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Full Length Research Paper

Effect of *Plantago major* sap on Ehrlich ascites tumours in mice

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Among the various known therapeutic effects of *Plantago major* (L.), a few recent studies have shown that preparations of the crude extracts of some plant leaves could prevent or regress the growth of certain tumours. In this study, the effect of *P. major*, an anticancerogenic, against Ehrlich ascites carcinoma in mice was investigated. The animals were separated into five groups: two groups for control (HC, healthy control; TC, tumour control) and three groups for experiments E1 (25 µg/ml extract given after tumour inoculation), E2 (50 µg/ml extract given after tumour inoculation) and E3 (75 µg/ml extract given after tumour inoculation). Ehrlich ascites tumours (1x10^6 cells) were injected intraperitonally into the mice's of E1, E2, E3 and TC groups. *P. major* extract was given in three different concentrations for 10 days orally. Following the administration, all animals sacrificed and their intestine and colon tissues taken out then stained with haematoxilen-eosin for pathological investigations. Pathological findings stated out that *P. major* extract had inhibitory effect against Ehrlich ascites carcinoma. Therefore, our results show that *P. major* could be proposed as an effective agent for cancer prevention.

Key words: Plantain, experimental, anti-cancer, crude extract.

INTRODUCTION

Plantago major L. is a perennial plant that belongs to the Plantaginaceae family (Samuelsen, 2000). It is renowned as a traditional herbal medicine throughout the world. *P. major*, a popular traditional cure that has been used for many diseases varying from cold to viral hepatitis (McCutcheon et al., 1995). The use of *P. major* in wound healing in Scandinavia, ulcers treatments in Turkey (Yesilada et al., 1993), against skin problems and gastro-intestinal disorders in Mexico (Samuelsen, 2000), is also rather old (Roca-Garcia, 1972). *P. major* leaf has been used as a diuretic agent in Guatemala (Caceres et al., 1987). Furthermore, teas of *P. major* have free radical scavengers (Campos and Lissi, 1995).

In the last decade, *P. major* preparation was reported to be effective in a screening system for prophylactic oncology and anti-metastasis effect on mice tumors (Yaremenko, 1990). In another study, an aqueous extract have been shown to have a prophylactic effect on mammary cancer in mice (Lithander, 1992). Methanolic extracts from seven *Plantago* species were used in traditional medicine for the treatment of cancer in USA. Then this treatment was evaluated for cytotoxic activity against three human cancer cell lines. Thus, the plant is registered and recommended by the National Cancer Institute (NCI, USA) too. According to the recommendation of NCI, *Plantago* extract have growth inhibitory and cytotoxic effects on breast adenocarcinoma and melanoma cell lines (Galvez et al., 2003).

Plantago extract have inhibitory effect against breast

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carcinoma *in vivo* as well as breast carcinoma and also melanoma cells *in vivo* and *in vitro*. Our goal is to investigate the anticancer effect of *P. major* against transplantable experimental tumor, Ehrlich ascites carcinoma (EAC), in mice.

METHODS

The preparation of crude extract

P. major leaves collected from the Botanical garden of the Gaziantep University. Ehrlich ascites carcinoma (EAC) tumor cells obtained from Physiology Department of Gaziantep University. Hot water extract of *P. major* was prepared from the leaves (Chiang et al., 2002). 25 µg sap was dissolved in 1 ml tap water. As the same procedure, 50 µg in 1 ml and 75 µg in 1 ml were prepared.

Determination of the effect *P. major* on ascites tumors

Male *Mus musculus* Balb/C mice (8 - 10 weeks old, 26 - 28 g) were used for this study. They were housed in wire cages with 12 h light/dark cycles at 23°C. The animals were fed with a standard pellet diet and tap water *ad libitum*. They were divided into 5 groups of six animals each (E1, E2, E3, HC and TC). Peritonitis carcinomatosa was formed with EAT at sterile conditions and ascites fluid were taken from mouse with paracenthesis (Ozaslan et al., 2007).

All groups of animals except group positive control 'HC' were transplanted i.p. with 1 x 10^6 EAC cells in 0.1 ml PBS. After 3 days, experimental groups were fed with *P. major* crude extracts (E1; 25 µg/ml, E2; 50 µg/ml and E3; 75 µg/ml crude extracts) per day orally. In the laboratory trials, 24 mice were inoculated with EAC. Then the drug administration were planned and continued for ten days. HC and TC groups were fed 0.9% NaCl for 10 days as like the rest of the mice fed *P. major* extracts in this period. At the end of the study, the animals were anesthetized with ether and sacrificed. Following scarification, intestine and colon tissues of all animals were removed and stained with the hematoxylin-eosin staining for histological examinations.

