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Abstract

Background: Oral squamous carcinoma is a head and neck cancer, which is one of the types of malignant cancers. Present study evaluates the anticancer activity of *Aster tataricus* (AT) on SCC-9 human oral squamous carcinoma.

Materials and Methods: Ethanol extract of AT was prepared by a standard procedure of maceration. AT extract was used in different concentrations like 10, 20, 40, 80, 160, 320 and 640 µg/ml for the evaluation of its anticancer activity. Effect of AT extract on SCC9 cells were observed by microscope and cytotoxicity by 3-(4, 5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Moreover, clonogenic assay was used for the estimation of effect of AT extract on colony forming ability of SCC9 cells.

Result: Result of the study suggested that treatment with AT extract causes cytotoxicity to SCC9 cancerous cells. In addition, AT extract treatment reduces clonogenic potential of SCC9 cell and it also inhibits the proliferation of cell significantly ($p < 0.001$) in G2/M phase. **Conclusion:** Thus, given study concludes that AT extract effectively attenuates the growth of SCC-9 cancerous cells by the virtue of its cytotoxic and anti clonogenic activity.

Key words: *Aster tataricus*, 3-(4, 5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, Human oral squamous carcinoma, SCC-9

Introduction

Oral squamous carcinoma is the 6th most malignant cancer across the globe (Krishna et al., 2013). Every year around 600000 cases reported and around 350000 deaths occur due to it. Squamous cell carcinoma is the commonest type of oral cancer (Forman et al., 2014). Head and neck cancer may originate from the different anatomical regions such as larynx, nasopharynx, lip, oropharynx and neck cavity. In 70 – 75 % cases of head and neck cancer the commonest cause is tobacco and alcohol (Anantharaman et al., 2013). Moreover, other risk factor that causes head and neck cancer is human papilloma virus (HPV) infection, poor oral hygiene, deficiency of vitamins and malnutrition (de Camargo Cancela et al., 2010).

However, there are various treatment options available for head and neck cancer such as radiotherapy, chemotherapy and surgery (Qi et al., 2010). Although these therapies possess beneficial effects but some time they cause severe toxicity and thereby affect the post treatment life of patient. Moreover, resistance of cancer cell from the therapy emerges new problem, which is required to develop an alternate therapy for the management of it (Saraswathy and Gong, 2013).

Aster tataricus (AT) traditionally used in China last from 2000 years for medicinal purpose. Roots of AS contain the chemical constituents like triterpenes and saponins (Dongliang and Yu, 1993). Other reported chemicals that were isolated from roots such as epifriedelinol, caffeoylquinic acids, astersaponins, shionone and aster peptides (Yu et al., 2015). Traditionally it has been used as antibacterial, antifungal, anticancer and management of chronic bronchitis and tuberculosis (Bown, 1995). Literature suggested that root extract of AS possess anti-inflammatory, expectorant and antitussive activity (Yu et al., 2015). Caffeoylquinic acids and epifriedelinol possess strong antioxidant and anti-inflammatory (Duke and Ayensu, 1985; Ma et al, 2011; Peluso et al, 1995). Thus, the given study evaluates the anti cancer activity of AT on human oral squamous carcinoma.

Material and Methods

Extraction of plant

Root of AS was procured from local supplier and authenticated from Institute of Medicinal Plant Development, Beijing (Specimen No: IMPD/10/2016). Extraction of AS roots was done by maceration. Dried root was powdered and kept it with ethanol for

72 hrs. Thereafter ethanol was evaporated from the extract at low temperature by rota vapor apparatus. Percentage yield of extract was found to be 8.2 % w/w.

Cell and culture media

SCC-9 and NIH/3T3 cell lines were procured from Shanghai Institutes for Biological Sciences, Shanghai, China and all the protocols were approved from Ethics Committee of the 306th Hospital of PLA, China. In a 5% CO₂ humidified incubator at 37 °C cells lines were incubated. DMEM medium was used with (10% v/v) fetal bovine serum as a culture medium for the present study. Medium used in this study was replaced at a specific interval of time and cell were trypsinized routinely till it grows up to 80-90% of confluency.

