Indian Journal of Natural Products and Resources
Vol. 12(1), March 2021, pp 34-42

Inhibition of metastasis and suppression of pERK1/2 and pFAK expression by *Solanum xanthocarpum* crude extracts in human lung cancer cell line A549 *in vitro*

Mandadi Narsimha Reddy and Mital Bhatt*

Department of Biosciences, Veer Narmad South Gujarat University, Udhana Magdalla Road, Surat 395007, Gujarat, India

Received 21 May 2019; Revised 06 November 2020

Solanum xanthocarpum is an important part of natural remedies for lung in Ayurveda. Lung cancer metastasis is a major challenge in the present time. Expressions and activation of focal adhesion kinase (FAK) and extracellular receptor kinases 1 and 2 (ERK1/2) are key checkpoints in cancer which lead to an invasion, epithelial-mesenchymal transition (EMT) and metastatic progression. In this study, the efficacy of the whole plant and root crude extracts prepared in ethyl acetate and chloroform on highly metastatic lung cancer cell line A549 was evaluated. The effect of crude extracts on cell migration was scrutinized using transwell migration assessment. The phosphorylated forms of proteins, pFAK and pERK1/2 levels, were estimated using ELISA. Zymography technique was utilized to evaluate the activities of MMP-2 and MMP-9 proteins. The EMT markers, vimentin and N-cadherin gene expressions were analyzed using semi-quantitative RT-PCR. The extracts significantly reduced single-cell migration. The expression levels of pFAK and pERK1/2 notably decreased and FAK and ERK1/2 remained unchanged. Moreover, gelatinase activity, vimentin and N-cadherin expression were also significantly reduced in treated cells compared to untreated cells. The crude extracts of *S. xanthocarpum* impaired the metastatic progression of A549 cells *in vitro.*

Keywords: A549, EMT, ERK, FAK, *Kantakari*, Lung cancer, Metastasis, *Solanum xanthocarpum*.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 125/00, A61P 35/00

Introduction

Solanum xanthocarpum Schrad. & H. Wendl. (syn. *S. virginianium* L.), is a traditional and scientifically acclaimed medicinal plant for its numerous therapeutic effects and immunomodulatory actions on lung-related ailments. *S. xanthocarpum* is known as *Kantakari* in Sanskrit[1-4]. The plant is an integral part of many alternative medicinal practices in Indian villages and folk cultures. Numerous ancient scriptures in ancient sciences such as *Ayurveda*, *Unani*, and *Siddha* mention the plant for its medicinally significant properties. The whole plant and different parts of the plant are useful in Ayurvedic formulations, such as *Dasamoolarishta*, *Chavanaprasha*, *Vyaghri tailam, Vyaghri ghrtam, Vyaghriyadi kwatha,* etc[1]. Interestingly, even the fume of the seeds has beneficial effects[5]. Literature is available for its anti-inflammatory and immunomodulatory properties apart from anti-asthmatic, anti fertility[4], antihyperglycaemic and anti-larvae[4] activities. *S. xanthocarpum* has extremely beneficial efficacy in respiratory tract related ailments[3,4]. Tribal practitioners in the South Gujarat region of India use the plant in the therapy of cancer. The plant is reported to have a wide variety of secondary metabolites such as alkaloids, phenolics, flavonoids, sterols, saponins and their glycosides, carbohydrates, fatty acids, tannins, and amino acids[2,6]. The secondary metabolites namely stigmasterol, carpesterol, diosgenin and lupeol are reported to be present in the plant and show immunomodulatory and anti-inflammatory effects[3,7]. In a previous study, authors reported *in silico* interaction of Lupeol, one of the active phytochemicals present in the plant, to ERK pathway proteins[8].

Metastasis is the most devastating hallmark of cancer with more than 90% of deaths worldwide[9-13]. Antimetastatic agents' development and resource findings are the most decisive in cancer research[14]. In the present time, back to nature approach is highly accepted. A large amount of plant-based molecules are developed and approved for the anticancer and antimetastatic therapies[15,16]. Many medicinal plants, *Ayurvedic*, *Siddha*, *Unani*, and other traditional formulations have proved their potential for cancer inhibition, suppression of metastasis, and easing many more illnesses for centuries[17].

*Correspondent author
Email: mital.bhatt14@gmail.com

Lung cancer metastasis is very difficult to handle[18]. A majority of lung cancer patients (80-85%) are diagnosed with non small cell lung carcinoma (NSCLC), which is highly metastatic and thus, has limited therapeutic options[19]. The Ras-Raf-MEK-ERK cascade is a major pathway and highly activated in NSCLC. Once activated, extracellular receptor kinases (ERK1 and ERK2) can facilitate epithelial to mesenchymal transition (EMT) by increasing the expression of EMT transcription factors, cell motility and invasion via increased repression of E-cadherin and activation of N-cadherin and matrix matallo proteinases MMPs[20]. Mitogen-activated protein kinase kinase (MAP2K or MEK) MEK1 and 2 carry out the phosphorylation of their sole target ERK1/2. However, the other factors, such as activated focal adhesion kinase (pFAK) can also activate ERK1/2[21] FAK plays a major role in cancer cell proliferation, survival, and metastasis by dynamically responding to changes in ECM, growth factors, and nutrient availability[22].

On the other hand, many reports of the therapeutic potential of *S. xanthocarpum* on respiratory and bronchial disorders exist, which provides a preliminary rationale for analyzing the plant's effects on a lung cancer cell. Thus, A549, a highly metastatic NSCLC cell line was selected for the analysis of the plant's effects. 5-Fu, a classical anticancer drug, was used as a standard. In a previous study, the authors reported the superoxide anion and nitrite reduction potential of the plant along with the collective migration ability of the A549 cells[2,23]. In the present study, the effects of the crude extracts of the whole plant and root were analyzed on the single-cell migration and the expression levels of the checkpoint proteins of the metastasis, FAK, pFAK, ERK, and pERK. The gelatinase activity is a measure of invasiveness in the cancer cells. The efficacy of the plant on the MMP-2 and MMP-9 proteins was accessed. Expression levels of EMT marker genes – vimentin and N-cadherin levels were scrutinized.

