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STUDY OF IMMUNOLOGICAL EFFECTS OF CRUDE EXTRACT OF *PORTULACA OLERACEA* L. IN THE TREATMENT OF TRANSPLANTED MAMMARY TUMOR IN FEMALE ALBINO MICE IMMUNIZED WITH *CANDIDA ALBICANS* Ag.

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ABSTRACT
This study was designed to evaluate the immunological effect of 70% ethanolic crude extract of *Portulaca oleracea* L in the treatment mice mammary adenocarcinoma cell line (*in vivo*). Immunological study was done on 4 animals groups, 200 mg/ kg .B.W subcutaneously of 70 % ethanolic extract of *Portulaca oleracea* injecting for tumor- bearing female mice (group II) and healthy female mice (group III) for 30 days. Tumor –bearing female mice (group I) and healthy female mice (group IV) were treated with D.W. killed *Candida albicans* were utilized for preparation of antigen to estimated the immune response. All groups (I-IV) were immunized at 10 and 20 days of the experiment with whole killed *Candida albicans* antigens subcutaneously. The results showed an increase in delayed type hypersensitivity reaction and antibody titer in tumor-bearing female mice which treated with 200 mg/kg B.W ethanolic extract of *Portulaca oleracea* S/C (groupII)(5.18±0.21mm, 618.0±2.22) respectively, compared with distilled water treated tumor –bearing female mice (group I) (2.55±0.06 mm, 34.67±1.48) respectively. While values of healthy female mice treated with ethanolic extract of *Portulaca oleracea* (group III) were (5.96±0.01 mm, 981.33±5.34) respectively, compared with distilled water treated healthy female mice (group IV) (3.58 ±0.21 mm, 128±9.60) respectively.

KEY WORDS: Immunological effect of Purslane on mammary tumor bering immunized mice.

INTRODUCTION

After a century of controversy, the notion that the immune system regulates cancer development is experiencing a new resurgence. For the last five decades, much of the debate centered on the validity of the cancer immunosurveillance hypothesis originally proposed by Burnet and Thomas[1,2]. Tumor have antigens that can be broadly classified into two categories: tumor specific antigens (TSAs), which are present only on tumor cells and not on any normal cells, and tumor-associated antigens (TAAs), which are present on tumor cells and on some normal cells[3]. Cell-mediated immunity (cytotoxic T lymphocytes, natural killer cells and magrophage) and humoral immunity can have antitumor activity; through recognize antigens that expressed on tumor cells[4]. Cytokines are proteins made by various cells, and most certainly involved in the activation of immune effectors mechanisms that limit the growth of the tumor[5, 6]. The use of medicinal plants wide spread now in order to prevent the side effects of chemical drugs. The specificity of medicinal plants is due to the presence of chemical compounds in their tissues which have beneficial effects on human and animal, these compounds may include alkaloids, glycosides compounds, aromatic oil and tannic substance[7]. Some of plants have been well studied in various experimental models of cancer, both *in vivo* and *in vitro* models [8]. They have shown significant inhibition of cell proliferation, some of them are in the phase of clinical trial or already available as food supplement. Cancer patients are specially exploring the use of Complement and alternative medicine (CAM), because of the high risk of mortality and long-term morbidity associated with surgical procedures of cancer management and high side effects of chemotherapy [9]. *Portulaca oleracea* (purslane) is a rich source of omega-3 fatty acids, which are beneficial in congenital heart disease (CHD) and certain cancers[10] carbohydrates, lipids, glycosides, alkaloids, sterols, triterpenes, and flavonoids [11]. *Portulaca oleracea* plant has different useful medical properties, as analgesic, antiarthritic, antiarteriosclerotic, anticancer (Colon, forestomach, liver, skin) activities[12]. but there are no data that have been published regarding the antitumor activity of *Portulaca oleracea* even the relationship with cancer in Iraq or in the worldwide therefore, the present study was designed to investigate the immunological effects of *Portulaca oleracea*, treatment of transplanted mammary tumor in female mice.

