Anti-Proliferative Effects of Licochalcone A and Paclitaxel on Human Oral Squamous Cell Carcinoma Cell Line (In Vitro Study)

Mohamed Osama Mostafa[1], Sawsan Naguib Abdel-Bary[2], Nermine Raouf Amin[3]

[1] Ph.D. Candidate at the Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University
[1] Assistant Lecturer of Oral Pathology, Faculty of Dentistry, Beni-Suef University
[2] Professor of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University
[3] Professor of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University
Corresponding author E-mail: nermine.raouf@dentistry.cu.edu.eg

ABSTRACT

Aim: To study the anti-proliferative effect of Licochalcone A (LCA) compared to Paclitaxel (PTX) on cultured OSCC cell line (SCC-15) and to determine the role of Licochalcone A in enhancing the anti-proliferative effects of Paclitaxel.

Methodology: The cultured squamous cell carcinoma cell line (SCC-15) was obtained and divided into three treatment groups: Licochalcone A treated group, Paclitaxel treated group, combined drugs treatment group (Licochalcone A + Paclitaxel) and untreated control group. The all groups were submitted to immunohistochemical examination assessed by IPO-38 proliferation maker.

Results: Our findings revealed that the untreated control group of SCC-15 cells showed the highest area percent for IPO-38 immunoexpression while the area percent for IPO-38 decreased in the Licochalcone A group. The Paclitaxel and combination groups of SCC-15 cells showed the lowest area percent of IPO-38 immunoexpression.

Conclusion: The combination treatment of LCA and PTX showed the maximum antiproliferative effect on SSC-15 cell line through downregulating IPO-38 proliferation marker.

DOI: 10.48047/ecb/2023.12.11.19

INTRODUCTION

It is believed that oral squamous cell carcinoma (OSCC) is the most prevalent malignant tumors in humans. Due to its local destruction and distant lymph node metastases, it has a poor prognosis and a low survival rate **(Pereira et al., 2007).**

Low levels of expectations for prognosis and survival rate come with standard treatment methods including surgery, radiation, and chemotherapy. **(Jemal et al., 2006)** On the other hand, severe chemotherapeutic side effects include kidney damage, gastrointestinal distress, and bone marrow suppression continue to be risks that cannot be avoided. **(Carr et al., 2008)** Thus, finding new strategies to treat OSCC is essential, as it is figuring out how to make chemotherapy more efficient and less necessary.

A further treatment option for many cancers is complementary and alternative medicine (CAM) **(Brauer et al., 2010; Lewith et al., 2010)**. As potent treatment agents for OSCC, traditional medicinal herbs and their derivatives have been widely used.

According to **Zhang et al. (2021)**, licorice root-containing medications have been shown to be cost-affordable, safe, and effective replacements and alternatives for the treatment of inflammatory illnesses as well as complementary therapy for primary cancer in its early stages.

One of the several natural extracts of the licorice (Glycyrrhiza inflata) plant is Licochalcone A (LCA). It has been used to treat cancer **(Rafi et al., 2002)**, infections (**Chen et al., 2001; Ziegler et al., 2004),** and inflammation. **(Shibata et al., 1991; Kwon et al., 2008)**

A chemotherapeutic drug of the Taxol type called Paclitaxel (PTX) is used to treat lung, ovarian, and prostate cancer. In addition, it is used to treat leukemia, sarcoma, breast, head and neck, and other cancers. Recent investigations and research have revealed that Paclitaxel has a

significant role in the activation and production of mitotic arrest, which ultimately results in cell death **(Weaver, 2014).**

Paclitaxel not only demonstrated a palliative treatment of many types of advanced cancers, including carcinomas of ovary, bladder, lung and head and neck, but has also revealed effectiveness in the initi.al treatment of early-stage cancers. **(Huang et al. 2004)**

MATERIAL & METHODS

1. Material:

a- Squamous cell carcinoma cell line (SCC-15) obtained from Veterinary Serum & Vaccine Research Institute (VACSERA).

b- Licorice extract (Licochalcone A) obtained from Cayman Chemical company.

c- Paclitaxel chemotherapeutic reagent is received from The Egyptian Pharmaceutical Trading Company.

d- IPO-38 proliferation marker obtained from US Biological life science - Abcam company for immunohistochemical staining.