Materials and Methods

Plant collection and identification

Whole plants of *S. xanthocarpum* were collected from the Girls' hostel campus of Veer Narmad South Gujarat University, Surat, India during the March-April period of 2015. The identity of the collected plant was authenticated by Prof. M. H. Parabia, Advisor, Bapalal Vaidya Botanical Research Centre (BVBRC), VNSGU, Surat. A voucher specimen (BVBRC1204) was deposited at BVBRC, Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India.

Plant extracts preparation and Cell culture

The crude extracts of *S. xanthocarpum* whole plants and roots were prepared as described previously[2]. Five different dilutions of all the extracts (50, 100, 200, 300, 500 µg/mL) were used for the assays. Extracts of the whole plant and roots with ethyl acetate were abbreviated as SEW, SER respectively and chloroform extracts of the whole plant and roots were abbreviated as SCW, SCR respectively[2]. The human lung carcinoma A549 cells were obtained from NCCS, Pune and maintained in humidified conditions (5% CO_2 and 37 °C). The cells were propagated up to 70-80% confluence for each assay.

Transwell cell migration assay

Effect of the plant crude extracts on the migration ability of A549 cells was observed using transwell chamber migration assay. Exactly 200 µL of the serum-less medium along with 1×10^4 cells, consisting of an appropriate extract were seeded in the polycarbonate transwell membrane assay plate, upper chamber consisting pore size 8 µm (TCP152, Himedia, growth area of the well = 0.33 cm^2). Lower wells were filled with 750 µL of 10% FBS containing a medium. The plate was incubated for 12 hours at 37 °C in 5% CO_2. More than five different fields were analyzed and migrated cells were counted (Corning life science, 2008). Results were expressed as percentage migration considering the field of view of the microscope. The equations given below were used for calculating % migration.

$$\text{Number of migrated cells} = \frac{\text{Avg migrated cells}}{\text{Field of view}} \times \text{area of well}$$

$$\%\ \text{Migration} = \frac{\text{Number of migrated cells}}{\text{Number of inserted cells}} \times 100$$

Cell lysis and total protein extraction

Cell lysates for protein expression analysis were prepared for cells treated with *S. xanthocarpum* plant extracts, SCR, SCW, SER, SEW (300 and 500 µg/mL), and untreated cells (control). Cells were scraped off their respective plates re-suspended, centrifuged, and washed twice using chilled PBS. Cell extraction buffer (Invitrogen, FN0011) supplemented with PMSF and protease inhibitor cocktail was added into cell palate after discarding the supernatant. The complete process of vortexing the samples for

30 minutes with 10 minutes intervals was performed on ice. Then samples were centrifuged at 13000 rpm at 4 °C for 10 minutes. Lysates were then stored at -40 °C for further use.

Expression analysis of total FAK, ERK1/2, pFAK and pERK1/2 using ELISA

The samples were checked for protein contents using the Lowry method[24]. Cell lysates were diluted as per total protein contents. The effects of treatments on quantitative levels of crucial proteins were analyzed using a respective ELISA kit as per the instructions given in the kit. Anti-pan FAK and anti-phsopho- FAK (Y397) antibody-based ELISA kit (PEL-FAK-Y397, RayBiotech) was used For FAK and pFAK estimation. For pan ERK1/2 and pERK1/2, the ELISA kit (PEL-Erk-202-T, Ray Biotech) comprising anti-pan-ERK1/2 for total Erk1/2, human, mouse and rabbit anti-phospho-Erk1 (Thr202/Tyr204) and human, mouse and rabbit anti-phospho-Erk2 (Thr185/Tyr187) was used. Absorbance was measured at 450 nm.

Gelatine zymography

Gelatinase activities of cells were observed using zymography. MMP-2 (ENZ-769) and MMP-9 (ENZ-438) standards were obtained from GenxBio. The complete procedure was followed as available in the protocol with some modifications[25]. SDS PAGE gel (8% Resolving) was co-polymerised with 0.1% gelatine. The 6.5% stacking gel comprising an appropriate lane was made. After adding an equal amount of protein samples in the appropriate lanes, the gel was subjected to electrophoresis. After that, the gel was washed and incubated for 36 hours. Next, 0.25% Coomassie brilliant blue was used to stain the gel. The gel was de-stained with acetic acid and ethanol mixture. Bands were observed and analyzed using Image J 1.46r (NIH, USA).

Determination of expression levels of EMT markers-Vimentin and N-cadherin

Cellular RNA was isolated using the RNA extraction kit (MB602, Himedia, India) and cDNA synthesis from the extracted RNA was carried out using cDNA synthesis kit (MBT076, Himedia, India) according to the manufacturer's instructions. Relative expression of EMT markers (Vimentin-F-GAGA ACTTTGCCGTTGAAGC R-TCCAGCAGCTTCC TGTAGGT, N-cadherin-F-ACTGGGCCAGGAGC TGACCA R-GTGCCCTCAAATGAAACCGGGCT) was determined by the semiquantitative RT-PCR method and the absorbance relative to control was analyzed by ImageJ ver.1.46r (NIH, USA).

