et al., 2021). Most of the studies showed that MAPK and NF-κB signaling were primarily targeted by CP and their constituents (Liu et al., 2014; Tang et al., 2018). Paeonol was explored for its pulmonary protection through counteracting the hypoxia in the lungs due to pulmonary hypertension via ERK-1/2 mediated mechanisms (Zhang et al., 2018). Management of vascular hypertension also provides an idea about the effect of CP on cardiovascular complications. Cardioprotective role of CP against atherosclerosis, renovascular complications were studied by several researchers and major mechanisms reported were modulation of ion channels for vasodilation, macrophage uptake of oxidized LDL, and anti-inflammation. Through calcium transport and regulated mechanisms, CP and the phytoconstituents were reported to influence the vasodilation function (Li et al., 2010; Zhang et al., 2017), while macrophage-based clearance of ox-LDL was upregulated by paeonol which resulted in interference in the pathogenesis of atherosclerosis by inhibition of macrophage-based foam cell formation. Furthermore, the effect of CP and paeonol in the preservation of lipid profiles (Total cholesterol, LDL, and triglycerides levels) seem to be the foremost observation that could aid in halting several cardiovascular disease progressions. Though most of the studies are in their preliminary stages, promising observations are claimed in favor of CP and their phytoconstituents (Lu et al., 2018; Wu et al., 2021).

5.2 Gastrointestinal and Hepatobiliary Diseases

Gastrointestinal diseases such as ulcerative colitis, inflammatory bowel syndrome, liver damage, and complications like ascites were successfully treated by CP and the compounds isolated from it (Sun et al., 1993; Zong et al., 2017). Patients with clinical presentations of different forms of gastritis were successfully treated by CP extract and some of those patients were identified to have developed H. pylori infection (Chen et al., 2020). CP was also helpful in curing some lower GI tract conditions like abdominal pain, diarrhea, or constipation. CP was also very effective against certain metabolic abnormalities in the liver such as chronic hepatitis, alcoholic/non-alcoholic liver fibrosis, cirrhosis, and cancer (Kong et al., 2013; Sun et al., 2018; Wu et al., 2019; Kong et al., 2020). Viral infections like HBV were also effectively controlled by CP which resulted in halting its progression to cirrhosis and cancer (Xie et al., 2005). Paeonol from CP extract was reported to inhibit the proliferation of hepatic stellate cells, that are responsible for upregulation of MMPs and TGF-β/Smad-3 signaling required for liver fibrosis and cirrhosis which predispose for hepatocellular carcinoma (Wu et al., 2019).

5.3 Nephrological and Neurological Complications

Renal complications due to oxidative stress, ER stress, nephrotoxicity, nephrotic syndrome, and inflammatory disorders in uterus and Fallopian tubes were successfully treated with the extract of CP or their phytochemical constituents (Wu et al., 2017; Liu et al., 2018). Neuroprotective effect of CP was also well-established against glutamate-induced neuronal damage through inhibition of pathogenic signaling pathways like NF-κB/MAPK. CP also was effective against microglia-induced inflammation and oxidative stress. Paeonol was reported to reduce inflammation and helped in managing ischemia-induced brain injury. It was also effective against depression induced by LPS administration in vivo (Weon et al., 2012; Weon et al., 2014; Liao et al., 2016).

6. ROLE OF CP IN CANCER TREATMENT

Effect of CP (plant extract) on various malignancies was not claimed as much it should have been because several in vitro and in vivo studies were reported where CP was effective against tumor development, progression, and metastasis with inflammation as the major target mechanism (Chen et al., 2020). However, the effect of paeonol was extensively reported regarding inhibition of several types of cancer progression. Paeonol showed the ability to induce apoptosis in esophageal cancer cell lines through regulation of Bax/Bcl2 balance and arrest the cells in the S phase of the cell cycle (Sun et al., 2008; Wan et al., 2008). The expression of caspases was also upregulated during these events. Similarly, apoptosis-mediated treatment of gastric cancer by paeonol was reported in vitro and in vivo (Li et al., 2010; Lyu et al., 2017; Fu et al., 2018). Major observation in this was the downregulation of MMP-2 & 9, which are the primary mediators in gastric tumor cells to acquire metastatic potential through the EMT pathway (Lyu et al., 2017). Anti-inflammatory potential of paeonol was also very helpful during anti-cancer activities and this was reported by Fu et al., 2018, where they showed downregulation of epidermal growth factor receptor by paeonol, played a significant role in inhibition of cancer metastasis and tumor growth (Fu et al., 2018).

