International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.1244.1251

Research Article
Biological Evaluation of Chinese Herbal Compounds as Anticancer Agents in Fighting Against Lung Cancer

[1]Xi Zhao and [2]Salam Pradeep Singh

[1]Health School of Nuclear Industry, The Affiliated Nanhua Hospital, Hengyang Medical School, University of South China, Hengyang, Hunan, 421001, China
[2]Suchee Bioinformatics Institute, Imphal West 795001, Manipur, India

Abstract

Background and Objective: The survival rate for lung cancer has not improved in recent years despite various treatments and therapy. Therefore, the present study attempt to investigate compounds of natural origin and natural source as a new lead for anti-lung cancer therapy. **Materials and Methods:** A549 cancer cell line assay was carried out against seven Traditional Chinese Herbal Compounds. It was followed by an interleukin assay against IL-10, IL-1β and IL-17A. The compounds were further checked for their molecular interaction at the active site of these enzymes using molecular docking simulation. **Results:** Scutellarin, glycyrrhizic acid, ginsenoside and astragaloside could inhibit the growth of A549 cells growth from 68-89%. These herbal compounds also inhibited IL-10, IL-1β and IL-17A with an IC value ranging from 8.41±0.014 (scutellarin in IL-1β) to 17.44±0.019 μM (astragaloside in IL-17A). The result of the molecular docking simulation observed that these four herbal compounds docked at the active site of IL-10, IL-1β and IL-17A with favourable docking scores. **Conclusion:** Scutellarin, glycyrrhizic acid, ginsenoside and astragaloside reduced the growth of A549 lung cancer cells. The ELISA assay also confirms the inhibition of IL-10, IL-1β and IL-17A which is consistent with the molecular docking result.

Key words: A549 cells, docking, ELISA, herbal compounds, interleukins, lung cancer, astragaloside

Citation: Zhao, X. and S.P. Singh, 2022. Biological evaluation of Chinese herbal compounds as anticancer agents in fighting against lung cancer. Int. J. Pharmacol., 18: 1244-1251.

Corresponding Author: Salam Pradeep Singh, Suchee Bioinformatics Institute, Imphal West 795001, Manipur, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chemical and herbal compounds which are present in Traditional Chinese Herbal Medicine (TCHM) are becoming common for the treatment of cancer and tumour among Asian populations, especially the Chinese population[1]. In many such cases, cancer patients usually supplement Chinese herbal compounds or replaced them with their prescribed therapy or treatments[2]. The TCHM is becoming popular in the healthcare system among the Chinese population as well other Asian populations for the treatment of cancer as well as other diseases such as diabetes[3]. On the other hand, lung cancer is becoming a leading cause of cancer deaths among the Chinese population and a majority of the cause is due to smoking habits coupled with alcohol consumption[4]. It is also categorized as fatal cancer with Non-Small Cell Lung Cancer (NSCLC) as the most common type. Majority of the lung cancer is due to tobacco smoking and caused by the carcinogens present in it which produce ROS (Reactive Oxygen Species) which alters the genome[5]. Despite various cancer treatments and therapy, the survival rate for lung cancer is very poor and had not shown any good progress in the past 5-10 years[6]. Therefore, more focus has been given to investigating the natural compounds and active substances of numerous medicinal plants. In recent years, special focus has also been given by various clinical doctors and clinical researchers on the potential use of TCHM as effective ant-cancer agent, especially in lung cancer patients[7]. The use of TCHM has been extensively investigated using *in vitro* and *in vivo* techniques against various types of cancer and explored their anti-cancer properties for therapeutic use[8]. On the other hand, it is eminent that proinflammatory cytokines such as interleukins play a key role in inflammation and the progression of lung cancer[9]. There were reports which evidenced the positive association of pro-inflammatory cytokines and their subsequent risk for lung cancer[10]. Increased concentrations of cytokines, such as interleukin-10 (IL-10), interleukin (IL)-6, IL-8, IL-1β, IL-17A were observed in patients associated with lung cancer who were diagnosed for more than 2 years after blood collection[11]. The IL-10 is mainly considered an important cytokine that plays a major role in cancer related immune response and inflammation. Whereas IL-1β is reported to have inflammation-associated tumour invasiveness and IL-17A which was previously called Cytotoxic T Lymphocyte antigen 8 (CTLA-8) is known to involve in various roles of tumour progression[12,13]. Therefore, inhibiting these enzymes may be crucial for the progression of lung cancer cells. The present investigation attempts to evaluate certain Chinese herbal compounds as anti-cancer agents in fighting against lung cancer using cell line assay against the A549 lung cancer cell line.