RESULTS AND DISCUSSION

The aim of present study was to examine the anti-tumoral effect of *P. major* aqueous crude extract against EAC in mice. It was observed that intestine and colon tissues from animals of only tumor inoculated TC group were invaded by tumor cells (Figures 1 and 2). It was determined that tissues from tumor-not inoculated HC group were normal architecture. Intestine tissues from 25 µg/ml dose crude extract-treated group E1 were invaded by tumor cells as the same with that of group TC (Figure 3). In spite of this, three colon tissue samples from group E1 were normal architecture (Figure 4a) and 3 of them were invaded on seroza layer by tumor cells (Figure 4b). Assessment of colon tissues belong to group E1 showed that *P. major* crude extract (25 µg/ml dose) had a tumor inhibitory effect. Intestine tissues preparations belonging to E2 were invaded by tumor cells like that of E1 (Figure 5). In assessment of colon, tissue preparats belonging to this group, two of them were normal colon architecture (Figure 6a), one was invaded serosa layer (Figure 6b)

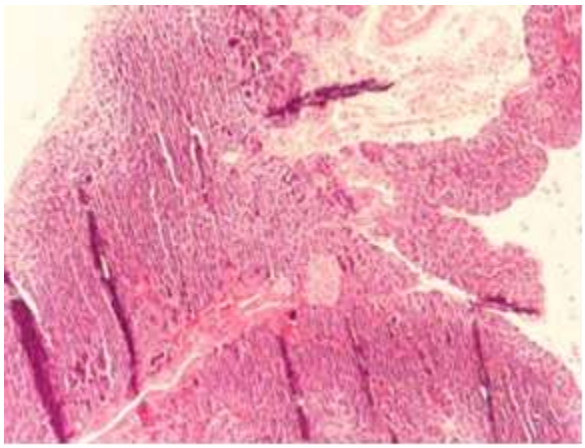

Figure 1. Necrosed intestine tissue from group TC (tumour control).

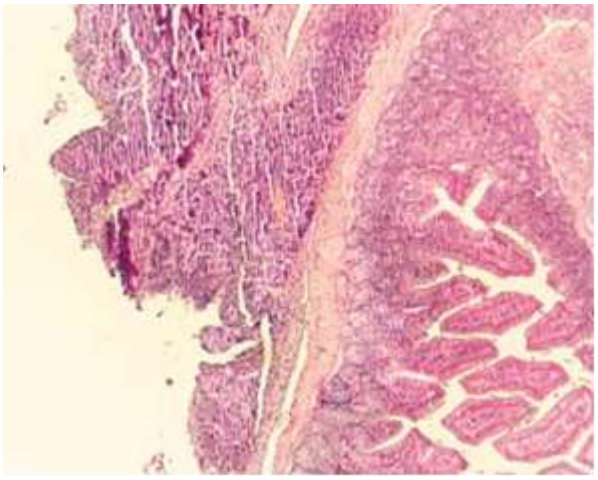

Figure 2. Completely invased colon tissue from group TC (tumour control).

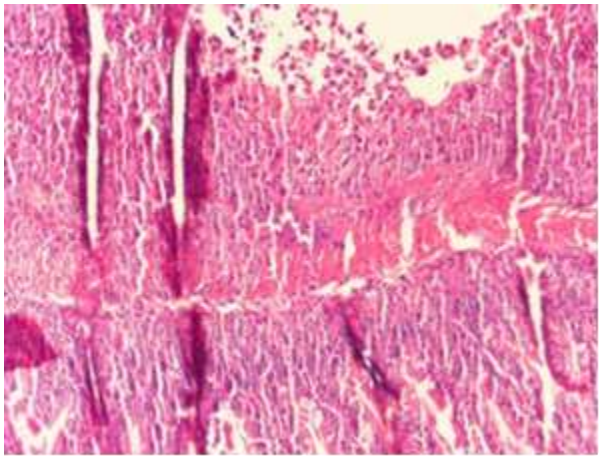

Figure 3. Completely necrosed intestine tissue from group E1 (25 µg/ml *P. major* crude extract given after tumour inoculation in mice).