Aster tataricus extract treatment

Extract of AT used for the treatment after diluted in the culture medium to achieve serial concentrations. Culture media with 1% DMSO was used to treat control cells.

Evaluation of morphology of SCC-9 cell lines

Light microscope was used to observe the changes of SCC-9 cells morphology after the treatment with AT extract. Cell lines (5×10^4 cells/well) were kept in 96 well plate and for one day treated with AT extract with different concentrations of 0 to 640 µg/ml. Moreover, as an additional control fibroblast cells (NIH/3T3) of mouse embryo was used in this study. Morphological changes in the cell line were monitored by the help of phase contrast microscope.

Estimation of cell viability by MTT assay

MTT assay was used to assess cellular viability. In which cell (5×10^3 cells/well) were placed at 96 well plate and treated with AT extract at a different concentration for the period of the duration of 3 days. MTT assay was performed as described by Xiao et al. MTT (50µl) was added to the well plate and keep it for incubation for 4 hr. at 37°C. Thereafter from the reaction mixture supernatant was washed out and subsequently dimethylsulfoxide (200µl) was added to all the well plate at room temperature. The absorbance was estimated at 570 nm wavelength (Xiao et al, 2015).

Estimation of AT extract effect on stages of SCC-9 cell cycle

SCC-9 cells (1×10^6 cells/well) were seeded in a 6 well plate and incubated for 32 hr. After a specific period of incubation DMEM media was replaced by serum (1%) and incubated it for synchronization of cell cycle. These cells were treated with 10, 20, 40, 80, 160, 320 and 640 µg/ml of AT extract for 24 hr and thereafter PBS was used to wash all the cells, later 70% ethanol at 4° C was used to fix it for 24 hr. Later cells were washed and incubated with DNase free-RNase-A (100 µg/ ml) for one hour at 37°C and propidium iodide was used for the staining of it. Cell cycle was estimated by using flowcytometer (Becton Dickinson, USA) (Chen et al., 2012).

Clonogenic assay

cells were (5×10^3 cells/ well) poured in to a 6 well plate and incubated for one day at 37 °C. These cells were treated with AT extract at 50, 100, 200 and 400 µg/ml for one day. Thereafter culture media was exchanged by DMEM containing FBS (10%) and this process of replacement of media with fresh one repeated after 5 day. On 21st day cells were washed with PBS and crystal violet (0.25%) was used for staining for 30 min. Number of colonies were counted by stereomicroscope and its plating efficiency was determined (Franken et al., 2006).

Statistical analysis

Data of given manuscript represented as mean \pm SD (n=10). All the results were analyzed statistically by one way ANOVA and post hoc study by Dunnett. In this study values $p < 0.05$ was considered as significant.

Result

Effect of AT extract on cell morphology

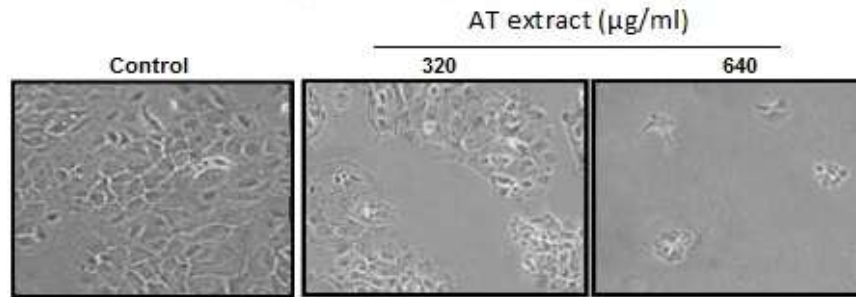
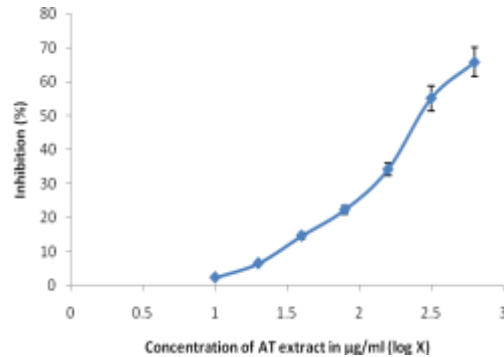