The study also involves the inhibitory potential of these herbal compounds against three interleukins viz., IL-10, IL-1β and IL-17A using ELISA assay.

MATERIALS AND METHODS

Study location: The study was carried out at Suchee Bioinformatics Institute, Imphal West 795001, Manipur, India and Affiliated Nanhua Hospital, University of South China, Hengyang 421000, Hunan, China from September, 2020 to December, 2021.

Ethical standards and clearance: The study does not include any human participants or animals study.

Cells: Human lung cancer cell line A549 which is a non-small cell was purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS supplemented with 1% of antibiotic (streptomycin) at 35°C in a 5% carbon dioxide incubator. All the media and chemical reagents were purchased from Sigma-Aldrich (Merck KGaA, USA).

Chemical compounds: A set of seven Chinese herbal plants which is known for possessing anti-lung cancer properties is investigated in this study. Herbal compounds viz. Z-ligustilide (Source: *Angelica sinensis*, Chinese name: *Dang Gui*), astragaloside IV (Source: *Astragalus membranaceus*, Chinese name: *Huang Qi*), glycyrrhizic acid, (Source: *Glycyrrhiza uralensis*, Chinese name: *Gan Cao*), vanillic acid, (Source: *Oldenlandia diffusa*, Chinese name: *Bai Hua She She Cao*), ginsenoside, (Source: *Panax* species, Chinese name: *Ren Shen*), dihydrotanshinone (Source: *Radix salvia*, Chinese name: *Dan Shen*) and scutellarin (Source: *Scutellaria barbata*, Chinese name: *Ban Zhi Lian*) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

A549 cell line assay and interleukin assay: In this study, an interleukin assay was carried out against IL10, IL-1β and IL-17) A549 cells were seeded using a 96-well plate and allowed to settle and adhere. The plates were incubated for 14-16 hrs. The A549 cells were then treated with the herbal compounds at a concentration of 15 μM each. The A549 cells were also treated with 20 μM each of DMSO and alectinib, a known anti-lung cancer drug that acts as the control. The treated cells were then incubated for 16-32 hrs in an incubator at 37°C in a 5% carbon dioxide incubator. The herbal compounds treated cells in the well plates were collected and centrifuged for 45 min at 9000 rpm. The cell supernatant was

carried forward for 1L-10, IL-1β and IL-17 Assay using Invitrogen's IL-10 Human ELISA Kit (Catalog # BMS215-2, ThermoFisher Scientific, MA, USA) IL-1β Mouse ELISA Kit (Catalog # BMS6002, ThermoFisher Scientific, MA, USA) and IL-17A Human ELISA Kit (Catalog # BMS2017, ThermoFisher Scientific, MA, USA) based on the manufacturer's instruction. The inhibitory potential of IL-10, IL-1β and IL-17A in the A549 cells was calculated by measuring the absorbance at 450 nm.