MATERIALS & METHODS

1- Collection and extraction of plant

Portulaca oleracea plant was obtained from field of College of Veterinary Medicine, University of Baghdad. Representative specimens (leaves and stems) were taken to the College of Science, Botany Department, University of Baghdad and identified by Professor Dr. Ali- AL-Mosawy as *Portulaca oleracea* L, Family Portulacaecea. Plant extraction was done According to [13].

2- Median lethal dose

Graduated doses of *Portulaca oleracea* ethanolic extract were dissolved in 10 ml distill water and administered S/C as 0.1 ml for each 10 gm of animal body weight. The range was of S/C single doses used in the determination of LD50 of the extract was (5000- 9500) mg /kg B.W. Mortality was recorded after 24 hrs and LD50 was calculated according to up and down method described by [14].

3- Animals treated with ethanolic extract of *Portulaca oleracea*

By returning to the results of LD50, and value reported in some references[15], the dose was adjusted in this study was (200 mg/ kg B .W for S/C injection daily for 30 days.

Candida albicans Stock was obtained from Department of Pathology /College of Veterinary Medicine / University of Baghdad.

4- Preparation of *Candida albicans* antigens

A-Culture media preparation for *Candida albicans* was according to technique used by [16].

B-Whole killed *Candida albicans* antigens were prepared according to [17].

***C*-Whole *Candida albicans* sonicated antigens** prepared according to[18]. Then protein concentration of sonicater antigen measured according to [19] using Biuret Kit (1.85 mg/ml).

5- Delayed type of hypersensitivity (DTH-Skin reaction) test was done according to [20].

6-Passive Heamagglutination Test (PHA) was done according to [21].

7-Transplantation of tumor cells in mice: was performed according to [22].

8-Experimental animals: Female adult albino mice (BALB/c), 8-10 weeks aged, their weight were ranged 25-30g, kept in well Air-Conditioned rooms at the animal house in Iraqi Center for cancer & Medical Genetics Research (ICCMGR)/ University of AL-Mustanseraia, and given pellets of balanced specially prepared animal feed and water *ad libitum.*

The effect of ethanolic extract on immune system in mice:

Eighteen female adult mice were used for immunological and pathological studies. These animals were divided into 4 groups, as follows:

I- Four adult female albino mice bearing tumor mass injected S/C daily with D.W for 30 days (control group).

II-Four adult female albino mice bearing tumor mass injected S/C daily with 200 mg / kg B. W of ethanolic extract of P. O for 30 days (treated group).

III- Five adult female albino mice (healthy animals) injected S/C daily with 200 mg / kg B. W of ethanolic extract of P. O for 30 days.

IV- Five adult female albino mice (healthy animals) injected S/C daily with D.W for 30 days and served as control.

At tenth day of experiment, all animals were immunized by inoculation with 0.25 ml of whole killed antigen of *Candida albicans S/C* (9×10^8 CFU / ml), and the booster dose was 0.5 ml injected S/C at 20 the day of experiment. At 27^{th} day skin test was done for all groups, at 30 the day all animals were sacrificed and blood collected directly from the heart for passive Heamagglutination test

RESULTS & DISCUSSION

Immunological study

1-Skin test

Cell mediated immunity was detected in different treated groups by increase in the footpad skin thickness of mice after 24 hrs.

Figure (1) shows a highly significant increase in right footpad thickness after 24hrs ($P \leq 0.01$) in healthy mice treated with 200mg/ kg. B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days and immunized with killed antigen (group III) (5.96±0.01 mm), compared with healthy mice treated with distill water and immunized with killed antigen (group IV) (3.58 ±0.21mm). However, highly significant increase was recorded ($P \leq 0.01$) in tumor-bearing mice treated with 200mg/kg .B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days and immunized with killed antigen (group II) (5.18±0.21mm), compared with tumor-bearing mice treated with distill water and immunized with killed antigen (group I) (2.55±0.06 mm).

