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Anticancer Effect of Food Supplements on Saos-2 Osteosarcoma Cell

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Abstract

Objective: Nowadays medicinal plants have been considered as complementary medicine in cancer treatment by many researchers. Osteosarcoma, one of the most common malignant tumors, which is more common at a young age; It is one of the most common types of cancer that occur in mesenchymal bone-forming cells and affect the musculoskeletal system. Today instead of cancer drugs due to their numerous side effects that can suppress or prevent cancer treatment, food supplement products that contain plant extracts may be preferred. In this study, the anticancer effects of five different food supplements were investigated on osteosarcoma Saos-2 cancer cell line.

Material-Method: Application doses were as 1000, 2000, 3000, 4000, 5000, 6000 µg/ml and IC50 values were calculated by creating two working groups of 24 and 48 hours.

Results: Extracts of five different herbal product showed cytotoxic effect on Saos-2 cancer cell line due to their mixture of medicinal plants, however REGULIN® showed the most cytotoxic effect among them.

Conclusion: It was determined that REGULIN® used in the remember regeneration therapy method (RTM), had the most anticancer effect in each of the 24 and 48 hour application groups. Cytotoxic effect of these food products on other cancer cell lines and their use in the pharmaceutical and health industries is recommended in future studies.

Keywords: Osteosarcoma, Anticancer, Saos-2, Food Supplement.

INTRODUCTION

Cancer is a serious health problem with a high mortality rate worldwide.[1]. Approximately 300,000 children die every year due to in childhood cancer. Osteosarcoma is primary bone cancer and one of the most common types of bone cancer in childhood and adolescence. Chemotherapy, surgery and postoperative chemotherapy were used as a standard treatment in the treatment of osteosarcoma [2–4]. Many different molecules are currently being investigated in cancer treatments, and the source of such molecules is products with herbal ingredients [5]. Although the mechanism of action of many medicinal plants is still uncertain, herbal products are widely used in traditional medicine as a supplement [6]. The combination of active phytochemicals from more than one plant shows a strong anticancer activity. There are many studies are proving this situation in Chinese medicine that medicinal plants can the improve quality of life in patients with bone cancer [7]. Single-drug approach was only for a period of time, however now the development of multi-drug and conventional pharmaceutical should be approached in the treatment strategy [6].

Food supplements are defined as products used to support substances which are missing in the diets of people such as minerals, vitamins, etc., [8]. Medicinal plants have been increasingly used as traditional and complementary medicine products from east to west. According to WHO, 21.000 plants are suitable for medication [9]. The widespread use of these products also increases the value of the market. It is known that foods have positive effects on human health. Therefore, the use of food supplements is increasing in societies. Urtica contains different components such as lignans, polysaccharides and, lectins. These components decline prostate enlargement. It has anti-inflammatory properties and prevents cancer cell growth. Juniper is a powerful antioxidant with high anti-inflammatory effect [10].

Studies have shown that it has a healing effect for diseases such as respiratory diseases, cough, bronchitis and, asthma. In addition, chemotherapeutic drugs have been found to reduce the side effects [11]. It has been revealed that it has protective properties for the liver and kidneys. Turmeric has an effect that strengthens the immune system, reduces or eliminates the risks in diseases such as diabetes and heart diseases that seriously affect human life. It is effective for liver diseases [11]. It contributes to the cleaning of toxins in the body. It speeds up the blood circulation and maintains the body temperature. It reduces the risk of getting rheumatological conditions such as arthritis, it is an effective antidepressant, it helps to lose weight. In addition, studies show that it is effective on colorectal cancer in rodents [12]. Ginger has high antioxidant properties. It has been revealed that it has anti-inflammatory, anti-apoptotic, antihyperglycemic properties. Studies have shown that ginger given orally has an anti-thrombolytic effect. In diabetes studies, it has been shown to protect against lipid peroxidation. Also, in the study performed on the transgenic mouse, it was found that it suppresses Alzheimer disease, it has significantly reduced symptoms in stomach ulcers [13–16]. On the anticancer property of rosemary, it has been revealed in the literature that it has healing effects in many cell lines such as colon cancer, breast cancer, prostate cancer, cervical adenocarcinoma and ovarian cancer [17]. The effectiveness of *Peganum harmala*, which has been tested in cells such as leukemia, breast cancer, ovarian cancer and stomach cancer, has been demonstrated by previous studies [18–20]. DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN®, these food supplements with herbal products are used in remember regeneration therapy method (RTM) [21].