Results

Transwell cell migration

Cell migration towards chemo-attractant is one of the most important properties of metastasizing cells and transwell migration assay is an accurate method for scrutinizing single-cell migration capacity. In the present study, Fig. 1a shows the number of cells migrated from the upper chamber to the lower chamber, towards the chemo-attractant during 15 hours under the influence of the testing samples, plant extracts. The total percentage of migration was found for each of the wells with the counted cells. The results are presented in Fig. 1b for the plant extract-treated cells. It indicates the effect of the plant extracts on the migration of the cells. Control well exhibited the heavily migrated cells. The well treated with 5-Fu did not show a migrated cell. The plant extract treatment at 300 and 500 µg/mL affected cells migration percentage and exhibited a huge impact on

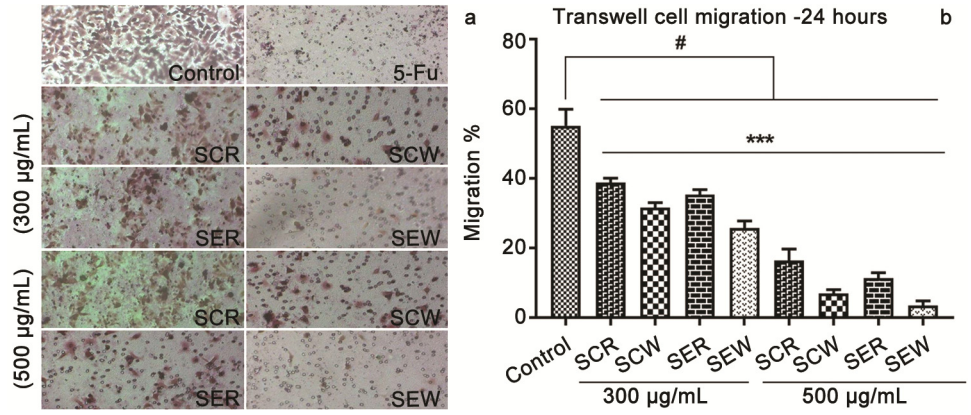

Fig. 1 — A549 cells migration, a) Control and treated with the plant extracts and b) A549 cells migration (in %)–Control and the plant extract (300 and 500 µg/mL) treated cells. Statistical significance is shown as *p <0.05, ***p <0.005, #p <0.001.

cell migration. SEW 300 µg/mL treated cells showed the most impaired transwell migration. The migration was significantly reduced in the cells treated with crude extracts at 500 µg/mL concentration.

Estimation of the proteins (FAK, pFAK, ERK, pERK)

ELISA estimates expression levels of the crucial proteins in the cell lysates. The present study aimed to examine the levels of key proteins in the A549 cells metastasis. Fig. 2 explains the effects on the amount of proteins [FAK and pFAK] quantified in the differently treated cells with the plant extracts, SCR, SCW, SER, SEW (300 and 500 µg/mL). The results suggest that the plant extracts show a noticeable decrease in FAK and pFAK expression. It is important to note that total FAK is decreased in SCW, SER, and SEW treated cells, whereas SCR treated cells showed increased FAK. Besides, no dose-dependence in the decrease of total FAK was observed, except in the SEW treated cells. In the case of pFAK concentration, except SCR treatment, SCW, SER, and SEW showed a dose-dependent effect on the A549 cells. SEW decreased pFAK secretion more significantly.

ERK1/2 is the key protein complex in metastatic progression. The activation, phosphorylation of the proteins is the crucial checkpoint in many biological events. Fig. 3 shows the effects of the plant extracts on ERK1/2 and pERK1/2 levels in the A549 cells. It is clearly observable, that pan ERK1/2 level is not affected significantly, but pERK1/2 levels are decreased drastically under the treatment of all the plant extracts. The plant shows effects in a dose-dependent manner on pERK1/2 expression. The lowest expression was observed in SEW 500 µg/mL treated cells.

Zymography (MMP-2 and MMP-9 estimation)

Determination of MMP-2 and MMP-9 - gelatinase enzymes provide an important understanding of the invasiveness of the cells. In the present study, Zymography technique based results are obtained. Fig. 4 shows the zymogram in the treated A549 cells. Fig. 4a shows the MMP-2 and MMP-9 expression in control and the plant extract treated cell lysates. Co

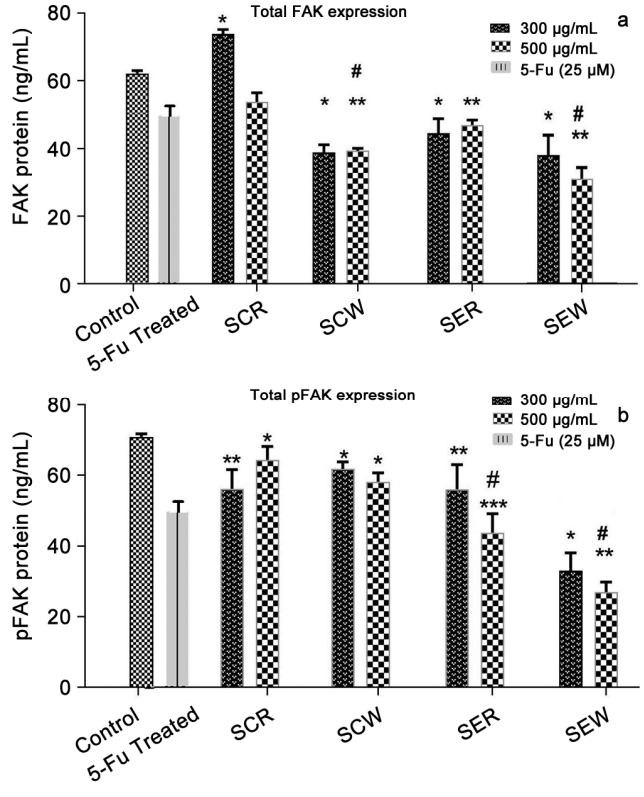

Fig. 2 — Total FAK and pFAK expression in A549 cells treated with the plant extracts, a) Total FAK expression in the cells treated with the crude extracts and b) pFAK expression in the treated cells. Statistical significance is shown as **p* <0.05, ***p* <0.01,*** *p* <0.005, #*p* <0.001.