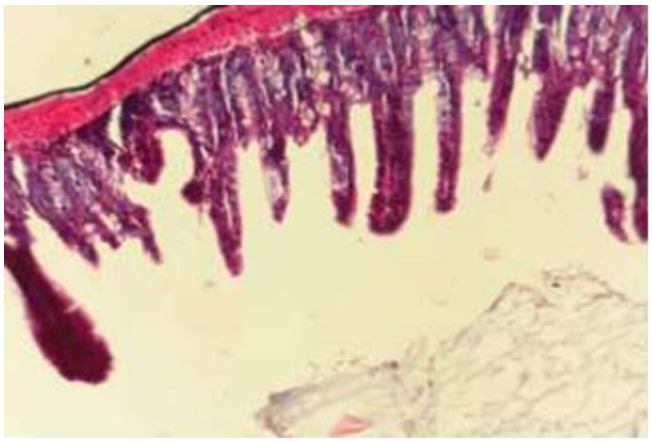

Figure 4a. Normal colon tissue from group E1 (25 µg/ml *P. major* crude extract given after tumour inoculation in mice).

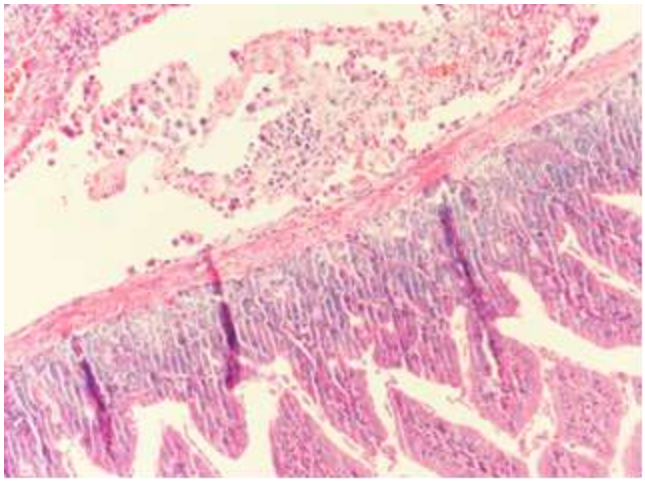

Figure 4b. Only serosal invased colon tissue from group E1 (25 µg/ml *P. major* crude extract given after tumour inoculation in mice).

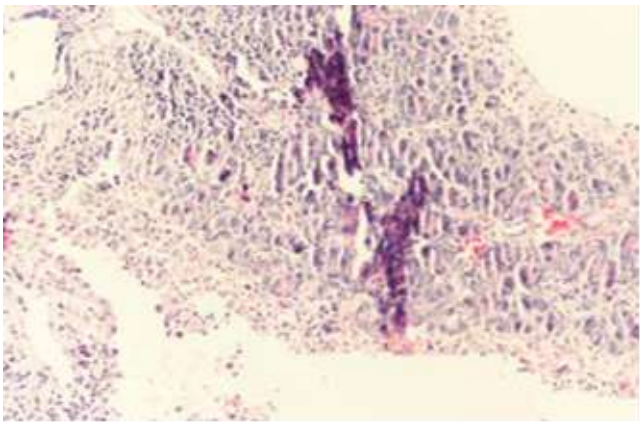

Figure 5. Completely necrosed intestine tissue from group E2 (50 µg/ml *P. major* crude extract given after tumour inoculation in mice).

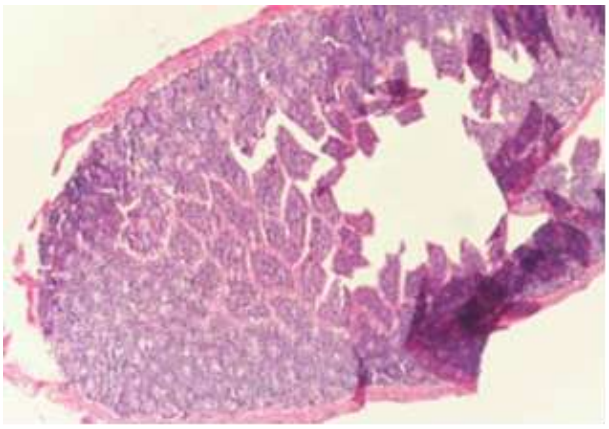

Figure 6a. Normal colon tissue from group E2 (50 µg/ml *P. major* crude extract given after tumour inoculation in mice).