The aim of current study is to investigate the anticancer effect of food supplements formed with herbal products with anticancer properties on osteosarcoma.

MATERIALS AND METHODS

Preparation of herbal extracts

Five different herbal product that is being sold in the market in Turkey; DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN® were evaluated in our study (Table 1). Dry extracts of plants were obtained from Naturin Nutraceutical in Turkey. 1 g of dry extract was dissolved in 5 ml of water in a 70 ˚C water bath for 90 minutes. The extracts were then centrifuged at 2500 rpm for 20 minutes and their supernatant removed. The solution was diluted with DMEM (Dulbecco's Modified Eagle's Medium) culture medium at 1000, 2000, 3000, 4000, 5000, 6000 µg/ml using 0.22 mm filters for sterilization of the solution. Preferred dose ranges of this mixture are preferred over the dose ranges in which these herbal mixtures are known to be effective [22–25].

Table 1. Herbal product contents

Herbal Products	Product Contents
REGULIN®	*Curcuma longa extract* (118 mg), *Silybum marianum extract* (118 mg), *Rosmarinus officinalis extract* (118 mg), *Juniperus communis* extract (118 mg), *Fumaria officinalis extract* (59 mg), *Cichorium intybus extract* (59 mg)
IST-GLIO®	*Curcuma longa* (67 mg), *Curcuma longa seed* (237 mg), *Peganum harmala* (133 mg), *Silybum marianum* (89 mg), *Zingiber officinale* extract (74 mg), *Nigella sativa seed* (68 mg), *Juniperus communis* fruit (44 mg), *Thymus sp* (15 mg), *Foeniculum vulgare* (4 mg), *Pimpinella anisum* (3 mg), *Cassia acutifolia* (3 mg), *Eugenia caryophyllata* (3 mg).
ROMX®	*Curcuma longa* extract (44 mg), *Juniperus communis* extract (158 mg), *Zingiber officinale* extract (127 mg), *Peganum harmala* extract (114 mg), *Thymus sp.* extract (95 mg), *Nigella sativa* extract (38 mg), *Foeniculum vulgare* extract (19 mg), *Pimpinella anisum* extract (15 mg *Cassia acutifolia* extract (15 mg), *Eugenia caryophyllata* extract (15 mg).
IST-REM®	*Curcuma longa* extract (68 mg), *Urtica sp.* seed (272 mg), *Silybum marianum* seed (102 mg), *Peganum harmala* seed (102 mg), *Nigella sativa* extract (68 mg), *Zingiber officinale* extract (68 mg).
DVD-ARD®	*Curcuma longa* extract (118 mg), *Silybum marianum* extract (118 mg), *Rosmarinus officinalis* extract (118 mg), *Juniperus communis* extract (118 mg), *Fumaria officinalis* extract (59 mg), *Cichorium intybus* extract (59 mg).

Cell culture

Experimental study was carried out at Düzce University Experimental Animals Application and Research Center. In the study, Saos-2 cell line was obtained from Bolu Abant İzzet Baysal University. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma) with 10% Fetal Bovine Serum (Sigma) and 1% penicillin + streptomycin (Sigma) broth containing mixture, 37 ˚C in medium containing 5% CO_2 and 95% humidity 25 cm^2 incubated in flasks.

MTT cell viability test

3-(4,5-dimethyltriazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is frequently used as a method for determination of cell viability [26]. Viable cells are easily able to be determined by absorbance of color due to the mitochondria-dependent reaction. MTT reduction property of the cells and MTT assay results obtained in the color density correlates with the number of viable cells. 10 ml MTT (5mg/ml) was added to each well. Cells were incubated at 37 ˚C for 4 hours after treatment with MTT. By removing the supernatant from each well, 100 ml of DMSO was added to the wells and the plate was kept in the incubator for 10 minutes and absorbance was measured at 570 nm by using microplate arrow (Cytation ™ Biotek, USA). Each reading was calculated according to the absorbance x 100/control absorbance formula of the cells. IC 50 values were calculated using the curve by plotting the percentage of extracted different concentrations and cell viability.