2. Methods:

A- Cell culture (Sample) Preparation:

- The stored cell line was removed from the liquid nitrogen container and thawed in a water bath at 37ºC.
- Cells were cultured in modified Eagle's medium (MEM) supplemented with heat inactivated fetal bovine serum (FBS), sodium bicarbonate and 2% streptomycin penicillin in flasks at 37ºC in a 5% CO_2 incubator.

The culture flasks were examined under the inverted phase contrast microscope to assure viability and sterility.

B- Sub-culturing:

- Trypsin was added to the flask and turned over for 15 seconds- 2 minutes. (*To breaks down the proteins that enable the cells to adhere to the flask wall)*
- Minimum Essential Medium (MEM) was then added to neutralize the action of trypsin.
- Cultured cells were re-suspended in fresh medium and centrifuged at 2000 rpm for 10 minutes to obtain a cell pellet.
- Cell pellet was dispersed and re-suspended in fresh complete culture media and diluted to the appropriate seeding concentration in the culture flask.
- Cells sub-cultured in a 96-well plate and incubated at 37ºC in the 5% CO2 incubator.
- The well plates were treated for 72 hours as follows:
- Group1: no treatment (control)
- Group 2: Licochalcone A treatment with concentration of (100 µM)
- Group 3: Paclitaxel treatment with concentration of (0.1 µM)

Group 4: combination treatment (Licochalcone A and Paclitaxel) with concentration of (100 µM).

C- Paraffin embedded cell line pellet preparation:

- Five ml of fixative with dilute Eosin for coloration was added and vortexed mildly, then fixed for 15 minutes.
- Cell suspension was spanned at 1800 rpm by centrifuge for 10 minutes at room temperature.
- Five ml of 70% ethanol was added for 30 minutes and vortexed mildly.
- Suspension is spanned at 1800 rpm for 10 minutes at room temperature.

- Five ml of 100% ethanol was added for 30 minutes and vortexed mildly. Overnight incubation in 100% ethanol at 4°C to make a very solid pellet.
- Suspension was spanned 1800 rpm for 10 minutes at room temperature.
- Using a clean wooden applicator stick, cell pellet was pulled out carefully of the tube and into a cassette lined with black biopsy filter paper.
- The cassette was placed in a tissue processor.
- The pellet sample was processed and embedded in paraffin within 24 hours of preparation. *(This technique was done following the National institute of environmental and health sciences (NIH).*

D- Steps of immunostaining procedure (Ramos-Vara 2005):

1- Serial sections (4 microns thick) were cut from each pellet paraffin block and were placed on the positively charged slides for the staining procedure. These slides provided better adhesion of the tissue sections and prevented them from sliding during staining.

2- Deparaffinization and hydration of the pellet sections were done in descending grades of alcohol (10 minutes in each concentration)

3- Staining for the formalin fixed cell pellet required boiling the pellet sections in 10 mM citrate buffer, pH 6.0 for 10-20 minutes followed by cooling at room temperature for 20 minutes (antigen retrieval step).

4- The sections were then incubated in 0.3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity.

5- The sections were washed before application of 100 microns of IPO-38 mouse monoclonal antibody at dilution of (1:25 -1:300) under incubation temperature of 30 °C for 80 minutes followed by application of secondary antibody for 30 minutes.

6- DAB (3, 3′ diaminobenzidine) was applied to the sections for 15 minutes at room temperature.

7- Counter stain with Mayer's Hematoxylin was applied for 8 minutes and post counter stain bluing reagent for 4 minutes.

8- Slides were extracted and arranged in racks.

9- Slides were washed in tap water for 5 minutes and then dehydrated in ascending grades of alcohol for 5 minutes in each concentration.

10-Slides were then cleared in xylene and cove slips were applie and mounted using Distyrene Plasticizer Xylene mounting agent.