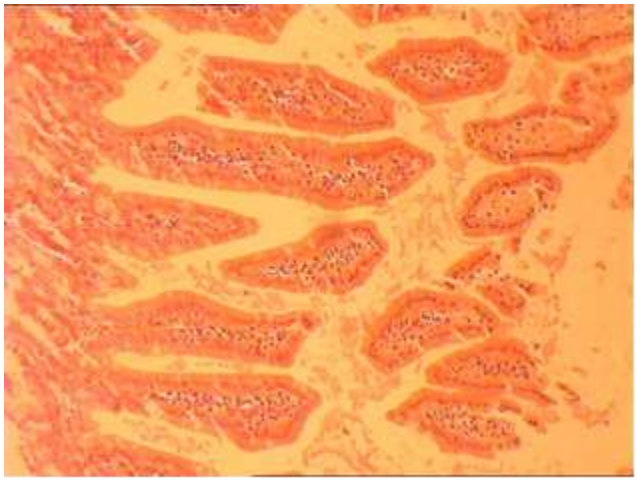

Figure 6b. Only serosal invased colon tissue from group E2 (50 µg/ml *P. major* crude extract given after tumour inoculation in mice).

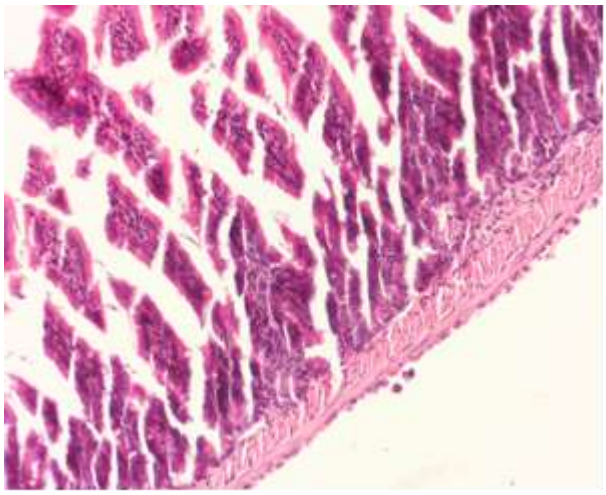

Figure 6c. Completely invased colon tissue from group E2 (50 µg/ml *P. major* crude extract given after tumour inoculation in mice).

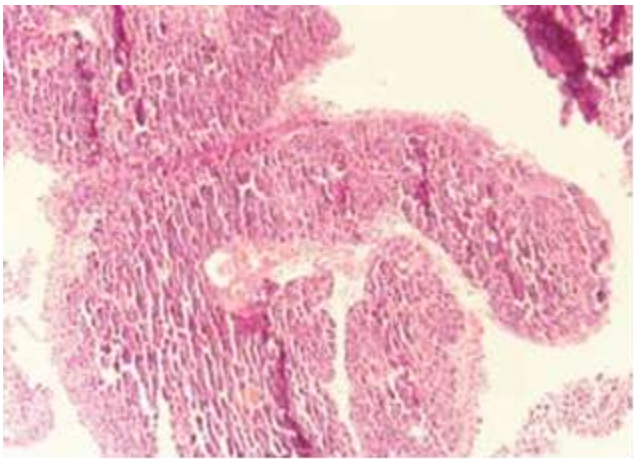

Figure 7. Completely necrosed intestine tissue from group E3 (75 µg/ml *P. major* crude extract given after tumour inoculation in mice).

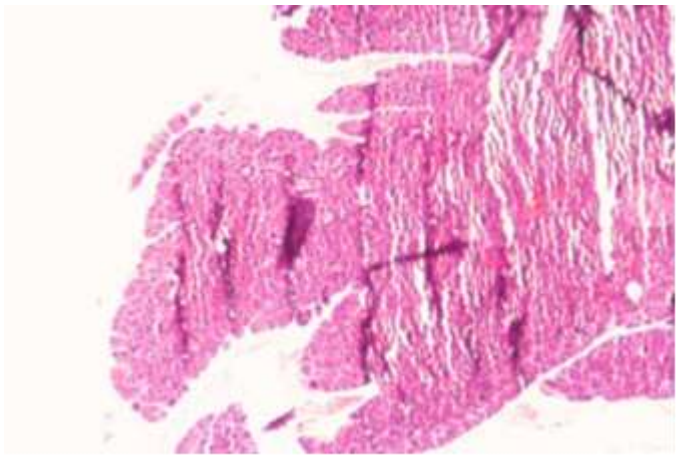

Figure 8a. Completely invased colon tissue from group E3 (75 µg/ml *P. major* crude extract given after tumour inoculation in mice).

and three were invaded in all layers (mucosa, sub mucosa, muscularis externa and serosa) of tissue (Figure 6c) as detected. These results showed that tumor relative inhibition was in group E2. In group E3, intestine tissues were also invaded completely like the others (Figure 7). In screening of colon tissue histological assessment belong to group E3, two of them were invaded all layers of colon (Figure 8a), two invaded with serosa layer of colon (Figure 8b) and two with normal colon architecture (Figure 8c) was determined.

