of fried foods, food preservatives, synthetic aroma for foods and beverages, carbonated drinks, foods that rapidly cooked, overuse of animal foods and inadequate chewing of foods and etc.

**Environmental factors:** In addition to dietary factors which have just been mentioned, there are more important factors which are not usually considered, of which inappropriate use of industrial life tools can be mentioned such as: abnormal noises, cordless phones and mobile phones radiation, radiation of bulbs and especially cold lamps or florescent lamps, unnecessarily use of lighting during the day, modern life, sedentary lifestyle, comfort-seeking, low physical activity and lack of exercise. These factors create the conditions for Souda incidence in human body.

**Psychological factors:** Different mental conflicts, persistent sadness, confusion, indecision, hesitancy in the course of life distrust in God and the lack of seriousness in changing the fate, surrender to setbacks, jealousy, greed, cutting friendly, romantic and family relations are psychological factors that definitely enhance the level of souda humour in human body. Also, incomplete sexual actions have an important role to play in the incidence breast and prostate cancer.

**Cancer treatment:** The first strategy of Iranian traditional medicine against cancer is cleansing the body of sauda humour and its attendant humours, and then other treatments such as medical management, surgery and diet, was prescribed in next phase. In traditional Iranian medicine, common methods to cleans body of sauda humour and attendant humours includes: vomiting (gastric cleansing), blood-letting and cupping (vessels and liver cleansing), depilatory (lymph nodes cleansing) and etc. It may be noted that although there is no complete cure, treatment of cancer at early stages is easier than advanced stages, so treatment of cancer must be given high priority as soon as the disease is diagnosed.

**Keywords:** Cancer, Traditional medicine, Homours, Souda, Prevention

**Cytotoxic effect and cell attachment of IL-12 antimicrobial peptide on A549 epithelial cells**

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Extended Abstract

**Introduction:** Antimicrobial peptides are diverse group of molecules that are produced by invertebrate, plant and animal species.

Their amino acid composition, amphipathicity, cationic charge and size allow them to attach and insert into membrane bilayers to form pores by barrel-stave, carpet or toroidal-pore mechanisms. These peptides are potent, broad spectrum antibiotics which demonstrate potential as novel therapeutic agents. The majority of conventional antibiotics it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators. The venoms of social wasps can also contain some tetra-, penta-, hexa- and hepta-peptides, but just a few of them have been structurally and functionally characterized up to now. Protonectin (ILGTILGLKGL-NH2, IL-12) is a polyfunctional peptide, presenting mast cell degranulation, release of lactate dehydrogenase (LDH) from mast cells, antibiosis against Gram-positive and Gram-negative bacteria and chemotaxis for polymorphonucleated leukocytes. Aerobic organisms are susceptible to the damaging actions by small amounts of O2 •, •OH and H2O2. These species are inevitably formed during the metabolism of oxygen, especially in the reduction of oxygen by the electron transfer system of mitochondria. ROS is a collective term that includes oxygen radicals and also some non-radical derivatives of O2 like H2O2, hypochlorous acid (HOCl), peroxynitrite (ONOO-), and O3 and singlet oxygen. Enormous efforts are still being devoted to understand the role played by partially reduced oxygen species (O2 • and •OH) in mediating a variety of pathological conditions, including toxicity due to several chemicals and ionizing radiation. Oxygen-derived species are mutagenic and may act as promoters of carcinogenesis. The main objective of this study was to examine cell attachment and evaluation of ROS produced in A549 cell line in the presence and absence of IL-12.
Material and methods: The peptide was synthesized by GL biochem (Shanghai, China). The crude peptide was purified by semi-preparative reverse-phase HPLC using C8 column. A549 cell line was prepared from Pasteur Institute of Iran (IPI).

Human lung carcinoma cell line A549 (ATCC CCL-185) was cultured in 25 cm² tissue culture flasks. The cells were maintained in RPMI 1640 medium (Biosera, East Sussex, UK) supplemented with L-glutamine, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 % heat-inactivated FBS (Biosera, East Sussex, UK), 100 μg/mL streptomycin and 100 U/mL penicillin (Bio-sera, East Sussex, UK). A549 cells were grown in a 5 % CO2-humidified atmosphere at 37 °C. The cells were passaged at 60–70 % confluence using trypsin-EDTA (Gibco, Grand Island, NY, USA). To examine the amount of ROS, the cell was incubated for 24 and 48 hours in the presence of 12 μg/ml and 50 μg/ml of the IL-12 peptide. A sample without peptide was considered as control. After adding DCF-DA as fluorescence probe, the amount of ROS was measured by flow cytometry. Results showed that at 50 μg/ml of the peptide, ROS was 26.7% and 84.7% over 24 h and 48 h- treatment, respectively; whereas, the amount of ROS value in control cells was 0.2%. To evaluate the adhesion of the peptide to desired cells, cell attachment technique was used. In this experiment, peptide at 12, 50 μg/ml were added to 96-well plates and stored overnight at 4 °C. Then a certain amount of RPMI-1640 2% and A549 cells were added to the plate. After 1 h, the plates were washed with PBS (0.1%) and the attached cells were stained with crystal violet (0.5x) and photographed with optical microscope. Finally, by the use of acetic acid (10%), the crystal violet was washed and the cells

Results and discussion: The aim of this study was to evaluate ROS and cell attachment of A549 cell line treated by IL-12 at 12 μg/ml and 50 μg/ml. Results of ROS measurement showed that at 50 μg/ml of the peptide, ROS was 26.7% and 84.7% over 24 h and 48 h- treatment, respectively; whereas, the amount of ROS value in control cells was 0.2%. Cell attachment experiments using crystal violet staining revealed that at 12 μg/ml and 50 μg/ml of the IL-12 peptide, the cell attachment values were 101% and 102%, whilst, this value for control cell was 26.1%. Our results suggest that the peptide possesses the binding ability to the cells leading to cell attachment. Furthermore, treatment of A549 cell with IL-12 peptide increased ROS.