E- Immunohistochemical interpretation:

The positive immunoreaction of IPO-38 was detected by nuclear brown staining and assessed by the following:

1- Transmission light microscope:

The stained sections were examined under the light microscope using high-power magnification of (1000x oil immersed lens) to assess the prevalence of immunopositivity for IPO-38 in the studied sections.

2- Image analysis computer system:

Area percent and the mean value of proliferation marker IPO-38 immunohistochemical staining in the three treatment groups was measured by image analyzer computer system which applying to (Image J) software analyzing program. The most homogenous areas of the reaction were chosen for evaluation to avoid edge artifact and transferred to monitor's screen. The most intense areas were masked by red binary color using software analyzing program. Mean area percent of IPO-38 in each field was measured and compared with normal control.

F- Statistical analysis:

Data was coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation. Comparison between all groups was done using analysis of variance (ANOVA). Pair wise comparisons between each two groups was performed by post hoc Tukey test ***(Chan, 2003)***. For comparison of serial measurements within each group repeated measures ANOVA was used ***(Chan, 2004)***. P-values less than 0.05 were considered statistically significant and P-values less than 0.0001 were considered highly significant.

RESULTS

1. Immunohistochemical findings:

A- Untreated SCC-15 cell line (control):

Microscopic examination of IPO-38 immunostained sections in untreated control group of tongue carcinoma cells (SCC-15) showed positive nuclear as well as faint immunoexpression staining of IPO-38**. Figure (1)**.

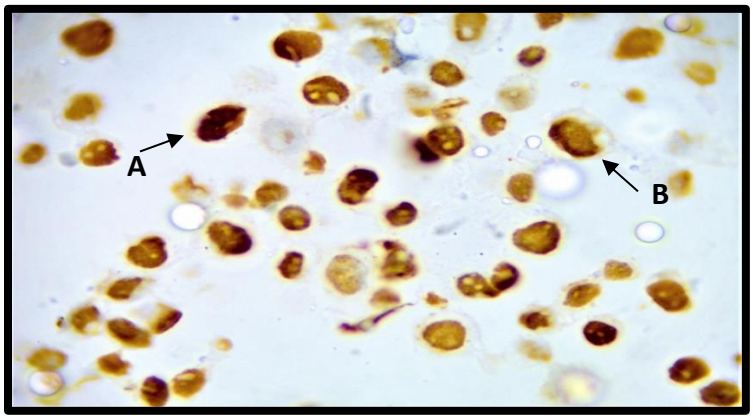

Figure (1): Photomicrograph showing positive nuclear (A) and faint cytoplasmic (B) immunoexpression of IPO-38 in untreated SCC-15 cells. (IPO-38 X 1000).

B- Licochalcone A treated SCC-15 cell line:

Microscopic examination of IPO-38 immunostained sections in LCA treated tongue carcinoma cells (SCC-15) at concentration of 100 µM for 72 hrs. showed positive nuclear immunoexpression staining in most of the carcinoma cells. Shrunken nuclei of apoptotic cells also showed positive IPO-38 immunoexpression. **Figure (2).**

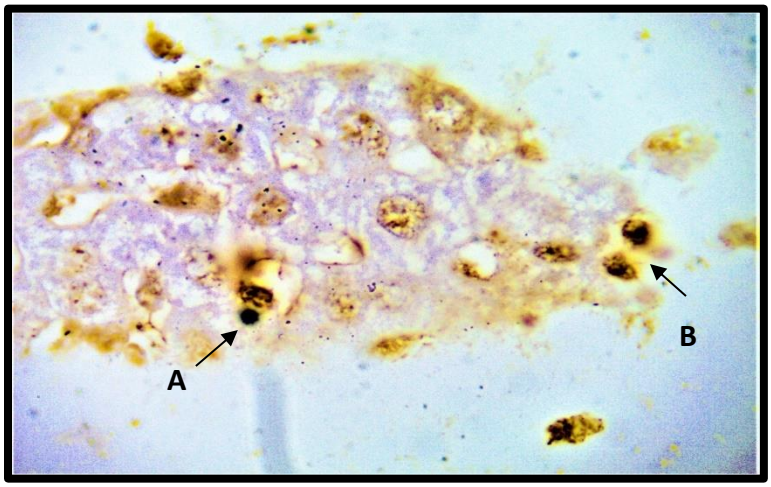

Figure (2): Photomicrograph of LCA treated SCC-15 cells at concentration of 100 µM for 72 hrs. showing positive nuclear IPO-38 immunoexpression in most of carcinoma cells (A). Shrunk nuclei of apoptotic cells also showed positive IPO-38 immunoexpression (B). (IPO-38X 1000)