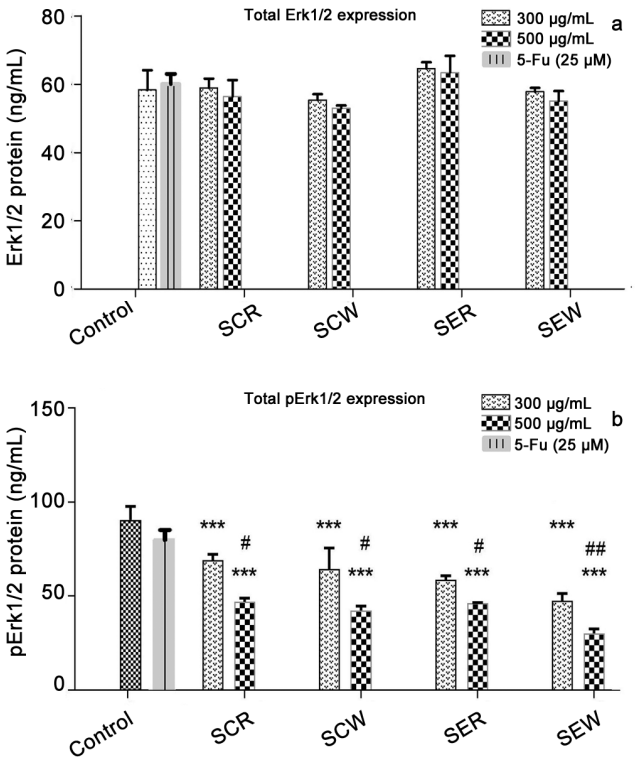

Fig. 3 — Total ERK1/2 and pERK1/2 expression in A549 cells treated with the plant extracts, a) Total ERK1/2 expressions in the cells treated with the crude extracts and b) pERK1/2 expression in the treated cells. Statistical significance is shown as *** *p* <0.005, #*p* <0.001.

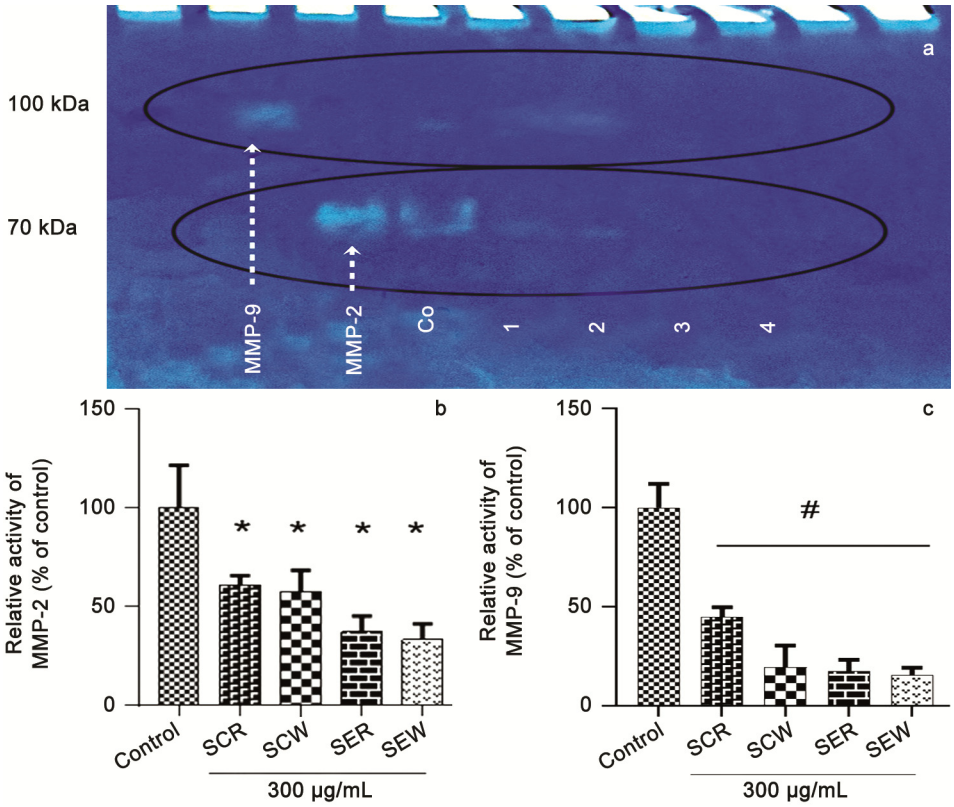

Fig. 4 — Zymography: A549 cells treated with plant extracts a) Zymogram- gelatinase activity is shown as colorless bands, b) MMP-2 activity quantified using ImageJ in the treated cells, and c) MMP-9 activity quantified by ImageJ in the treated cells. Statistical significance is shown as *p <0.05, **p <0.01, ***p <0.005, #p <0.001.

=Control, 1= SCR, 2=SCW, 3= SER, 4= SEW treated cell lysates. The decolourized area of the gel indicates the gelatinase activity. The decolourized bands were measured with the help of ImageJ 1.46r (NIH, USA) and intensities were calculated relative to control. Fig. 4b & c shows the MMP-2 and the MMP-9 activity in the cells treated with 300 µg/mL of SCR, SCW, SER and, SEW respectively.