Molecular docking study of herbal compounds against Interleukins 10, 1β and IL-17A: Computational molecular docking simulation was carried out against IL-10 (PDB Code: 4X51), IL-1 Beta (PDB Code: 2MIB) and IL-17A (PDB Code: 6HGA). The docking experiment was carried out using MVD 6.0 (Molex Software, Denmark). The potential ligand-binding site was identified using cavity detection toolkit and the binding coordinates were set at X: -3.49, Y:-26.39, Z: 53.06 for IL-10 (PDB Code: 4X51), X: 41.12, Y:-12.81, Z: -23.44 for IL-1β (PDB Code: 2MIB) and X: -43.33, Y:15.84, Z: -4.41 for IL-17A (PDB Code: 6HGA). Flexible docking was carried out setting the strength at 0.80 au with a tolerance of 1.0 au for the amino acid residues surrounding the potential ligand-binding site. For better accuracy, at least 1000 docking engine runs were carried out for each ligand and the most excellent pose was considered for future analysis. Furthermore, the binding affinity of the most excellent docked pose was calculated using a multiple linear regression equation using MVD Data Modeller (Molex Software, Denmark).

Statistical analysis: The SPSS 20.0 (SPSS, Inc, USA) was used for carrying out the statistical analysis and comparative analysis was carried out using Student's t-test. Data are presented as Mean±Standard error. Data were considered significant with values of $p<0.05$.

RESULTS

Screening of herbal compounds against A549 lung cancer cell: The study observed that DMSO (Fig. 1a) had the poorest inhibition of the A549 cells. Fig. 1b represents the control (alectinib) which represents a good inhibition. The mixture of alectinib and DMSO (Fig. 1c) and ligustilide (Fig. 1d) also showed poor inhibition. Astragaloside (Fig. 1e) and glycyrrhizic acid (Fig. 1f) also had a good inhibition of the A549 cells. However, vanillic acid (Fig. 1g) had a very poor inhibition whereas ginsenosides (Fig. 1h) had a good inhibition by reducing the cell growth. Lastly, dihydrotanshinone (Fig. 1i) showed the poorest inhibition of A549 cells and scutellarin (Fig. 1j) had the strongest inhibition. The study also revealed that 20 µL each of scutellarin (Fig. 1j), glycyrrhizic acid (Fig. 1f), ginsenosides (Fig. 1h) and astragaloside (Fig. 1e) could reduce cell growth with the least proliferation compared to Alectinib (control) (Fig. 1b-c). The DMSO (Fig. 1a), vanillic acid (Fig. 1g), dihydrotanshinone (Fig. 1i) and ligustilide (Fig. 1d) showed poor inhibition of the A549 cells. Scutellarin inhibited A549 cell growth to 89% and the least inhibition with dihydrotanshinone with 9%. The control drug Alectinib inhibited A549 cell growth to 63% whereas, glycyrrhizic acid, ginsenoside and astragaloside inhibited A549 cell growth to