C- Paclitaxel treated SCC-15 cell line:

Microscopic examination of IPO-38 immunostained sections of Paclitaxel treated tongue carcinoma cells (SCC-15) at concentration of 0.1 µM for 72 hrs. showed nuclear and cytoplasmic IPO-38 immunoexpression in few carcinoma cells. **Figure (3).**

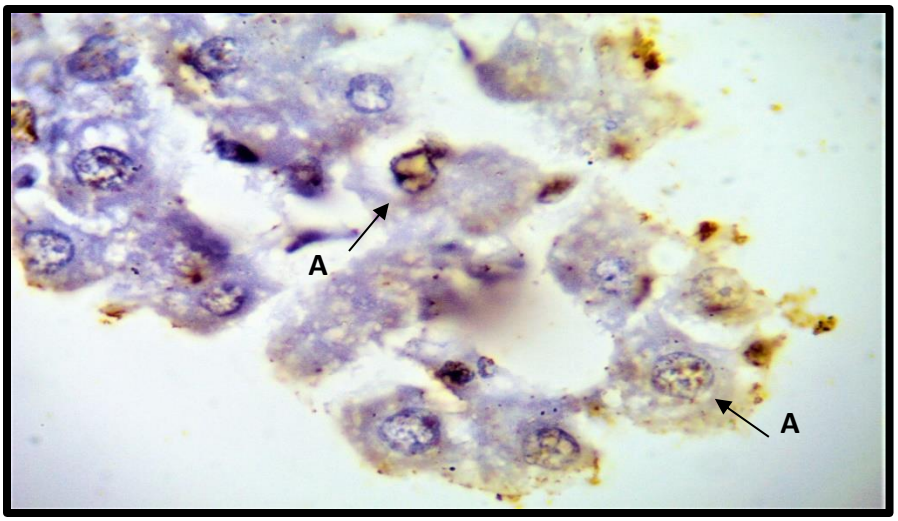

Figure (3): Photomicrograph of Paclitaxel treated carcinoma cells (SCC-15) at concentration of 0.1 µM for 72 hrs. showing nuclear and cytoplasmic IPO-38 immunoexpression in few carcinoma cells (A). (IPO-38 X 1000).

D- <u>Combination drugs (Licochalcone A and Paclitaxel) treated SCC-15 cell line:</u>

Microscopic examination of IPO-38 immunostained sections in combination (Licochalcone A and Paclitaxel) group of tongue carcinoma cells (SCC-15) at concentration of 100µM for 72 hrs. showed immunoexpression of IPO-38 in few carcinoma cells having a focal granular staining pattern in nuclei and cytoplasm. **Figure (4).**

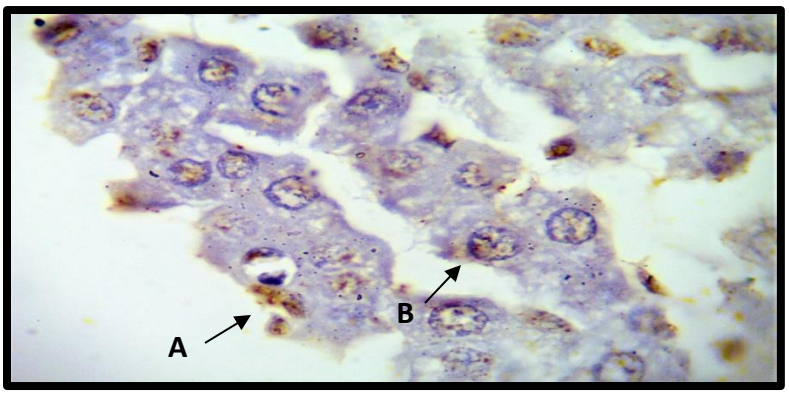

Figure (4): Photomicrograph of combination (Licochalcone A and Paclitaxel) group treated SCC-15 cell line at concentration of 0.1 µM for 72 hrs. showing IPO-38 immunoexpression in few carcinoma cells having a granular staining pattern in nuclei (A) and in the cytoplasm of carcinoma cells (B). (IPO-38 X 1000).