Table 2. Protective role of CP in different diseases conditions in the human system and the target mechanism

Different types of diseases	Target mechanism	References
Pulmonary	Downregulation of NF-κB signalling, TGF-β1, induced MAPKs signalling	Li (2006); Liu et al., (2014), Tang et al., (2018); Zhou et al., (2021)
Cardiovascular	Downregulation of mTOR pathway, Reduction of lipid profile (total cholesterol, triglycerides, and LDL)	Li et al., (2010); Zhang et al., (2017)
Kidney	Reduction of oxidative stress, inhibition of AMPK, GSK-3 pathway	Wu et al., (2017); Liu et al., (2018)
Hepatobiliary and gastrointestinal	Inhibition of NF-κB pathway, reduced activation of stellate cells, inhibition ofTGF-β mediated EMT and fibrosis	Sun et al., (1993); Kong et al. (2013); Zong et al. (2017); Sun et al., (2018); Wu et al., (2019); Kong et al., (2020)
Neurological	Downregulation of NF-κB, MAPK pathways	Weon et al., (2012); Weon et al., (2014); Liao et al., (2016)
Cancer	Activation of apoptosis, downregulation of NF-κB pathway, EMT inhibition, downregulation of MMPs, PI3K/Akt pathway, Epidermal growth factor	Sun et al., (2008), Wan et al., (2008); Li et al., (2010); Li et al., (2013); Yin et al. (2013); Lyu et al., (2017); Xu et al., (2017); Zhou et al., (2017); Fu et al., (2018)

Hepatocellular carcinoma is the most common type of cancer and paeonol has reduced liver cancer progression in vivo by reducing its oxidative injury through anti-oxidant and immunomodulatory effects (Chen et al., 2012). In the case of downregulation of TGF-β/Smad signaling, paeonol was reported to reduce the progression of pancreatic adenocarcinoma through inhibition of EMT, which influenced the anti-metastatic potential of the cancerous cells. This provides a very important observation with respect to the metastatic potential of cancer cells and the effect of CP to negatively influence metastasis through inhibition of EMT (Cheng et al., 2020). Paeonol also caused a significant upregulation of apoptosis process in other cancer conditions like colon, ovarian, and prostate cancers. Ovarian cancer cells were directed toward apoptosis through downregulation of surviving proteins along with activation of apoptotic pathway (Yin et al., 2013; Zhou et al., 2017). On the other hand, an influx of excessive Ca^{2+} to the colon cancer cells was considered as the mechanism by which paeonol has induced apoptosis (Li et al., 2013; Xu et al., 2017). The ability of CP and its constituent (Paeonol) to inhibit inflammation, induce apoptosis, and inhibit EMT has shown the potential of this medicinal plant to suppress growth, progression, and metastasis in the management of cancer.

7. CP FOR BREAST CANCER: AN UPDATED LITERATURE

Breast cancer management through CP is still in the infantry stages and significant research has to be carried out for the identification of potential compounds in this regard. CP has been effective against several diseases and inflammation and immunomodulation were some of the specific activities that were regulated during CP supplementation (Chen et al., 2020; Zhou et al., 2020). NF-κB is the major player in organizing the inflammatory signaling during several metabolic diseases and CP shows potential inhibition of this pathway and downstream signaling. Similarly, MAPK is another important pathway that is targeted by CP during treatment of many cancer conditions, including breast cancer. Macrophage activation is also considered as an important process during cancer development and CP has shown a significant effect on this process (Saahene et al., 2018).