Semi-quantitative RT-PCR for detection of vimentin and N-cadherin expressions

EMT is a major hallmark through which cancer cell disseminates from the primary site and migrate. Expression levels of mesenchymal markers- Vimentin and N-cadherin were examined for the cells treated with the extracts. Fig. 5a explains the gene expression of vimentin and N-cadherin in **(C)** Control cells, **(1)** SCR 300 µg/mL, **(2)** SCW 300 µg/mL, **(3)** SER 300 µg/mL, **(4)** SEW 300 µg/mL, **(5)** SCR 500 µg/mL, **(6)** SCW 500 µg/mL, **(7)** SER 500 µg/mL, and **(8)** SEW 500 µg/mL treated cells. The expression was semi-quantified using Image J 1.46r, the expression of the gene relative to control was presented in Fig. 5b & c. Vimentin expression was significantly low in all the treated cells. N-cadherin also reduced in a significant amount. More decrease was observed in the cells treated with a higher concentration of the plant extracts. SEW again showed the maximum decrease in the expression of both genes.

Discussion

The major characteristic of metastasis is cell migration. Evaluation of A549 cell migration under the influence of the plant in the previous experiment showed the efficacy of the crude extracts. Especially SEW treatment reduced the wound healing- collective cell migration effectively[22]. While wound healing assay is the most convenient and accurate method to analyze cell mass or group migration, transwell cell migration provides a step further to study single-cell motility (mesenchymal and amoeboid migration)[26]. Fig. 1a is a representation of cells migrated transwell in the direction of chemo-attractant. The cells in untreated conditions clearly show the ability of A549 cells to migrate. In contrast, a huge decrease in cell migration in the SCR, SCW, SER, and SEW (300 µg/mL) treated wells illustrate the clear conditions. Despite maintaining the viability, cells did

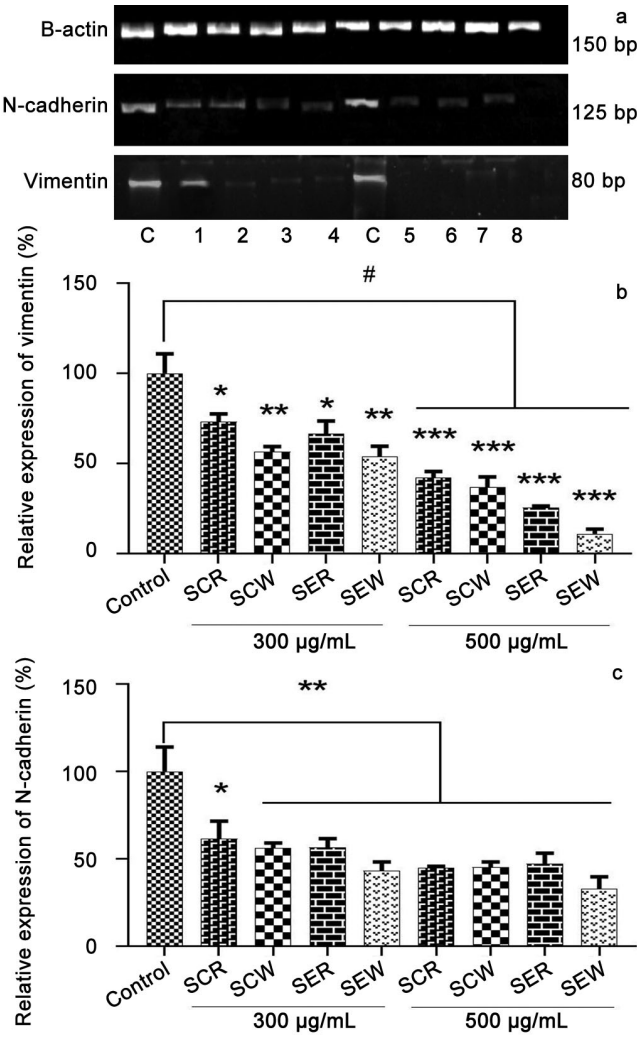

Fig. 5 — Gene expression analysis: vimentin and N-cadherin, a) Gene expression in differently treated cells, b and c) Semi-quantitative representation of the genes expressed. Statistical significance is shown as *p <0.05, **p <0.01, ***p <0.005, #p <0.001

not manage to migrate through the membrane, which is a critical and noteworthy observation. Fig. 1b shows the % migration of the cells treated with two different dilutions of the crude extracts. SCW and SEW treatment showed the impact and the higher dilution treatments exhibited a drastic decrease in the ability of the cells to migrate transwell. A huge cell migration reduction in the whole plant treatment is a salient finding of the study. Interestingly, no cytotoxicity was observed at this concentration of plant[2]. Many reports are available suggesting the anti-fertility properties of the plant[3,4]. It is reported that the plant seed shows the depletion of sperm motility in animals[27]. Although sperm motility depends on major sperm protein (MSP) and has no biochemical or structural relationship to actin-myosin functions, a study on *C. elegans* showed that the 'molecular clutch' principles apply to the MSP cytoskeleton system also along with actomyosin-based cell motility[28]. Hence, the clutch concept remains robust in cell motility for different cytoskeletons. However, the possible correlations between cancer cells and sperm motility remain open for speculations.

FAK and ERK signalling events play active roles in actin-myosin cytoskeleton modulation and motility[29-31]. FAK and autophosphorylated FAK at tyrosine 397 serve as the prognostic markers of metastatic cancer cells[32-34]. FAK and pFAK (y397) estimation in the cells treated with the plant extracts was carried out. The plant extracts SCW, SER, and SEW showed a significant decrease in total FAK as well as pFAK expression as shown in Fig. 2a & b. SEW treatment decreased the FAK expression the most. These results correlate with the anti-migratory effects of the plants.