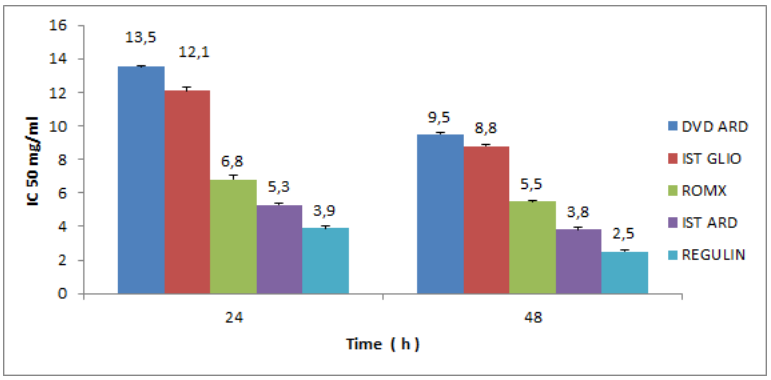
Statistical analysis

Statistical analysis was performed using ANOVA test by SPSS version 24 program and (P value<0.05) was regarded as significant. Experiments were performed in triplicate and expressed as the means ± standard deviation. Experiments were performed in triplicate and expressed as the means ± standard deviation values in each column showed significant differences (P<0.05).

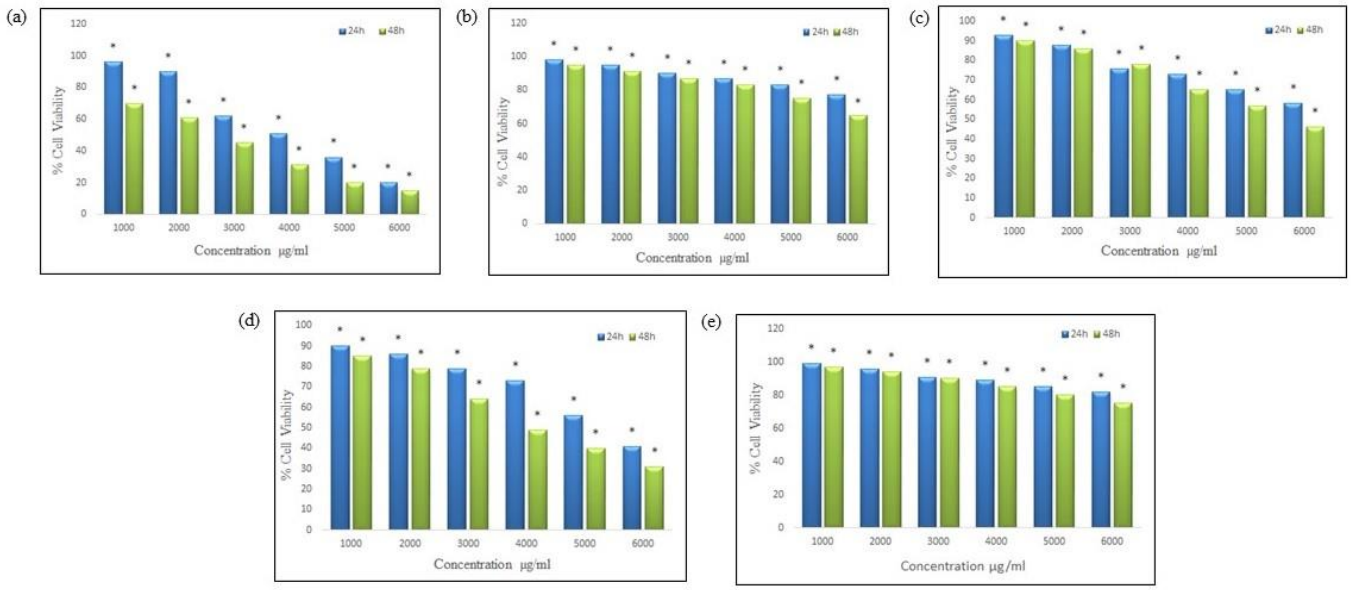
RESULTS

According to the results of graphic 1 the lowest IC_{50} values that shows the most effective extracts belonged to REGULIN® extract at the concentration of 6000 µg/ml, within 48h which was about IC_{50} = 2.5 ± 0.001 mg/ml with 15% cell viability of Saos-2 cells.

The effect of various concentrations of extracts on proliferation of Saos-2 cells within 24 and 48 hours was evaluated by MTT assay. According to the data statistical analysis of Graphic 2, extracts showed a dose-time dependent inhibition of cell viability which means cytotoxic effect of extracts increased with increasing concentrations of extracts also bypassing exposure time of the cells to the extracts. The most cytotoxic effect of extracts belonged REGULIN®>IST ARD®>ROMX®>IST GLIO®>DVD ARD® respectively.



Graphic 1. Mean IC50±SD (mg/ml) values of Saos-2 cell lines treated with different concentrations of DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN® extracts (1-6 mg/ml) for 24 and 48 h.