2-Passive haemagglutination test (PHA):

The results showed highly significant increase ($P \leq 0.01$) in treated groups compared with their controls. Figure (2) shows highly significant increase ($P \leq 0.01$) in antibody titer in tumor-bearing mice treated with 200mg/kg .B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days and immunized with killed antigen (group II) (618.0±2.22) compared with tumor-bearing mice treated with distill water and immunized with killed antigen (group I) (34.67±1.48). And there was highly significant increase ($P \leq 0.01$) in antibody titer in healthy mice treated with 200mg/ kg. B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days and immunized with killed antigen (group III) (981.33±5.34) compared with healthy mice treated with distill water and immunized with killed antigen (group IV) (128±9.60).

Cell mediated immunity is the dominant antitumor mechanism *in vivo*, although antibodies can be made against tumor after administration of monoclonal Ab against tumor cells[4]. The results of skin test and heamagglution test indicate that *Portulaca oleracea* have capability to enhance the immune system, observed in group tumor-bearing female mice treated with P.O (group II) and healthy female mice treated with P.O (group III), compared with tumor-bearing female mice of control group (group I) and healthy female mice (group IV) both of them treated with distill water.

Non treated tumor-bearing female mice (group I) showed severe immune suppression in the animals which indicate that tumor cells have greatest effect on immune system .It is commonly observed that tumor –bearing animals are immune suppressed [23]. Researchers mentioned that tumor cells have ability to secrete immunesupression factors [24]. Tumor cells like ovarian carcinoma, B- cell lymphoma and melanoma secreting IL-10 which was decrease dendritic cells activity and development and as general immunsuppressor[25]. [26]Also mentioned that malignant brain tumor and others secret immunsuppression protein called Transforming growth factor –β (TGF-β) which causes inhibition of T cell CD^{+8} and B lymphocyte development[27]. Mammary adenocarcinoma cell line secretion in this study had ability to secrete

immunesuppression factors and decreased T cell proliferation when incubation with these cells *in vitro* [22] and mammary tumor suppressed the population of, T-Lymphocyte and cytotoxic T cells compared with mice not carrying tumor[28]. Group II and III showed high cellular and humoral immunity ,especially group III compared with group I and IV ,this referred that P.O may fight cancer by increasing immunity against its antigens. *Portulaca oleracea* have chemical compound may increase immune response. As a defense mechanism, the body produces a number of endogenous antioxidants capable of scavenging the harmful reactive oxygen species (ROS) to maintain an optimal oxidant, antioxidant balance, thereby maintaining normal cellular function and health. However, under conditions of high oxidative stress, the ability of these antioxidants to eliminate ROS is often exceeded and, therefore, dietary sources of antioxidants or drugs are required. The most widely used dietary antioxidants include vitamin E, vitamin C, carotenoids, flavanoids, zinc and selenium. The harmful effects of ROS are not unique to immune cells but affect all cell types. However, immune cells are particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of polyunsaturated fatty acid (PUFA) and they generally produce more ROS[29]. Antioxidant and carotenoid increased immunity by production Igs have been used in humoral immune response[30], increase lymphoblastogenesis *in vivo*[31] increasing innate and adaptive immunity against infection and tumor by increase lymphocyte cytotoxic activity, cytockines production [30] and increased delayed type hypersensitivity (DTH) response[32]. Many earlier studies focused on ß-carotene [33,34] reported a marked stimulatory action of ß-carotene on the growth of the thymus gland and a large increase in the number of thymic small lymphocytes. The stimulatory activity of ß-carotene on lymphocyte blastogenesis has similarly been demonstrated in rats[35], pigs[36], and cattle [37]. Dietary ß-carotene stimulated DTH response, the number of CD4+Th cells, and IgG production in dogs, Cats fed ß-carotene [38]. [39]Also showed heightened DTH response, higher Th and B cell subpopulations, and increased plasma IgG concentrations. Also dopamine has an ability to regulate B and T cell proliferation *in vivo* [40].