2. Statistical Analysis:

Comparing the studied groups by ANOVA and according to the different concentrations for each treatment group among duration of 72 hours, the untreated control group of SCC-15 cells showed the highest area percent for IPO-38 immunoexpression while the area percent for IPO-38 decreased in the Licochalcone A group. The Paclitaxel and combination groups of SCC-15 cells showed the lowest area percent of IPO-38 immunoexpression. The P value was statistically highly significant for all compared groups. **Table (1). Figure (5).**

Table (1): Values of area percent of IPO-38 immunoexpression of SCC-15 cell line in different treatment groups at concentration of 72 hrs. using ANOVA test.

	Control group	Licochalcone A group	Paclitaxel group	combination group	P value
Area % for IPO-38 immunoexpression	12.11 ± 0.97	4.06 ± 0.79[a]	2.27 ± 0.63[b]	0.83 ± 0.26[b]	< **0.001****

****Statistically highly significant**

By comparing each two groups together by Post hoc Tukey Pairwise test, the P value of IPO-38 immunoexpression was statistically highly significant for each treatment group compared to the control group **(P < 0.001).** The P value was statistically significant between Licochalcone A group and Paclitaxel group **(P<0.024)**, while it was highly significant between Combination group and Licochalcone A group.

By comparing Paclitaxel group and combination group, the P value was statistically insignificant. **(P=0.087). Table (2)**

Table (2): Values of area percent of IPO-38 immunoexpression area percent using Post hoc pairwise comparison test (P value between each two groups).

Groups	Licochalcone A group	Paclitaxel group	combination group
control group	< 0.001**	< 0.001**	< 0.001**
Licochalcone A group	----------	0.024*	< 0.001**
Paclitaxel group	0.024*	----------	0.087 (insignificant)

***Statistically significant** ****Statistically highly significant**

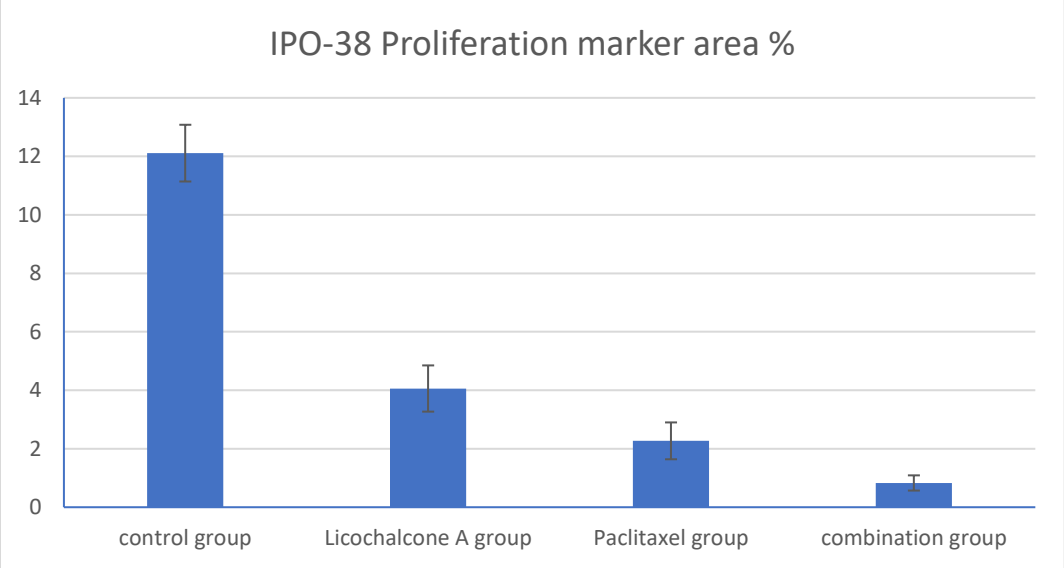

Figure (5): Bar chart illustrating area percentage of IPO-38 immunoexpression in different treatment groups of SCC-15 cell line.