The observations were stupendous in the case of ERK and pERK expressions**.** Fig. 3a presents the total ERK1/2 expression in the cells when treated with the plant extracts. It is clear from the results that ERK1/2 expression remains unaffected in each treated group. Nevertheless, the results shown in Fig. 3b are intriguing. The expression of phosphorylated ERK1/2 decreased in all the cells treated with the plant extracts. ERK1/2 phosphorylation is the process, which is carried out by many possible activators. ERK1/2 is a downstream component of the Ras-Raf-MEK-ERK cascade and its activation leads to many other sequential events[35]. Upon activation by Ras, Raf triggers MAP2K (MEK1/2). MEK1/2 are the only identified ERK1/2 activators that can phosphorylate ERK1/2 on both tyrosine and threonine residues, thus named dual-specificity protein kinases. Their only substrate is ERK1/2 [36]. Hence, the decreased pERK1/2 instantly correlates with the less activity of MEK. ERK activation is also affected by other activators such as FAK action, reactive oxygen species, nitrites etc[37-39]. In the present results of FAK and pFAK expression, a reduction was observed when the cells were treated with the plant extracts. Thus, reduced activity of FAK also might have contributed to depletion in ERK1/2 phosphorylation. More than a two-fold decrease in the expression of pERK was noticed in the cells treated with 500 µg/mL dilution of all the crude extracts. SEW (500 µg/mL) treated cells reduced pERK1/2 levels up to three folds. These

results showed a salient connection with the other observations in the study, viz. superoxide anion and nitrite reduction, collective cell migration reported earlier and FAK-pFAK expressions[2].

Successful inhibition of FAK and ERK1/2 activation can directly reflect upon the anti-invasive properties of the cells as the pFAK and pERK1/2 are positively correlated with MMP-2 and MMP-9 activity[40-42]. MMP-2 and MMP-9 gelatinase activity is closely related to apoptosis, ECM remodelling, migration, and invasion[43]. Thus, following the FAK and ERK1/2 estimation, Zymography was utilized for checking the activity of gelatinase enzymes MMP-2 and MMP-9. Fig. 4a shows the gelatinase activity bands wherein, standards of MMP-2 & 9 are shown in the first two tracks. MMP-2 bands are clearly observed in the plant extract-treated cells, while MMP-9 bands are inconspicuous. A drastic decrease in MMP-2 activity was observed in the bands in comparison to control. Fig. 4b & c show the gelatinase activity measured in comparison to control. The bands in ethyl acetate extract-treated cells showed fewer gelatinase activities.

All of these proteins pFAK, pERK, MMP-2, and MMP-9 are associated with various signalling pathways such as TGF-β signalling, receptor tyrosine kinase, wnt-notch signalling etc., which all lead to epithelial-mesenchymal transition (EMT)[44]. The transition of epithelial to mesenchymal cell types is a reversible process and is called the mesenchymal-epithelial transition (MET). EMT is the central regulator of metastatic progression[45]. Typically, epithelial cells are immobile, organized in a layer, with well-defined junctions, in the fashion of apical-basal polarity. The transition of these cells into mesenchymal cell types enables them to exhibit motility and invasiveness by adopting an elongated shape with front-back polarity[46,47]. EMT is the fundamental process found in embryogenesis, where it is required for organogenesis. In adults, the process is exploited in a controlled manner for wound healing or in the case of cancer[47]. High expression levels of N-cadherin and vimentin are signatures of a mesenchymal phenotype and increased during EMT in various cancer cells including NSCLC[47-50]. In the present study, confirmation of the levels of vimentin and N-cadherin provided an insight into the *S. xanthocarpum* extracts' effects on A549 cells. Semi-quantitative RT-PCR was used to evaluate the levels of the mRNA expressed for vimentin and N-cadherin after an equal number of cycles in untreated versus treated cells. β- actin gene was used as a reference housekeeping gene. Fig. 5 shows the expression of the cells treated with the plant extracts and control cells. It is evident from the result that, both the mesenchymal markers gene expression reduced supremely. For semi-quantitative estimation, ImageJ 1.46r plot values were utilized for the calculation of expression relative to β- actin. The expression data suggest the decrease in vimentin and N-cadherin expression levels in comparison to control following the treatment of plant extracts Fig. 5b & c.

Vimentin is an intermediate filament found in mesenchymal cells. It is important to note here that the vimentin expression is necessary for the cancer cells to maintain the ERK1/2 phosphorylated and the pERK1/2 is essential to maintain the fidelity of signalling pathways leading to proliferation and metastasis events. Additionally, vimentin does not bind with ERK1/2 but only binds to the phosphorylation lip of pERK1/2, covers and protects it from dephosphorylation[49]. In the present study, increased ERK1/2 and decrease in the pERK expression correlates with the decrease in the expression of vimentin. Hence, this suggests a definite loss of vimentin–pERK reciprocal complex. In the same way, N-cadherin is also associated with sustained MAPK-ERK activation by FGF-2. Cadherin switch, i.e., E-cadherin to N-cadherin is a crucial hallmark; and increased N-cadherin is associated with escape from anoikis, cell migration, and invasion[47]. Thus, a decrease in two major mesenchymal markers expression levels are indicators of the plant's action on the metastatic potential of the A549 cells. The plant extracts modulate several events through inhibition of pFAK, pERK, MMP-2, MMP-9, vimentin and N-cadherin expressions and thus affecting the migratory ability of the cells. Especially, the whole plant crude extracts showed outstanding efficacy in all the analyses. Thus, the traditionally important and noted plant, *S. xanthocarpum*, has significant efficacy on the metastatic cell line of lung cancer. Further investigations on the other molecular and metabolical pathways within the cells treated with the plant can provide promising outcomes in the direction of antimetastatic treatments.