In our previous study with CP water extract, we showed the differential effect of our extract on different types of breast cancer cells, where cell migration of ER/PR negative breast cancer cells was significantly inhibited which might correlate to possible inhibition of EMT. Interestingly, the medium obtained from cells treated with CP extract showed better inhibition cell migration compared to direct treatment of the extract. This shows the extracellular secretions from the cells play a crucial role in cell migration and EMT in breast cancer patients. Further, triple-negative cells were sensitive to CP extract and showed poor survival above the LC_{50} concentration (Yang et al., 2021). As a part of that experiment, we studied the cell migration of MDA-MB-231 (triple-negative breast cancer cells) and MCF-7 (HER2 negative cells) in the presence of CP extract and the conditioned medium collected from CP extract treated cells.

7.1 Effect of CP on Cell Migration

The effect of direct CP treatment and CP treated conditioned medium treatment on cell migration of MCF-7 (Fig. 2) and MDA-MB-231 cells (Fig. 3) was tested using the scratch assay. The concentration of CP in each treatment was selected based on our previous observation, where, the toxicity and LC_{50} of CP extract to the specific cells were identified and, in this study, we have used a slightly higher concentration of the extract than the CP extract, which is 3 µg/ml for MCF-7 cells and 2.7 µg/ml for MDA-MB-231 cells.

Briefly, the cells were grown at the density of $7 * 10^4$ cells with culture insert in respective medium supplemented with 10% FBS and changed to CP extract added in 5% exosome-free FBS medium while the insert was removed. The gap left after removing the insert, which resembled the scratch was photographed at 24 hr, 48 hr, and 5 days after the removal of insert. The four treatment groups are conditioned medium (sup-CP) obtained from cells treated with the CP extract and conditioned medium control (sup-Control) obtained from cells without CP extract, cells treated directly with CP extract (dir-CP), and the untreated cells were considered as its control (Control). The distance of migration was estimated by measuring the length of this line in the ImageJ software (NIH freeware). The average distance of all cells in the fields was calculated and compared among groups.

7.2 Effect of CP Extract and CP Extract Conditioned Medium on Migration of MCF-7 Cells

In the control and sup-control groups, the migration of MCF-7 cells was very aggressive and in sup-control treatment, significant migration was observed in 24 hrs than the normal control (Fig. 2), which infers clearly about the effect of extracellular contents, especially exosomes that has promoted intracellular signaling and accelerated the cell migration. In the case of both the sup-CP and dir CP treatment, significant inhibition of the cell migration was observed throughout the experiment period and this shows that the constituents of CP showed inhibition of cell migration by inhibiting the intracellular signaling (Fig. 2). Further, the autocrine signaling of the cells influenced through the exosomes was also observed to have interfered because the cells were supplemented with conditioned medium obtained from the same cells treated with CP extract.