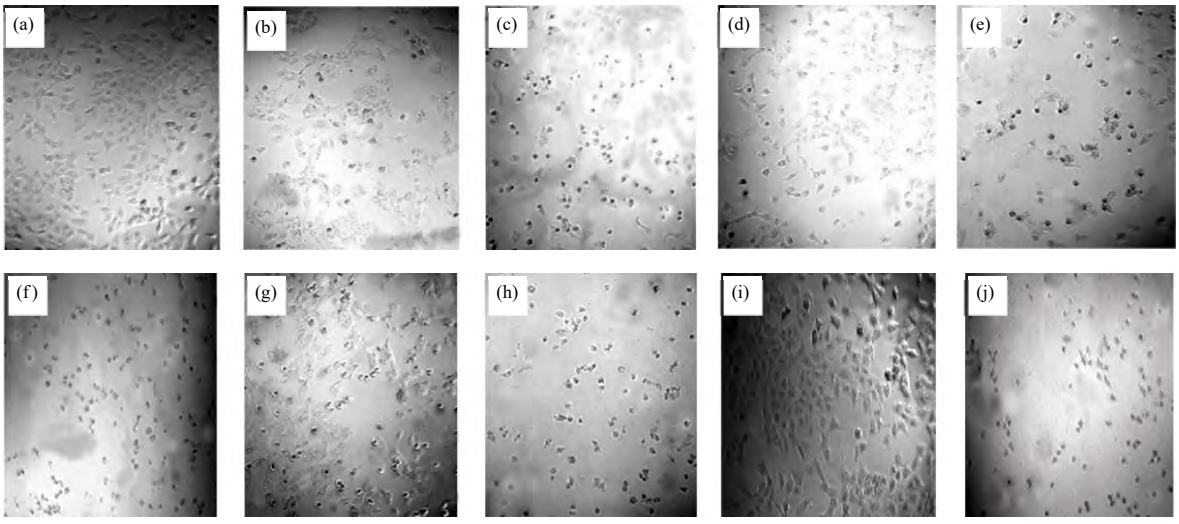

Fig. 1(a-j): Morphological changes of A549 lung cancer cells treated with 20 µL of each of the herbal compounds, (a) DMSO, (b) Alectinib, (c) Alectinib+DMSO, (d) Ligustilide, (e) Astragaloside, (f) Glycyrrhizic acid, (g) Vanillic acid, (i) Dihydrotanshinone and (j) Scutellarin

Table 1: ELISA assay of herbal compounds against IL-10, IL-1β and IL-17A indicating the inhibitory potential

Plant source and Chinese name	Compound (PubChem ID)	IL-10 (μM)	IL-1Beta (μM)	IL-17A (μM)
DMSO	DMSO (CID 679)	294.00±0.431	291.00±0.342	293.00±0.853
Positive Control (Kit)	NA	18.32±0.011	19.18±0.231	18.94±0.051
Alectinib (Reference)	Alectinib (CID 49806720)	18.55±0.014	15.41±0.082	21.19±0.048
Angelica sinensis (Dang Gui)	Z-ligustilide (CID 5319022)	38.81±0.021	43.82±0.011	34.38±0.018
Astragalus membranaceus (Huang Qi)	Astragaloside IV (CID 13943297)	16.31±0.042	14.91±0.025	17.44±0.019
Glycyrrhiza uralensis (Gan Cao)	glycyrrhizic acid (CID 14982)	12.81±0.041	10.53±0.051	11.83±0.032
Oldenlandia diffusa (Bai Hua She She Cao)	Vanillic acid (CID 8468)	58.33±0.024	69.83±0.039	48.41±0.084
Panax species (Ren Shen)	Ginsenoside 441923	12.11±0.061	14.23±0.081	13.15±0.031
Radix salvia (Dan Shen)	Dihydrotanshinone (CID 5316743)	84.33±0.013	81.84±0.038	85.83±0.033
Scutellaria barbata (Ban Zhi Lian)	Scutellarin (CID 185617)	9.34±0.023	8.41±0.014	10.92±0.021

Table 2: Docking scores of herbal compounds against IL-10 (4X51)

Ligands	MolDock score	Rerank score	Interaction	HBond	Binding affinity	Total
Astragaloside	-118.10	-105.78	-164.30	-2.50	-26.99	-417.67
Ginsenoside	-105.42	-94.04	-158.46	-6.84	-25.24	-390.00
Glycyrrhizic acid	-105.26	-93.76	-150.18	-4.90	-21.14	-375.23
Scutellarin	-113.02	-84.44	-136.32	0.00	-29.23	-363.01
Alectinib (Reference)	-87.79	-82.69	-122.85	0.00	-20.33	-313.67
Ligustilide	-67.58	-57.37	-71.78	0.00	-20.51	-217.24
Dihydrotanshinone	-63.96	-53.41	-91.63	0.00	-27.86	-236.86
Vanillic acid	-50.95	-44.66	-63.53	-2.50	-23.11	-184.75

Table 3: Docking scores of herbal compounds against IL-1β (2MIB)