Figure 1

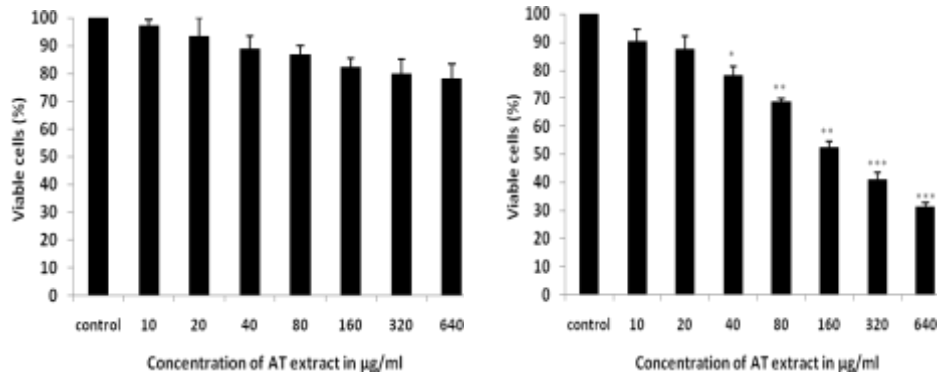
The effect of AT extract on morphological characteristics of SCC9 cell line was shown in Fig.1. AT extract treated SCC9 cells were lost the characteristics as contact between adjacent cell lost and cell shrunken in size. The effect was found in a dose dependent manner.

Evaluation of effect of AT extract on cell by MTT assay

Effect of AT extract on SCC9 cells was estimated by MTT assay as shown in Fig.2. It was observed that treatment with AT extract inhibit the proliferation of SCC9 cells in a dose dependent manner as at a low concentration (10-40 µg/ml) AT extract inhibit the proliferation by below 20%. Whereas at 80 and 160 µg/ml concentration it shows inhibition of proliferation between 20-30% and at higher concentration such as 320 and 640 µg/ml AT extract inhibits above 50% proliferation of SCC9 cell.



(A)



(B)

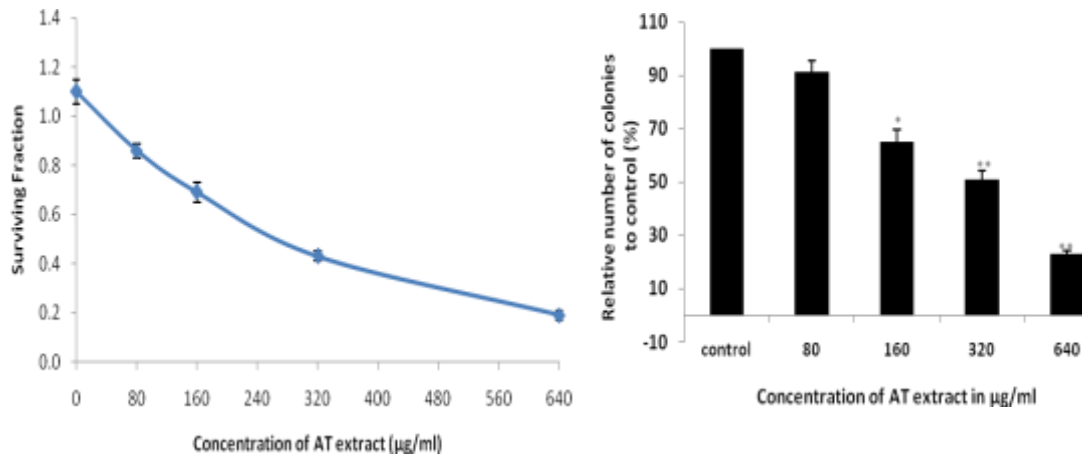
(C)

Figure 2: Cytotoxic effect of AT extract on SCC9 and NIH/3T3 cell by MTT assay. (A) Inhibitory effect of AT extract on SCC9 cells. (B) Percentage of growth inhibition of SCC9 cells (C) Percentage of growth inhibition of NIH/3T3 cell. Values are means ± SD (n=10); *p < 0.05, **p<0.01, ***p<0.001 (vs. Control group)

Effect of AT extract on SCC9 cell by clonogenic assay

Surviving fractions was found to be significantly decreases when SCC9 cells were treated with AT extract for the period of one day. This decrease in survival fraction of SCC9 cell with AT extract was found to be in a dose dependent manner as shown in Fig 3.A.