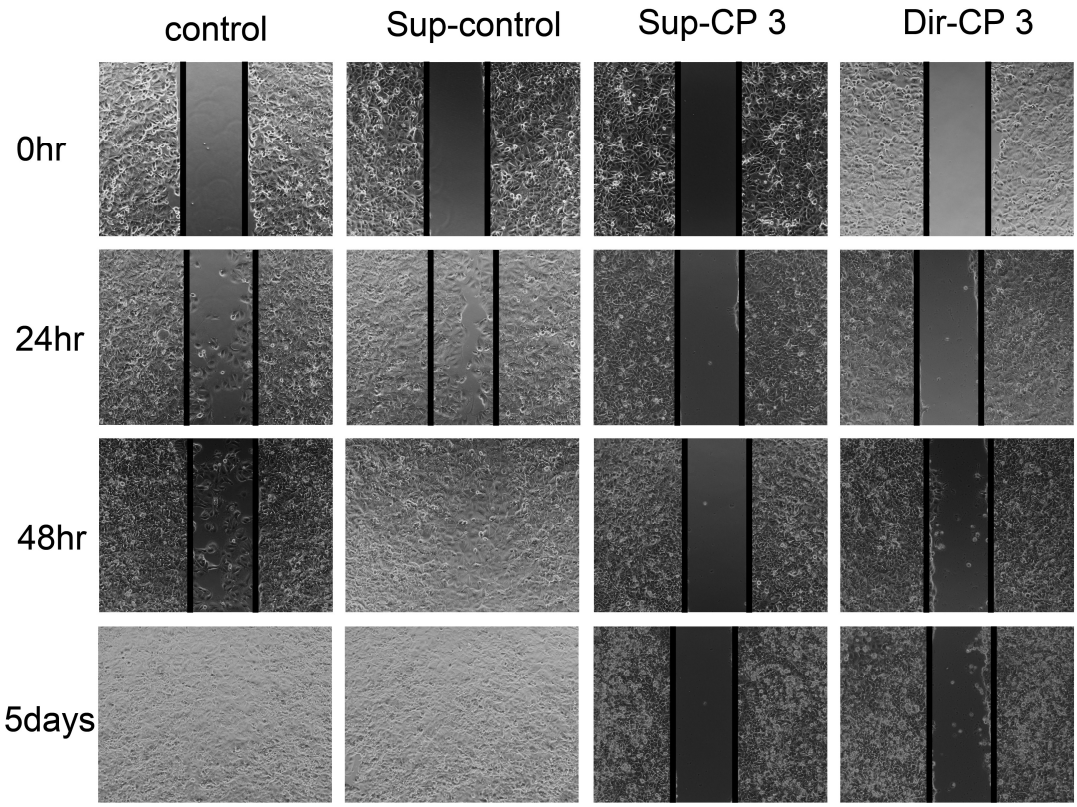

Fig. 2. Inhibitory effect of Cynanchum paniculatum (CP) extract on MCF-7 cell migration. Cell migration was inhibited by dir-CP and sup-CP treatment and shows that metastatic potential of MCF-7 cells was reduced by CP constituent directly and through modulating exosomal secretions

7.3 Effect of CP Extract and CP Extract Conditioned Medium on Migration of MDA-MB-231 Cells

In the case of migration of MDA-MB-231 cells, as shown in Fig. 3, the CP extract showed significant inhibition of the cell migration when it was directly applied to the cells (dir-CP). However, the sup-CP treatment showed a better ability to inhibit cell migration than dir-CP and this shows that more than inhibiting the cells, CP extract showed a better effect on exosome secretion or modulated their molecular pattern. Control and sup-control showed similar patterns like MCF-7 cells where cell migration was complete within 24 hrs (Fig. 3).

Our results clearly showed the inhibitory pattern towards cell migration in both the cells by direct effect and through the conditioned medium. Paeonol is the major constituent obtained from CP and there are several biological properties of paeonol reported already. Anti-cancer effect of paeonol against different types of cancer is studied by researchers and some of the mechanism by which paeonol inhibit cancer cell proliferation is through induction of apoptosis and anti-inflammation (Wang et al., 2020). The direct effect of CP was obvious from our previous study where we could find cytotoxicity of 4 different breast cancer cell types including the 2 of the cells used in this study. However, an appreciable effect of conditioned medium opens up several possible mechanisms, and all the relatable hypotheses point towards inhibition or dysregulation of the autocrine and paracrine mechanisms of the cancer cells through exosomes.

Among several pathways that compounds in CP could have targeted, TGF-β signaling was one of them. This might have resulted in impaired EMT signaling molecules that are secreted along with the exosomes and vital pathways for EMT and metastasis like Wnt pathway and Notch signaling might have been downregulated (Gonzalez and Medici, 2014). In our previous study, the inhibition of MMP-2 expression was observed in both the HER2 negative breast cancer cells and this was observed with conditioned medium treated cells. This observation was related to our study, as inhibition of MMP-2 plays a significant role in ECM modulation and facilitation of tumor invasion induced by EMT. This could have happened in our study, when the cells were treated with sup-CP, while sup-control facilitated the cell migration end EMT signaling with upregulated MMP-2 expression (Yang et al., 2021).