Ligands	MolDock score	Rerank score	Interaction	HBond	Binding affinity	Total
Scutellarin	-104.90	-99.70	-154.74	-13.48	-21.54	-394.36
Glycyrrhizic acid	-106.86	-89.78	-165.98	-9.05	-13.96	-385.64
Ginsenoside	-93.97	-97.60	-157.37	-11.04	-23.44	-383.42
Astragaloside	-99.70	-104.74	-149.65	-3.11	-24.48	-381.68
Alectinib (Reference)	-123.21	-94.17	-141.96	-2.45	-19.43	-381.24
Ligustilide	-91.50	-74.10	-96.47	0.00	-20.68	-282.75
Vanillic acid	-84.10	-73.62	-97.90	-8.18	-25.88	-289.67
Dihydrotanshinone	-75.89	-54.52	-103.56	-0.69	-28.18	-262.85

Table 4: Docking scores of herbal compounds against IL-17 (6HGA)

Ligands	MolDock score	Rerank score	Interaction	HBond	Binding affinity	Total
Scutellarin	-124.93	-113.75	-172.14	-11.32	-26.42	-448.56
Glycyrrhizic acid	-127.26	-111.81	-176.88	-13.49	-14.67	-444.10
Astragaloside	-136.88	-89.43	-185.58	-11.56	-6.32	-429.77
Ginsenoside	-128.82	-95.52	-183.06	-10.29	-1.91	-419.59
Alectinib (Reference)	-125.75	-92.88	-148.99	0.00	-28.24	-395.85
Dihydrotanshinone	-94.70	-78.91	-122.37	0.00	-27.86	-323.84
Ligustilide	-87.88	-72.86	-92.07	-3.29	-22.03	-278.14
Vanillic acid	-68.06	-58.82	-80.36	-6.79	-25.00	-239.04

68, 72 and 74%, respectively (Fig. 1e, f, h). Thus indicating that scutellarin, glycyrrhizic acid, ginsenoside and astragaloside could kill the A549 lung cancer cells.

Inhibition of Interleukins IL-10, IL-1β and IL-17A: The interleukin assay of the herbal compounds observed that scutellarin, glycyrrhizic acid, ginsenoside and astragaloside had a strong inhibition of IL-10, IL-1β and IL-17A. These four herbal compounds showed better inhibition compared to the control (kit) and Alectinib (Table 1). Scutellarin inhibited IL-10, IL-1β and IL-17A with the least IC indicating maximum inhibition with IC values ranging from 8.41-10.92 μM. (Table 1). Glycyrrhizic acid, ginsenoside and astragaloside also inhibited IL-10, IL-1β and IL-17A with an IC value ranging from 10.53-17.44 μM. (Table 1). Whereas, dihydrotanshinone, vanillic acid and ligustilide showed poor inhibiting with a much more threshold IC value than the control (kit) and alectinib (approved drug).

Docking study: The result of the molecular docking simulation of the herbal compounds against IL-10, IL-1β and IL-17A were presented in Table 2-4, respectively. In Table 2, astragaloside, ginsenosides, glycyrrhizic acid and scutellarin docked at the active site of IL-10 with excellent scores compared to the control drug alectinib. The in-depth molecular interaction analysis observed that astragaloside possessed molecular interaction with Leu48 (Fig. 2a). Ginsenoside interacts with Asp41, Leu48, His83 (Fig. 2b),