Conclusion

Solanum xanthocarpum, a member of Dashamularisth and numerous other Ayurvedic formulations is well known for its uses in different

aliment. However, its potential use on lung cancer is not reported. In the present study, anti-metastatic properties of the roots and whole plant crude extract were evaluated against lung cancer (A549 cells). The whole plant crude extracts treatments significantly hamper cell migration, reduce pERK1/2 and pFAK, decrease expression levels of EMT markers vimentin, N-cadherin, deplete the activities of MMP-2 and 9. Hence, the plant shows promising therapeutic potential for metastatic progression in human lung cancer.

Conflict of interest

The authors declare no conflict of interest.

References

1 Roshy C, Rangasamy I and Patgiri B J, Therapeutic potentials of kantakari *(Solanum xanthocarpum* Schrad. & Wendl.), *Ayurpharm Int J Ayur Alli Sci* 2012, **1**(2), 46-53.

2 Bhatt M H and Reddy M N, Antimetastatic, superoxide anion and nitric oxide reduction potential of *Solanum xanthocarpum* on human lung cancer cell line A549, *Indian J Tradit Know*, 2018, **17**(3), 468-473.

3 Reddy N M and Reddy R N, *Solanum xanthocarpum* chemical constituents and medicinal properties : A review, *SCh Acad J Pharm*, 2014, **3**(2), 146–49.

4 Singh O M and Singh T P, Phytochemistry of *Solanum xanthocarpum*: An amazing traditional healer, *J Sci Ind Res*, 2010, **69**(10), 732-740.

5 Pandey H P, Seed fume of *Solanum surattense*: A traditional panacea for teeth and gums, *Indian J Tradit Knowle*, 2004, **3**, 206-207.

6 Neelapu N, Naikwadi G D, Muvvala S and Jadhav K V, A preliminary phytochemical investigation on the leaves of *Solanum Xanthocarpum*, *Int J Res Ayurveda Pharm*, 2011, **2**(3), 845–850.

7 Sultana S, Asif H M, Nazar H M I, Akhtar N, Rehman J U, *et al.*, Medicinal plants combating against cancer--a green anticancer approach, *Asian Pac J Cancer Prev*, 2014, **15**(11), 4385-4394.

8 Bhatt M H, Prajapati C K and Reddy M N, *In silico* docking studies of Lupeol with MAPK pathway proteins- Raf-1, MEK & ERK, *J Exp Ther Oncol*, 2017, **12**, 137-140.

9 Bodenstine T M and Welch D R, Metastasis Suppressors and the tumor microenvironment, *Cancer Microenviron*, 2008, **1**(1), 1-11

10 Hanahan D and Weinberg R A, Hallmarks of cancer: The next generation, *Cell*, 2011, **144**(5), 646-674.

11 Valastyan S and Weinberg R, Tumor metastasis: Molecular insights and evolving paradigms, *Cell*, 2011, **147**(2), 275-292.

12 Seyfried T N, Flores R E, Poff AM and D'Agostino D P, Cancer as a metabolic disease: Implications for novel therapeutics, *Carcinogenesis*, 2014, **35**(3), 515-527.

13 Chaffer C L, Weinberg R A, A perspective on cancer cell metastasis, *Science*, 2011, **331**(6024), 1559–1564.

14 Iiizumi M, Liu W, Pai S K, Furuta E and Watabe K, Drug development against metastasis-related genes and their pathways: A rationale for cancer therapy, *Biochim Biophys Acta*, 2008, **1786**(2), 87-104.

15 Greenwell M and Rahman P K S M, Medicinal Plants: Their use in anticancer treatment, *Int J Pharm Sci Res*, 2015, **6**(10), 4103-4112.

16 Rane M H, Sahu N K, Ajgoankar S S, Teli N C and Verma D R, A holistic approach on review of *Solanum Virginianum* L., *Int J Pharm Sci*, 2014, **3**(3), 1–4.

17 Thanzami K, Pachuau V L T, Lalremruati C and Kakoti B B, Phytochemical screening, anti-oxidant and anti-inflammatory activities of ethyl acetate extract of *Combretum punctatum var. squamosum*, *Indian J Nat Prod Resour*, 2020, **11**(1), 38–45.

18 Zappa C and Mousa S A, Non-small cell lung cancer: Current treatment and future advances, *Transl Lung Cancer Res*, 2016, **5**(3), 288-300.

19 Lee S H, Jaganath I B, Manikam R and Sekaran S D, Inhibition of Raf-MEK-ERK and Hypoxia pathways by *Phyllanthus* prevents metastasis in human lung (A549) cancer cell line, *BMC Complement Altern Med*, 2013, **13**(1), 271.

20 Lamouille S, Xu J and Derynck R, Molecular mechanisms of epithelial–mesenchymal transition, *Nat Rev Mol Cell Biol*, 2014, **15**(3), 178-196.

21 Hu J, Liao H and Ma Z, Focal adhesion kinase signaling mediated the enhancement of osteogenesis of human mesenchymal stem cells induced by extracorporeal shockwave, *Sci Rep*, 2016, **6**, 1-12.

22 Zhang J and Hochwald S N, The role of FAK in tumor metabolism and therapy, *Pharmacol Ther*, 2014, **142**(2), 154-163.

23 Kim N H, Kim S K, Kim D S, Zhang D, Park J A, *et al.*, Anti-proliferative action of IL-6R-targeted antibody tocilizumab for non-small cell lung cancer cells, *Oncol Lett*, 2015, **9**(5), 2283-2288.

24 Lowry O H, Rosebrough N J, Farr A L and Randall R J, Protein measurement with the Folin phenol reagent, *J Biol Chem*, 1951, **193**(1), 265–275

25 Frankowski H, Gu Y H, Heo J H, Milner R and del Zoppo G J, Use of gel zymography to examine matrix metalloproteinase (Gelatinase) expression in brain tissue or in primary glial cultures, *Methods Mol Biol*, 2012, **814**, 221-233.