DISCUSSION

HNSCC is known to be one of the most fatal cancers worldwide and shows a high frequency of recurrence. **(Huang et al., 2013)** Although curative clinical management strategies for HNSCC exist, including surgical interventions, radiotherapy, and chemotherapy, the 5-year survival rate of HNSCC patients is approximately only 50–60%. **(Osthus et al., 2013)** Moreover, survivors experience temporal or permanent side effects including osteoradionecrosis as well as discomfort in chewing, swallowing, and speaking. **(Bossi, et al., 2019)**

One of the well-studied drugs in recent history is PTX. PTX is a natural product that is used in various cancer treatment regimens. It is administered to patients with breast, lung, and ovarian cancers, and is currently being studied for the treatment of squamous cell carcinoma of the oral cavity and tongue. **(Ledwitch et al., 2013)**

Significant advancements have been witnessed in pharmacological research over the past decades regarding natural extracts like licorice flavonoids such as LCA. LCA inhibits the proliferation of epithelial carcinoma and mesenchymal sarcoma cells such as hepatocellular carcinoma, breast and colon cancer of epithelial origin, and melanoma of mesenchymal origin. **(Li et al., 2022)** Furthermore, LCA demonstrates various pharmacological properties, including anti-inflammatory, antibacterial, and antioxidant effects. **(Li et al., 2022)**

IPO-38 antigen is present in the nuclei of proliferating cells such as carcinomas and lymphomas and it is a useful marker of cell proliferation during monitoring of tumor progression. It can be used in immunohistochemical studies as well as immunofluorescence protocols. **(Guo et al., 2010)**

Regarding immunohistochemical expression in our study, we assessed IPO-38 monoclonal proliferation marker for all treatment groups after 72 hours.

According to our results, the LCA treated cells group showed positive area percent of nuclear reaction of IPO-38 proliferation marker in SCC-15 cells which was obviously lower than the expression of SCC-15 cells of the control group. These results are in accordance with the results of **Gao et al. (2021)** who studied the LCA effect on non-small cell lung cancer (NSCLC) in xenograft tumor model animals. Their data indicated that LCA delayed the tumor growth and showed a decrease in the expression of Ki-67 proliferation marker protein compared to the untreated xenograft tumor. This could be due to the pharmacological ability of LCA to inhibit proliferation by blocking the cell cycle at different transition phases via regulating specific mRNAs and protein levels, such as nuclear protein kinase (Wee1), P21, cyclins, mouse double minute 2 (MDM2), Survivin, and cyclin-dependent kinase 1 (CDK1) and hence delaying of tumor growth. **(Li et al., 2022).**

Regarding the PTX-treated group, the positive reaction of proliferative marker IPO-38 was detected in few nuclei of SCC-15 cells and the expression was low. These findings are in the same line with **Hu et al. (2015)** who stated that proliferative cell nuclear antigen (PCNA) levels were significantly decreased in PTX-treated oral cavity squamous cell carcinoma cells **(tea8113)** compared to the untreated group according to western blot analysis. This could be explained by the inhibiting effect of PTX on cancer cell proliferation through multiple signaling pathways such as epithelial growth factor receptor EGFR and PI3K/AKT signaling pathways. **(Hu et al., 2015)**

The addition of LCA to PTX showed the strongest effect on reducing IPO-38 immunoexpression as seen by microscopic examination of immunostained sections. This could be explained by the synergistic effect of LCA when added to PTX. The microscopic findings were confirmed by statistical analysis performed in this study.

It is also evident from statistical analysis that PTX was significantly more effective on IPO-38 inhibition compared to LCA alone. Moreover, the addition of LCA to PTX reduced IPO-38 immunoexpression compared to PTX alone. This is in accordance with **Wu et al. (2018)** who revealed that the level of the key factor of tumor invasion and development of metastases (urokinase plasminogen activator protein- uPA) in hepatocellular carcinoma (HCC SK-Hep-1) cells was significantly reduced when these cells were treated with combination of LCA with different doses of Sorafenib (2.5 and 5 μM).the

This result sheds light on the ability of natural products to enhance the effect of chemotherapy and produce better effects and reduce the chemotherapeutic doses to reduce its possible side effects.