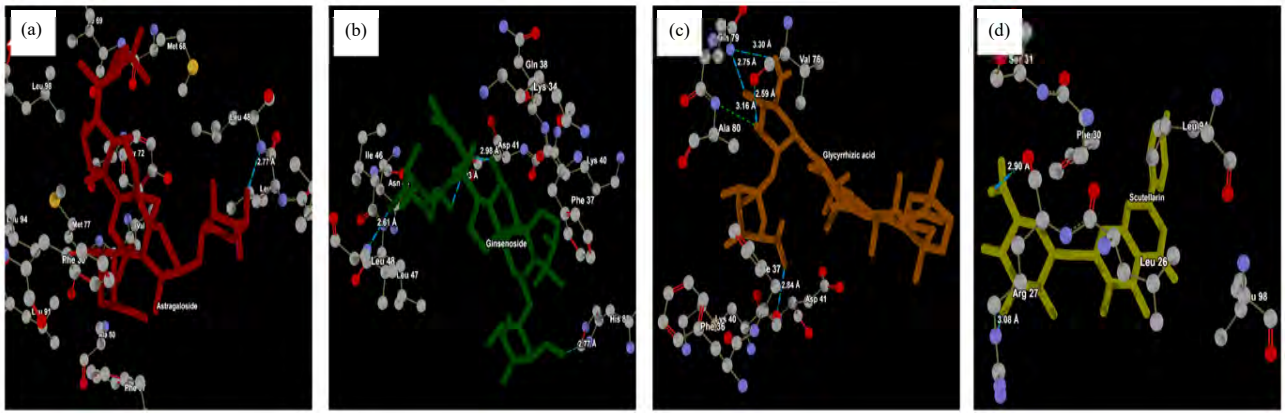

Fig. 2(a-d): Molecular docking interaction of the top docking hits at the active site of IL-10, (a) Astragaloside interacting with Leu48, (b) Ginsenoside interacting with Asp41, Leu48 and His83, (c) Glycyrrhizic acid interacting with Ph37, Val76, Gln79 and Ala80 and (d) Scutellarin interacting with Arg27 residues

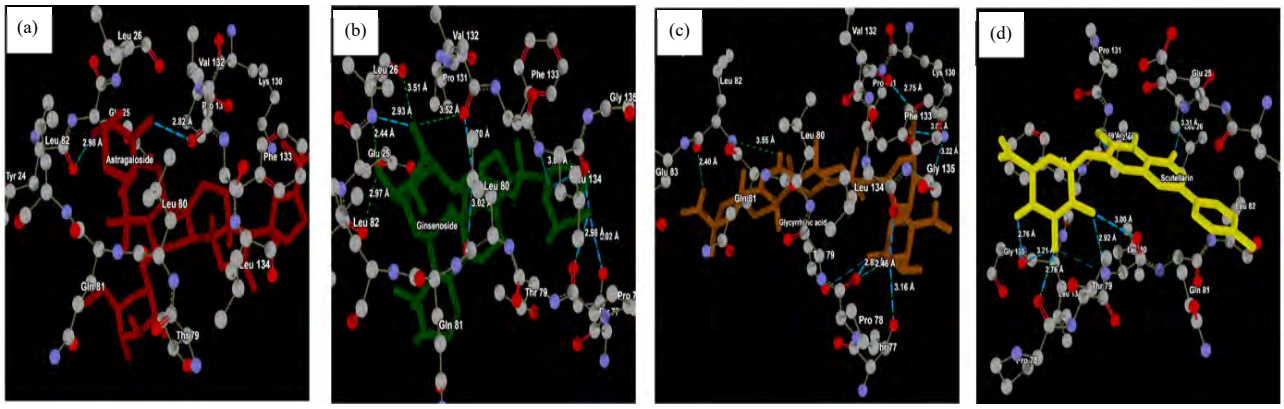

Fig. 3(a-d): Molecular docking interaction of the top docking hits at the active site of IL-1β, (a) Scutellarin interacting with Leu26, Pro78, Leu80, Val132 and Leu134, (b) Glycyrrhizic acid interacting with Thr77, Pro78, Thr79, Leu82, Lys130 and Pro131, (c) Ginsenoside interacting with Tyr24, Leu26, Thr77, Pro78, Leu80, Val132 and Leu134 and (d) Astragaloside interacting with Tyr24, Val132 residues

glycyrrhizic acid with Ph37, Val76, Gln79, Ala80 (Fig. 2c) and scutellarin have interaction with Arg27 (Fig. 2d) of IL-10. In Table 3, scutellarin, glycyrrhizic acid, ginsenoside and astragaloside docked at the active site of IL-1β with the best scores compared to alectinib.