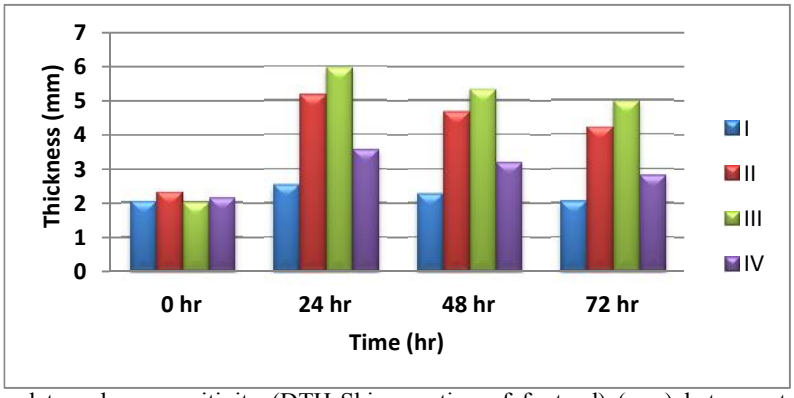

FIGURE1: Delayed type hypersensitivity (DTH-Skin reaction of footpad) (mm) between tumor-bearing female mice treated with distill water and immunized with killed antigen (group I), tumor-bearing female mice treated with 200mg/kg. B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days and immunized with killed antigen (group II) , healthy female mice treated with 200mg/ kg. B.W S/C of ethanolic extracts of *Portulaca oleracea* for 30 days and immunized with killed antigen (group III) and healthy female mice treated with distill water and immunized with killed antigen (group IV).

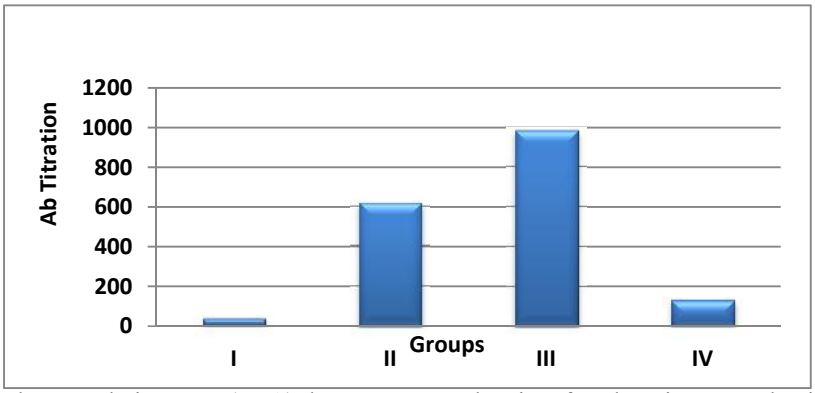

FIGURE 2: Passive heamagglution test (PHA) between tumor-bearing female mice treated with distill water and immunized with killed antigen (group I), tumor-bearing female mice treated with 200mg/kg .B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days and immunized with killed antigen (group II) , healthy female mice treated with 200mg/ kg. B.W S/C of ethanolic extracts of *Portulaca oleracea* for 30 days and immunized with killed antigen (group III) and healthy female mice treated with distill water and immunized with killed antigen (group IV).

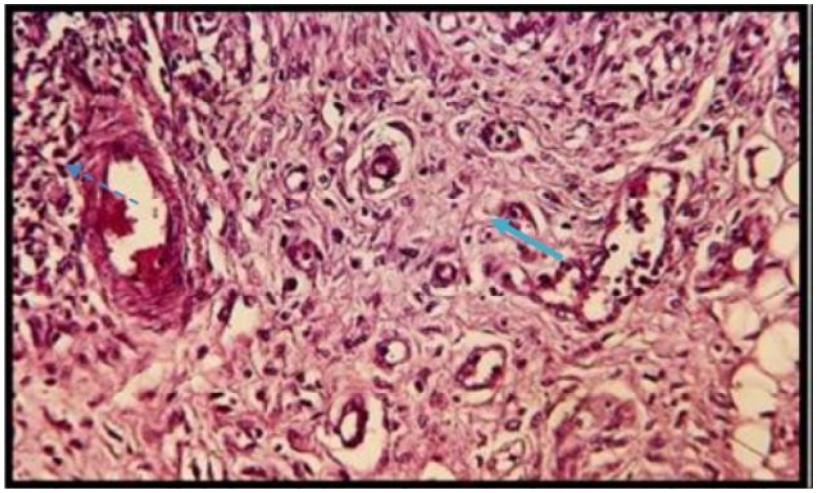

FIGURE 3: Histological section in Right footpad of tumor-bearing female mice treated with 200 mg/kg. B.W S/C of P.O for 30 days and immunized (group II) Showing granulation tissue formation (➡) with intravascular mononuclear cell infiltration (- - ➡) (200XH&E).