Graphic 2. Cytotoxic effect of (a) REGULIN®, (b) IST GLIO®, (c) ROMX®, (d) IST ARD®, (e) DVD ARD® (1000-6000 µg/ml) on cell viability of Saos-2 cancer cell line for 24 and 48 h. * In each column indicates significant differences between concentration of 1000-6000 µg/ml ($p<0.05$).

DISCUSSION

Osteosarcoma is a rare but highly malignant bone cancer that occurs in the skeletal system and generally affects children, adolescents and young adults [27]. Current treatments for osteosarcoma include chemotherapy, surgical interventions, or radiotherapy, but these treatments cannot provide adequate survival [28]. In our study, the anticancer effect on osteosarcoma cells was investigated by using food supplements with different herbal ingredients. IC50 values were calculated on Saos-2 cells. Among the five products that we evaluated their effectiveness, REGULIN® was the highest mixture with the IC50 value of 2.5 mg/ml.

There are various plants are that known to have anticancer effects in the content of each of the five different mixtures we used in our study. The anticancer properties of many of these plants have been proven and there is no study comparing the synergistic effects of herbal mixtures available in the market today, but the bioactive compounds of each plant are being investigated on various cancer cells. Plants contain secondary metabolites such as phenol and flavonoids with antioxidant properties, which have strong potential to clear free radicals to prevent diseases such as cancer [27].

There are various extracts that known to have anticancer effects in the content of each of the five different mixtures we used in the study. Anticancer activity of n-hexane turmeric extract from these extracts has been frequently demonstrated. The effect of turmeric on lung cancer was evaluated in the study in which telomerase activity was evaluated and its potential to be converted into drugs [29]. The anticancer effect of turmeric (*Curcuma longa L.*) aqueous extract on sarcoma and breast cancer has been demonstrated [30]. Rosemary (*Rosemarinus officinalis L.*) extract reveals the antitumor potential of prevention, development and drug response in various types of cancer, thereby emphasizing in previous studies that clinical studies should be initiated to confirm the possible use fulness of this extract as a complementary agent for certain cancer patients [31]. The anticancer effect of stinging nettle (*Urtica dioica*) has been investigated in breast cancer cells and it has been revealed that its use with cancer drug paclitaxel may be potential for treatment [32]. Positive results were obtained in prostate cancer, in which the apoptotic effects of nettle herb dichloromethane

extract were examined [33]. Juniper (*Juniperus phoenicea*) activities in human lung, breast, liver cells have been demonstrated [34]. Although the efficacy of more well-known extracts on breast cancer, such as harmal (*Peganum harmala L.*), ginger (*Zingiber officinale*), rosemary (*Rosamarinus officinalis L.*), juniper *(Juniperus communis*), has not been tested, its effectiveness on osteosarcoma has not been investigated [30,35–37]. The anticancer activity of *Caulis spatholobi* ethyl acetate extract, which is frequently used in Chinese medicine with such antioxidant properties, has been demonstrated on human osteosarcoma cell Saos-2 [22]. The anticancer effect has been revealed by targeting molecular mechanisms on osteosarcoma cell [38]. The compound of curcumin, which is an indispensable part of traditional medicine, in turmeric, has been known to have cytotoxic effects in many studies in vitro and in vivo. However, its instability and poor metabolic features limit its clinical application. There are studies investigating the effect of curcumin on Saos-2 [27,39,40]. The anticancer effect of *Tilia dasystyla* and *Polygonatum orientale* extracts on Saos-2 has been demonstrated [25].

In addition to investigating the anticancer effect of different herbal extracts on osteosarcoma, the molecular mechanisms of the synergistic effect of co-administration are planned in our future studies. These components are known to regulate epigenetic mechanisms at the cellular level. So, as mentioned in the literature, the RTM to traditional and complementary medicine treatments with herbal ingredients [21].

CONCLUSION

In this study, results indicate that extracts of five different herbal product including; DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN® showed cytotoxic effect on Saos-2 cancer cell line due to their mixture of medicinal plants, however REGULIN® showed the most cytotoxic effect among them and the reason of REGULIN® effectiveness requires more detailed investigation in subsequent studies. Cytotoxic effect of these food products on other cancer cell lines and their use in the pharmaceutical and health industries is recommended in future studies.

Additional Information

A part of this study was presented as a poster at the International 2. Traditional and Complementary Medicine Congress, 2019, 24-27 April, Istanbul/Turkey.

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