Control group of SCC9 cells has shown 100% cloning ability. Moreover, treatment with AT extract significantly (p<0.001) declines the cloning ability of SCC9 cells as compare to control group in a dose dependent manner as shown in Fig. 3B.



(A)

(B)

Figure 3: Effect of AT Extract on SCC-9 Cells clonogenic ability. (A) Survival curve (B) Number colonies. Values are means ± SD (n=10); *p < 0.05, **p<0.01 (vs. Control group)

Effect of AT extract on SCC9 cell cycle

Effect of AT extract on SCC9 cells at various stages of cell cycle was shown in Fig.4. It was observed that treatment with AT extract significantly (p<0.001) increases the cells at G2/M phase compared to control group. Whereas, the cell in G0/G1 phase decreases in AT extract treated group compared to control group.

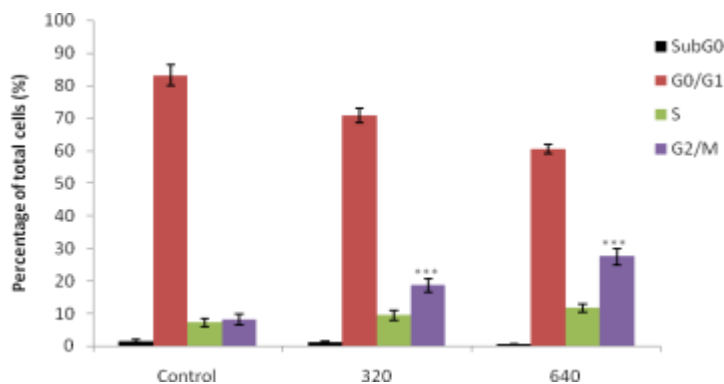


Figure 4: Effect of AT extract on SCC9 cells at various stages of cell cycle
Values are means \pm SD (n=10); ***p<0.001 (vs. Control group)

Discussion

Management of cancer becomes complex as due to resistance of cancer cell towards conventional treatment options. Literature reveals that phytomedicine possess strong anticancer activity and promises alternative therapy for the management of cancer (Xavier et al., 2009). This study evaluates the anticancer activity of *Aster tataricus* extract on SCC-9 cancer cells. Effect of AT extract on SCC9 cancer cells was evaluated by observing the morphology, MTT assay, clonogenic assay and cells in which phase of

cell cycle. Previous report suggested that phytomedicine do the morphological changes in oral cancer cell line and the result of the give study shows that treatment with AT extract causes similar changes in the morphology of SCC9 cell line (King et al., 2007). Report suggested that AT contain several chemically active substances that possess several medical properties. Caffeoylquinic acid is the chemically active compound that reported to have strong antioxidant and anti-inflammatory property (Duke and Ayensu, 1985; Ma et al, 2011; Peluso et al, 1995). Moreover, it has effect on cell viability and effective against lung cancer (In et al, 2016). Thus, cytotoxic potential of AT extract was estimated by MTT assay. In which mitochondrial dehydrogenase that is present only in viable cell reduces tetrazolium salt and converted it in to formazan (Mosmann, 1983). Our study shows the reduction of tetrazolim salt in a dose dependent manner in AT extract treated SCC9 cell line. Moreover, clonogenic ability of cancer cell is the important characteristics and estimation of it was dose by clonogenic assay (Katz et al., 2008). Cytotoxic drugs show anticlonogenic activity and thereby inhibit the growth of tumor cell. Result of this study suggests that treatment with AT extract significantly (p<0.01) decreases cloning ability of SCC9 cells in a dose dependent manner. In addition, effect of AT extract was observed over the cell cycle and observed that treatment with AT extract significantly (p<0.01) increases the no cells in G2/M phase compared to control group.

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Conclusion

This study concludes that *Aster tataricus* extract possess anticancer activity on human oral squamous carcinoma cell line.

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