P. major have long been used among the people as remedy against different diseases. Reports about traditional uses of *P. major* are generously found in literature. They are about anti-ulserogenic, antibiotic, antioxidant, anti-inflammatory and analgesic and diuretic effects of *P. major* (Caceres et al., 1987; Yesilada et al., 1993; Campos and Lissi, 1995; Guillen et al., 1997). *P. major* contains ferulic acid and caffeic acid which has been demonstrated to possess activity against herpes simplex virus 2 (HSV-2) *in vitro* (Bourne et al., 1999). In addition to studies about biological activities of *P. major*, its toxic effect was also studied. In conclusion, of these studies, *P. major* was determined to possess a low toxicity in rats at oral and i.p. administration (Angelov et al., 1980). Taking the claimed low toxicity and biological activities of *P. major* into consideration, *P. major* seems to be an active agent against different diseases.

Some researchers abundantly publish reports about traditional uses of *P. major* for cancer treatment. In a screening of anticancer activity of *P. major*, its crude extract has been used to treat cancer in Chile, Panama, Venezuela, and Canary Islands (Bhakuni et al., 1976).

This study was planned on antitumoral effect of *P. major*. At the end of the study, *P. major* was showed to have an antitumoral effect on transplantable, experimental tumor EAC. Intestine tissues of *P. major* treated-all experimental groups were completely distorted by tumor cells as compared to group negative control. *P.*

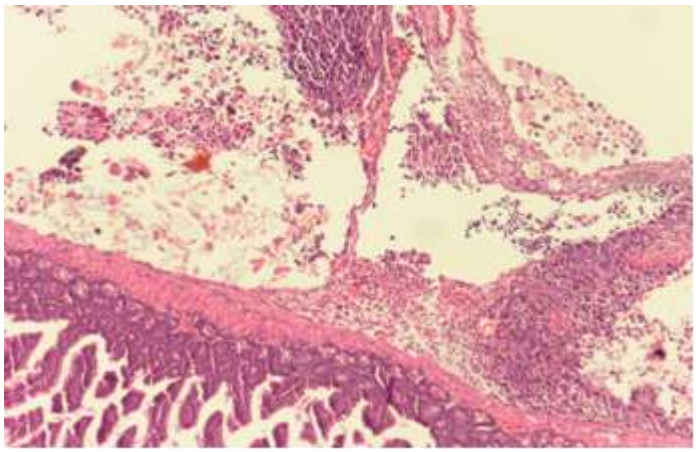

Figure 8b. Only serosal invased colon tissue from group E3 (75 µg/ml *P. major* crude extract given after tumour inoculation in mice).

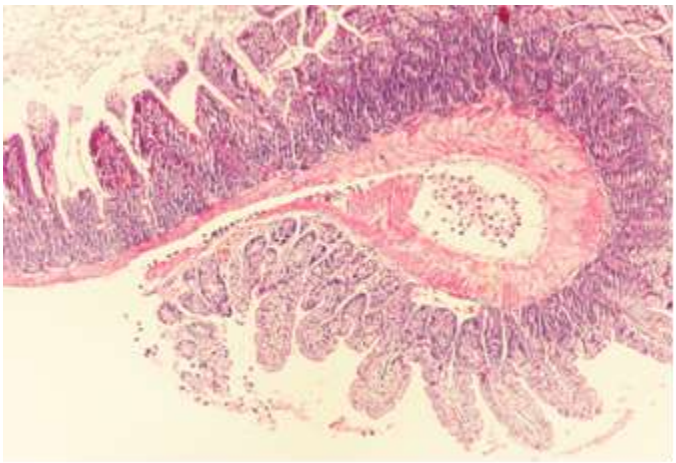

Figure 8c. Normal colon tissue from group E3 (75 µg/ml *P. major* crude extract given after tumour inoculation in mice).