26 Justus C R, Leffler N, Ruiz-Echevarria M and Yang L V, *In vitro* cell migration and invasion assays, *J Vis Exp JoVE*, 2014, **88**, 51046.

27 Thirumalai T, David E, Therasa S V and Elumalai E K, Effect of *Solanum surattense* seed on the oxidative potential of cauda epididymal spermatozoa, *Asian Pac J Trop Biomed*, 2012, **2**(1), 21-23.

28 Svitlana H, Mezanges X, Batchelder E and Plastino J, Extending the molecular clutch beyond actin-based cell motility, *New J Phys*, 2014, **16**(10), 105012.

29 Tang D D and Gerlach, B D, The roles and regulation of the actin cytoskeleton, intermediate filaments and microtubules in smooth muscle cell migration, *Respir Res*, 2017, **18**(1), 54.

30 Fabry B, Klemm A H, Kienle S, Schäffer T E and Goldmann W H, Focal adhesion kinase stabilizes the cytoskeleton, *Biophys J*, 2011, **101**(9), 2131-2138.

31 Tanimura S, Hashizume J, Arichika N, Watanabe K, Ohyama K, *et al.*, ERK signaling promotes cell motility by inducing the localization of myosin 1E to lamellipodial tips, *J Cell Biol Histol*, 2016, **214**(4), 475-489.

32 Thanapprapasr K, Nartthanarung A, Thanapprapasr D and Jinawath A, PFAK-Y397 overexpression as both a prognostic and a predictive biomarker for patients with metastatic osteosarcoma, *PLoS ONE*, 2017, **12**(8), 1–17.

33 Grigera P R, Jeffery E D, Martin K H, Jeffrey S, Hunt D F, *et al.*, FAK phosphorylation sites mapped by mass spectrometry, *J Cell Sci*, 2005, **118**(21), 4931–4935.

34 Salem I, Alsalahi M, Chervoneva I, Aburto L D, Addya S, *et al.*, The effects of CEP-37440, an inhibitor of focal adhesion kinase, *in vitro* and *in vivo* on inflammatory breast cancer cells, *Breast Cancer Res*, 2016, **18**(1), 37.

35 Roberts P J and Der C J, Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer, *Oncogene*, 2007, **26**(22), 3291–3310.

36 Wortzel I and Seger R, The ERK cascade: Distinct functions within various subcellular organelles, *Genes Cancer*, 2011, **2**(3), 195-209.

37 Hayashida T, Wu M H, Pierce A, Poncelet A C, Varga J, *et al.*, MAP-Kinase activity necessary for TGF beta1-stimulated mesangial cell type I collagen expression requires adhesion-dependent phosphorylation of FAK tyrosine 397, *J Cell Sci*, 2007, **120**(23), 4230–4240.

38 McCubrey J A, Steelman L S, Chappell W H, Abrams S L, Wong E W, *et al.*, Roles of the Raf/Mek/Erk pathway in cell growth, malignant transformation and drug resistance, *Biochim Biophys Acta*, 2007, **1773**(8), 1263-1284.

39 Saud K, Herrera M R and Von B R, Pro- and anti-inflammatory cytokines regulate the ERK pathway: implication of the timing for the activation of microglial cells, *Neurotoxicity Res*, 2005, **8**(3–4), 277–287.

40 Yang M H, Nickerson S, Kim E T, Liot C, Laurent G, *et al.*, Regulation of RAS oncogenicity by acetylation, *Proc Natl Acad Sci U S A*, 2012, **109**(27), 10843-10848.

41 Moulik S, Pal S, Biswas J and Chatterjee A, Role of ERK in modulating MMP 2 and MMP 9 with respect to tumour invasiveness in human cancer cell line MCF-7 and MDA-MB-231, *J Tumor*, 2014, **2**(2), 87-98.

42 Sen T, Ganguly K K, Biswas J and Chatterjee A, Focal adhesion kinase induces matrix metalloproteinase-2 by involving α5β1-mediated signaling in breast cancer cell, MCF-7, *Acta Med Int*, 2015, **2**, 29-39.

43 Kunz P, Sähr H, Lehner B, Fischer C, Seebach E, *et al.*, Elevated ratio of MMP2/MMP9 activity is associated with poor response to chemotherapy in osteosarcoma, *BMC Cancer*, 2016, **16**(1), 1-10.

44 Gonzalez D M and Medici D, Signaling mechanisms of the epithelial-mesenchymal transition, *Sci Signal*, 2014, **7**(344), 8.

45 Ye X and Weinberg R A, Epithelial-Mesenchymal plasticity: A central regulator of cancer progression, *Trends Cell Biol*, 2015, **25**(11), 675-686.

46 Gavert N, Ben-Ze'ev A, Epithelial–mesenchymal transition and the invasive potential of tumors, *Trends Mol Med*, 2008, **14**(5), 199–209.

47 Pradella D, Naro C, Sette C and Ghigna C, EMT and stemness: Flexible processes tuned by alternative splicing in development and cancer progression, *Mol Cancer*, **16**(1), 2017, 1-19.

48 Heerboth S, Housman G, Leary M, Longacre M, Byler S, *et al.*, EMT and tumor metastasis, *Clin Transl Med*, 2015, **4**(1), 6.

49 Perlson E, Michaelevski I, Kowalsman N, Ben-Yaakov K, Shaked M, *et al.*, Vimentin binding to phosphorylated Erk sterically hinders enzymatic dephosphorylation of the kinase, *J Mol Biol*, 2006, **364**(5), 938-944.

50 Bhatt M, Patel M, Adnan M and Reddy M N, Anti-metastatic effects of lupeol via the inhibition of MAPK / ERK pathway in lung cancer, *Anticancer Agents Med Chem*, 2021, **21**(2), 201-206.