The in-depth molecular docking analysis revealed astragaloside had interaction with Tyr24, Val132 (Fig. 3a). Ginsenoside possessed molecular interaction with Tyr24, Leu26, Thr77, Pro78, Leu80, Val132 and Leu134 (Fig. 3b). Glycyrrhizic acid had interaction with Thr77, Pro78, Thr79, Leu82, Lys130, Pro131 (Fig. 3c) whereas scutellarin possessed strong molecular interaction with Leu26, Pro78, Leu80, Val132 and Leu134 residues of IL-1β (Fig. 3d).

Similarly in Table 4, scutellarin, glycyrrhizic acid, astragaloside and ginsenoside docked at the active site of IL-17A with the best scores compared to alectinib. In the case of IL-17A.

Astragaloside had interaction with Trp239, Gln243, Gly244, Lys247 and Pro248 (Fig. 4a) and ginsenoside had interaction with Trp239, Gln243, Gly244, Pro246, Pro248 and Arg249 residues of IL-17A (Fig. 4b). Glycyrrhizic acid had interaction with Trp239, Gln241, Gln243, Gly244, Arg249 and Gln274 (Fig. 4c). Scutellarin possessed molecular interaction with Leu237, Asn240, Gln243, Lys247, Pro248 and Trp250 (Fig. 4d). Taking into account all the three interleukins investigated in this study, it is evidenced that scutellarin, glycyrrhizic acid, ginsenoside and astragaloside are potential inhibitors of IL-10, IL-1β and IL-17A. Therefore, these compounds possessed strong inhibitory potential against interleukins.

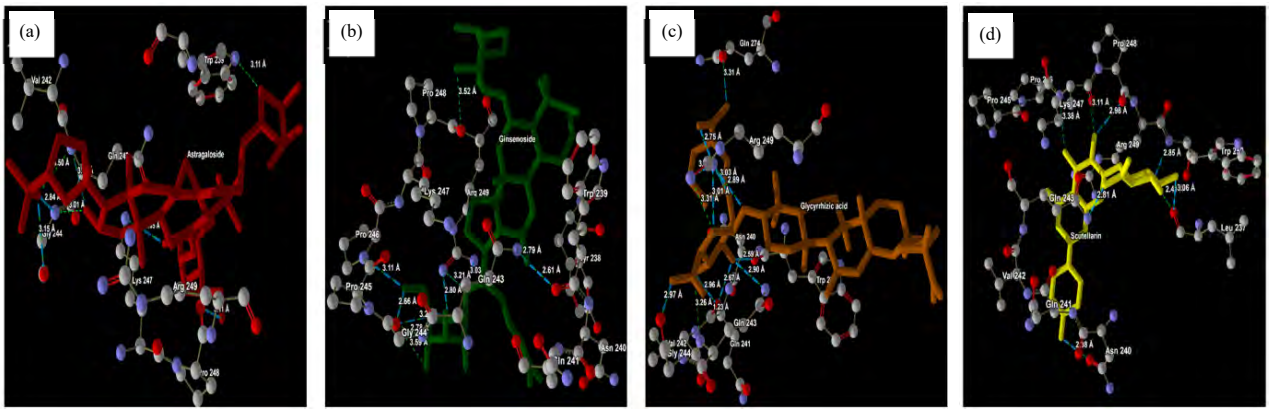

Fig. 4(a-d): Molecular docking interaction of the top docking hits at the active site of IL-17A, (a) Scutellarin interacting with Leu237, Asn240, Gln243, Lys247, Pro248 and Trp250, (b) Glycyrrhizic acid interacting with Trp239, Gln241, Gln243, Gly244, Arg249 and Gln274, (c) Astragaloside interacting with Trp239, Gln243, Gly244, Lys247 and Pro248 and (d) Ginsenoside interacting with Trp239, Gln243, Gly244, Pro246, Pro248 and Arg249 residues