major crude extract caused-inhibition was considered in a screening of colon tissue. Although all layers of intestine was completely distorted by EAC cells, some of colon tissue was distorted like intestine tissue, some of them invaded only serosa layer and the others had the normal architecture. Tumor inhibition degree among the groups could be compared because different pathologic conditions of colon tissue belong to each group. Three of the colonic sections from group E1 appeared to have normal histological architecture and three of them were distorted in only serosa layer. This group was the most inhibited observed-group. In the screening of pathologic conditions of group E2 colon tissue, two colonic sections appeared normal, three were invaded in only serosa and one was completely distorted. Tumor inhibition degree at this group was remarkable as compared to group TC but in comparison to group E1, the inhibition decreased significantly. In assessment of group E3 colonic sections, two of them were completely distorted, two were invaded only serosa and two appeared to have normal architecture. In comparison to obtained inhibition rates, tumor inhibition was less than the others but it was also significant compared to group TC.

According to results of our research work, it is concluded and proved that *P. major* prevented tumor extension. *P. major* crude extract showed tumor inhibitor activity on the EAC cells at the treated doses. The amount of each group's inhibition can be presented as follows; group E1 > group E2 > group E3. Furthermore, inhibition decreased due to increasing crude extract concentration and 25 µg/ml dose was determined to be the most effective dose rather than the other experimental doses. This can be due to unknown content of plant sap; some ingredients of *P. major* can have toxic effect and/or side effect on lower gastrointestinal tissues after the level of the acceptable concentration, that is, 25 - 50 µg/ml. Therefore, active compounds containing *P. major* should be determined and their direct effect against cancer needs further and detailed research works. We propose that *P. major* can be taken into consideration in cancer therapy.

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REFERENCES

Angelov A, Lambev I, Markov M, Yakimova K, Leseva M, Yakimov A (1980). Study of acute and chronical toxicity of dispergue of *Plantago major*. Med. Arch. 18: 47-52.

Bhakuni DS, Bittner M, Marticorena C (1976). Screening of Chilean Plants for antiancer activity. Lloydia 39: 225-243.

Bourne KZ, Bourne N, Reising SF, Stanberry LR (1999). Plant Products as Topical Mikrobicide Candidates; Assessment of in vitro and in vivo Activity Against Herpes Simplex Virus Type II.. Antiviral Res. 42: 219-226.

Campos AM, Lissi EA (1995). Evaluation of the Antioxidant Capacity of Herbal Teas by a Procedure Based on the Bleaching of ABTS Radical Eations. Boletin de la Sociedad Chilena de Quimica 40: 375-381.

Chiang LC, Chiang W, Chang MY, Ng LT, Lin CC (2002). Antiviral Activity of Plantago major Extracts and Related Compounds In vitro. Antiviral Res. 55: 53-62.

Galvez M, Martin-Cordero C, Lopez-Lazaro M, Cortes F, Ayuso MJ (2003). Cytotoxic effect of Plantago spp. on cancer cell lines. J. Ethnopharmacol. 88: 125-130.

Guillen MEN, Emim JAS, Souccar C, Lapa AJ (1997). Analgesic and Antiinflammatroy Activities of the Aqueous Extract of *Plantago major* L. Int. J. Pharmacognosy 35: 99-104.

Lithander A (1992). Intracellular Fluid of Waybread (Plantago major) as a Prophylactic for Mammary Cancer in Mice. Tumor Biology 13: 138-141.

Mc Cutcheon AR, Roberts TE, Gibbons E (1995). Antiviral Screening of British Colombian Medicinal Plants. J. Ethnopharmacol. 49: 101-110

Ozaslan M, Karagoz ID, Kalender ME, Kılıc IH, Sarı I, Karagoz A (2007). *In vivo* Antitumoral Effect of Plantago major L. Extract on Balb/C Mouse with Ehrlich ascites tumour. Am. J. Chin. Med. 35(5): 841-851.

Samuelsen AB (2000). The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review; J. Ethnopharmacol. 71: 1-21.

Yaremenko KV (1990). Adaptogens of the Natural Origin in Prophylactyc Oncology. J. Cancer Res. Clin. Oncol. 116: 82.

Yesilada E, Sezik E, Fujita T, Tanaka S, Tabata M (1993). Screening of Some Turkish Medicinal Plants For Their Antiulcerogenic Activities. Phytotherap. 7: 263-265.