Additionally, **Gloria et al. (2018)** found that PCNA protein expression was significantly inhibited by curcumin-paclitaxel combination on the (MDA-MB-231 cells) breast cancer cell line more than paclitaxel alone. Supporting this study, **Panji et al., (2021)** found that (25 and 50 μg/ml) of green tea combined with PTX (20 and 40 μg/ml) synergistically inhibited cell viability of ovarian cancer cells more than green tea alone or PTX alone after 24 hours of treatment.

Mao et al. (2022) also revealed a significant decrease in positive immunostaining of (PCNA) proliferation marker in (MDA-MB-231) mammary cancer cell lines when treated by combination treatment of curcumol and paclitaxel compared to groups treated with curcumol alone.

CONCLUSION

According to the results of the present study, we concluded that the combination treatment of LCA and PTX showed the maximum antiproliferative effect on SSC-15 cell line through downregulating IPO-38 proliferation marker.

REFERENCES

Bossi, P., Giusti, R., Tarsitano, A., Airoldi, M., De Sanctis, V., Caspiani, O., ... & Siano, M. (2019). The point of pain in head and neck cancer. *Critical reviews in oncology/hematology*, *138*, 51-59.

Brauer JA, El Sehamy A, Metz JM and Mao JJ. (2010): Complementary and alternative medicine and supportive care at leading cancer centers: a systematic analysis of websites***. J. Altern. Complement Med.,*** 16: 183–6.

Carr C, Ng J and Wigmore T. (2008): The side effects of chemotherapeutic agents. ***Curr. Anaesth. Crit. Care.*** 19:70–9.

Chan YH (2003): Quantitative Data – Parametric & Non-parametric Tests. ***Singapore Med J.,*** Vol 44(8): 391-396.

Chan YH (2004): Linear Regression Analysis. ***Singapore Med J,*** Vol 45(2): 55.

Chen M, Zhai L, Christensen SB, Theander TG and Kharazmi A (2001): Inhibition of fumaratereductase in Leishmania major and L.donovani by chalcones.. ***Antimicrob. Agents Chemother***.,45:2023–9.

Gao, F., Li, M., Yu, X., Liu, W., Zhou, L., & Li, W. (2021). Licochalcone A inhibits EGFR signalling and translationally suppresses survivin expression in human cancer cells. ***Journal of Cellular and Molecular Medicine,*** *25*(2), 813-826.

Gloria M., Richard Ponce-Cusi, and Flavio Carrión. (2018) "Curcumin and paclitaxel induce cell death in breast cancer cell lines." ***Oncology reports*** 40.4: 2381-2388.

Guo, X., Liu, W., Pan, Y., Ni, P., Ji, J., Guo, L., Yu, Y. (2010). Homeobox gene IRX1 is a tumor suppressor gene in gastric carcinoma. ***Oncogene,*** *29*(27), 3908-3920.

Hu, J., Zhang, N. A., Wang, R., Huang, F., & Li, G. (2015). Paclitaxel induces apoptosis and reduces proliferation by targeting epidermal growth factor receptor signaling pathway in oral cavity squamous cell carcinoma. ***Oncology Letters,*** *10*(4), 2378-2384.

Huang, G. C., Liu, S. Y., Lin, M. H., Kuo, Y. Y., & Liu, Y. C. (2004). The synergistic cytotoxicity of cisplatin and taxol in killing oral squamous cell carcinoma. ***Japanese journal of clinical oncology,*** *34*(9), 499-504.

Huang, Z., Wang, L., Wang, Y., Zhuo, Y., Li, H., Chen, J., & Chen, W. (2013). Overexpression of CD 147 contributes to the chemoresistance of head and neck squamous cell carcinoma cells**.** ***Journal of oral pathology & medicine****, 42*(7), 541-546.

Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C and Thun MJ (2006): Cancer statistics, ***CA Cancer J,*** 56:106–30.

Kwon HS, Park JH, Kim DH, Kim YH, Park JH and Shin HK. (2008): Licochalcone A isolated from licorice suppresses lipopolysaccharide stimulated inflammatory reactions in RAW264.7 cells and endotoxin shock in mice. ***J. Mol. Med.,*** 86:1287–95.

Ledwitch, K., Ogburn, R., Cox, J., Graham, R., Fritzsche, A., Gosnell, D., & Manning, T. (2013). Taxol: efficacy against oral squamous cell carcinoma. ***Mini reviews in medicinal chemistry, 13***(4), 509-521.

Lewith G, Britten N and Paterson C (2010): Complementary and alternative medicine (CAM) and cancer: the kind face of complementary medicine. ***Int. J. Surg.,*** 8:81.

Li, M. T., Xie, L., Jiang, H. M., Huang, Q., Tong, R. S., Li, X., & Liu, H. M. (2022). Role of licochalcone A in potential pharmacological therapy: a review. ***Frontiers in Pharmacology, 13.***

Mao, A., Qin, Q., Yang, W., & Wei, C. (2022). Synergistic anticancer mechanisms of curcumol and paclitaxel in triple-negative breast cancer treatment may involve down-regulating ZBTB7A expression via the NF-B signaling pathway. ***Iranian Journal of Basic Medical Sciences, 25***(5), 652.

Osthus, A. A., Aarstad, A. K., Olofsson, J., & Aarstad, H. J. (2013). Prediction of survival by pretreatment health-related quality-of-life scores in a prospective cohort of patients with head and neck squamous cell carcinoma. ***JAMA Otolaryngology–Head & Neck Surgery, 139***(1), 14-20.

Panji, M., Behmard, V., Zare, Z., Malekpour, M., Nejadbiglari, H., Yavari, S., & Shabanzadeh, M. (2021). Synergistic effects of green tea extract and paclitaxel in the induction of mitochondrial apoptosis in ovarian cancer cell lines. ***Gene, 787,*** 145638.

Pereira MC, Oliveira DT, Landman G and Kowalski LP (2007): Histologic subtypes of oral squamous cell carcinoma: prognostic relevance***. J. Can. Dent. Assoc.,*** 73:339–44.4.

Rafi MM, Vastano BC, Zhu N, Ho CT, Ghai G and Rosen RT (2002): Novel polyphenol molecule isolated from licorice root (Glycyrrhiza glabra) induces apoptosis, G2/M cell cycle arrest, and bcl-2 phosphorylation in tumor cell lines. ***J.Agric.Food.Chem***.,50:677–84.

Ramos-Vara JA. (2005): Technical aspects of immunohistochemistry. ***Vet Pathol.*** Jul;42(4):405-26.

Shibata S, Inoue H, Iwata S, Ma RD, Yu LJ, and Ueyama H (1991): Inhibitory effects of Licochalcone A isolated from Glycyrrhiza inflata root on inflammatory ear edema and tumour promotion in mice. ***Planta. Med., 57***:221–4.

Weaver B A. (2014): How Taxol/paclitaxel kills cancer cells. ***MolBiol Cell***. 15; 25(18): 2677–2681.

Wu J, Zhang X, Wang Y, Sun Q, Chen M, Liu S, Zou X. (2018): Licochalcone A suppresses hexokinase 2-mediated tumor glycolysis in gastric cancer via downregulation of the Akt signaling pathway. ***Oncol Rep.*** Mar;39(3):1181-1190.

Zhang Z, Yang L, Hou J, Tian S, Liu Y. (2021): Molecular mechanisms underlying the anticancer activities of licorice flavonoids. ***J Ethnopharmacol.*** 267:113635.

Ziegler HL, Hansen HS, Staerk D, Christensen SB, Hägerstrand H and Jaroszewski JW (2004): The antiparasitic compound Licochalcone A is a potent echinocytogenic agent that modifies the erythrocyte membrane in the concentration range where anti plasmodial activity is observed. ***Anti microb. Agents Chemother.***,48:4067–71.