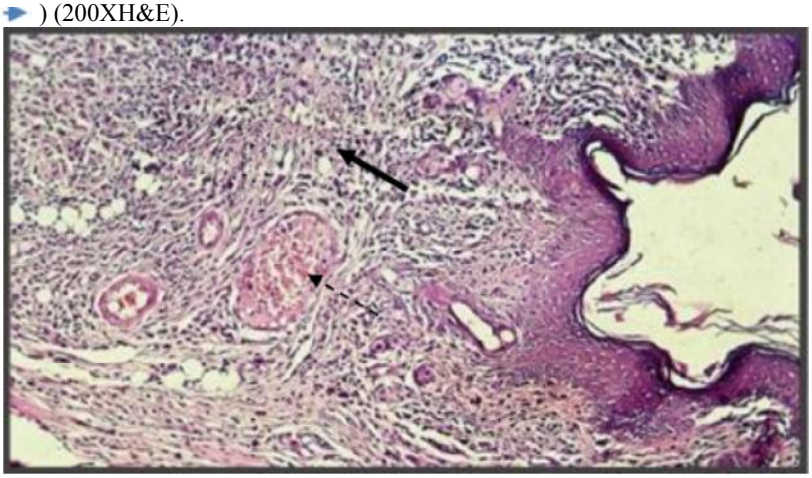

FIGURE 4: Histological section in footpad of healthy female mice treated with 200 mg/kg. B.W S/C for P.O for 30 days and immunized (group III) Showing granulation tissue formation (- - ➡) with sever congestion of B.V (➡) (200XH&E).

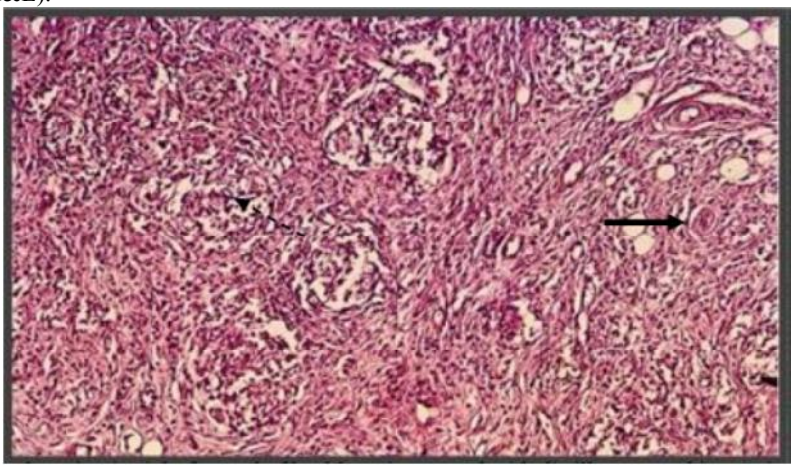

FIGURE 5: histological sections in right footpad of healthy mice treated with distill water and immunized (group IV), Showing granulation tissue formation (- - ➡) and congestion of B.V (➡) (200X H&E).

Pathology of delay type hypersensitivity (DTH)

Right footpad of tumor-bearing mice treated with distilled water and immunized (control group) (group I):

Histological section showed no lesion detected.

Right footpad of tumor-bearing mice treated with P.O and immunized (group II)

Histological section in this group showed granulation tissue formation in footpad with intravascular mononuclear cells infiltration (Figure: 3).

Right footpad of healthy mice treated with P.O and immunized (group III).
Showed granulation tissue formation and sever congestion of B.V in footpad (figure: 4). **Right footpad of healthy mice treated with distill water and immunized (group IV).** Section showed granulation tissue formation. (Figure: 5)

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