DISCUSSION

The study observed that scutellarin, glycyrrhizic acid, ginsenoside and astragaloside reduced the A549 lung cancer cell growth compared to the market-approved drug alectinib. These four compounds also inhibited IL-10, IL-1β and IL-17 from the ELISA assay compared to the control (kit) and alectinib (approved drug). The result of the molecular docking simulation of the herbal compounds against IL-10, IL-1β and IL-17A also evidenced the inhibitory potential of scutellarin, glycyrrhizic acid, ginsenoside and astragaloside. In the past decade, herbal compounds present in Chinese herbs have proved their effectiveness against various types of cancer and its disease progression. These herbal compounds also possessed superior pharmacological effects with lesser ADME-Toxicity parameters which are mainly because of their natural origin. There are around 500 Chinese herbal medicines that are registered and reported as having anti-cancer properties which are effective against lung cancer, colon cancer, liver cancer, breast cancer etc[14]. In the present study, the inhibitory potential of seven Chinese herbal compounds of anti-lung cancer properties using the A549 lung cancer cell line and interleukin assay was investigated. There are several reports which observed that interleukins are associated with lung cancer risk with elevated concentrations of interleukin IL-10, IL-1β, IL-17A, IL-35, IL-8, IL-6, IL-2 and IL-12[15]. The biological mechanism of interleukins such as IL-10, IL-1β and IL-17A are directly associated with cancer cell growth, metastasis and invasion. Therefore, herbal compounds such as scutellarin, glycyrrhizic acid, ginsenoside and astragaloside interfere and promotes A549 cancer cell inhibition as well as inhibit the biological mechanism of IL-10, IL-1β and IL-17A thereby reducing the cancer growth, invasion and promoting cancer cell apoptosis[16]. The molecular docking study against IL-10, IL-1β and IL-17A also confirmed the inhibitory potential of scutellarin, glycyrrhizic acid, ginsenoside and astragaloside. This molecular docking result is correlated with the cell line assay as well as the interleukin assay. The molecular docking simulation result also observed the in-depth molecular interaction of these herbal compounds at the active site of IL-10, IL-1β and IL-17A. The docking score used in the present investigation accounts for 89% accuracy and comprises of MolDock and Rerank score[17]. The MolDock score is based on piecewise linear potential with the approximation of steric energy whereas the Rerank score is based on the weighted combination of MolDock score coupled with certain energy terms such as LJ12-6, VDW energy etc[18]. Numerous studies have reported the association of interleukins in lung cancer progression. Zhang and Veeramachaneni[19] reported on the presence of high concentrations of IL-1β and IL-17A on the blood samples of lung cancer patients diagnosed for more than three years. Another study observed the association between IL-10 and tumorigenesis processes in lung cancer patients based on tumour immune surveillance thereby promoting the progression of tumour progression[20]. Therefore, it is quite sure that Chinese herbal medicine may serve as a substitute for the treatment of cancer or be prescribed in combination for therapeutic use[21]. Additionally, these herbal compounds can also reduce the toxic side effects of radiotherapy and chemotherapy.

CONCLUSION

The study observed that Traditional Chinese Herbal Compounds such as scutellarin, glycyrrhizic acid, ginsenoside and astragaloside could reduce the growth of A549 lung cancer cells compared to the control drug (alectinib). The ELISA assay of the interleukins also observed that scutellarin, glycyrrhizic acid, ginsenoside and astragaloside had a strong inhibition of IL-10, IL-1β and IL-17A compared to the positive control (kit) and control drug(alectinib). This finding is also consistent with the result of the molecular docking simulation where these four compounds were found to be the top docking compound with a favourable docking score.

SIGNIFICANCE STATEMENT

The study discovered that scutellarin, glycyrrhizic acid, ginsenoside and astragaloside could kill A549 lung cancer cells and stop their progression. These four compounds also inhibit interleukins IL-10, IL-1β and IL-17A which are based on the ELISA assay thereby interfering with the cancer cell growth, metastasis and invasion. Molecular docking data also confirmed the inhibition of these interleukins. Therefore, the study provides new leads for anti-lung cancer therapy using natural sources.

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