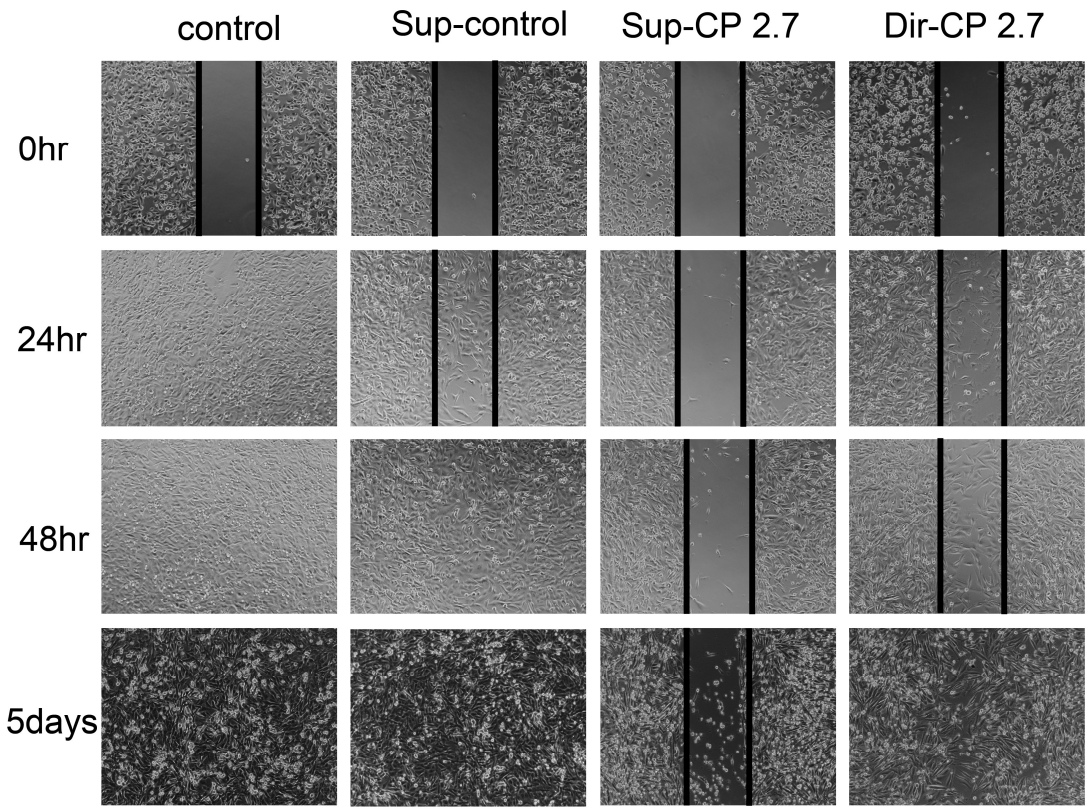

Fig. 3. Inhibitory effect of Cynanchum paniculatum (CP) extract on MDA-MB-231 cell migration. Both sup-CP and dir-CP showed equally good inhibition of cell migration and shows metastasis of MDA-MB-231 cells can be targeted for the triple-negative cell type of breast cancer treatment.

8. CONCLUSION

In conclusion, there are abundant literature on the biological properties of Cynanchum paniculatum and their anti-cancer properties are gaining the interest of researchers and oncologists. Our study with CP extract on cell migration is a significant observation and adds up to the scientific repository, that extract from CP containing several bioactive compounds has significantly inhibited the cell migration of MCF-7 and MDA-MB 231 cells, which are highly metastatic. Further, the conditioned medium obtained from respective cells, that are treated with CP extract showed equally good or better activity than direct CP treatment. This shows CP has the potential to interfere with the autocrine/paracrine signaling of these cells through exosomes. We assume that CP has modulated the biochemical profile of exosomes or reduced the number of exosomes, which carries signals for metastatic mechanisms in the in vitro condition. Hence, our observations report the ability of CP and its compounds to inhibit breast cancer metastasis and their invasion.

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