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STUDY OF PATHOLOGICAL 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 pathological effect of 70% ethanolic crude extract of *Portulaca oleracea* L. in the treatment mice mammary adenocarcinoma cell line (*in vivo*). Pathological 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. Histopathological sections shows hyperplasia of white pulp in the spleen with amyloid deposition, proliferation of megakaryocytes, mononuclear cells infiltration in liver and kidney parenchyma of group II, III, compared with non treated tumor bearing female mice (group I), which shows mild hyperplasia in white pulp vacuolar degenerative in hepatocyte and kidney parenchyma.

KEY WORDS: Pathological effect of purslane extract on Immunized mice bearing mammary tumor.

INTRODUCTION

Neoplasia, which literally means "new growth", is defined as an abnormal growth of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissue and persists in the same excessive manner after the cessation of stimuli, which evoked the change[1]. The process of conversion of normal cell to benign and malignant neoplasia is called carcinogenesis and agents which cause this are termed carcinogen. Carcinogenesis is multistep, multigenic and multicausal process[2]. 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[3]. Some of plants have been well studied in various experimental models of cancer, both *in vivo* and *in vitro* models[4]. 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[5]. *Portulaca oleracea* (purslane) is a rich source of omega-3 fatty acids, which are beneficial in congenital heart disease (CHD) and certain cancers[6], carbohydrates, lipids, glycosides, alkaloids, sterols, triterpenes, and flavonoids[7]. *Portulaca oleracea* plant has different useful medical properties, as analgesic, antiarthritic, antiarteriosclerotic, anticancer (Colon, forestomach, liver, skin) activities [8]. 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 pathological 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 [9].

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 [10].

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

By returning to the results of LD50, and value reported in some references[11], the dose was adjusted in this study

was (200 mg/ kg B .W for S/C injection daily for 30 days.

4- Preparation of *Candida albicans* antigens

A-Culture media preparation for *Candida albicans* was according to technique used by [12].

B-Whole killed *Candida albicans* antigens were prepared according to [13].

5-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. 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. All organs taken from the animals then fixed in 10%formaline in order to study histopathological changes [14].

RESULTS & DISCUSSION

Pathology of spleen

1. Spleen of tumor-bearing mice treated with distill water and immunized (control group) (group I) showed mild hyperplasia in white pulp region (Fig 1).
2. Spleen of tumor-bearing mice treated with P.O and immunized (group II) showed extensive hyperplasia of white pulp in the periarterial sheath (T-cell region), extensive hyperplasia of remainder region of white pulp (B-cell region) and amyloid deposition surrounding white pulp (Figure 2).
3. Spleen of healthy mice treated with P.O and immunized (group III) showed extensive hyperplasia of white pulp in the periarterial sheath (T-cell region), extensive hyperplasia of remainder region of white pulp (B-cell region) and amyloid deposition surrounded the follicles and proliferation of megakaryocytes. (Figure 3)
4. Spleen of healthy mice treated with distills water and immunized (group IV). Spleen of this group showed hyperplasia of white pulp periarterial region (T- cell region) and other region (B- cells region) (Figure 4).

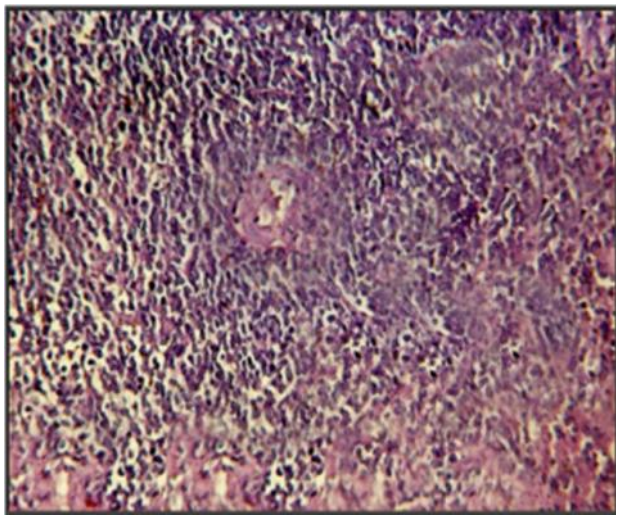

FIGURE 1: Histological section in spleen of tumor-bearing female mice treated with distills water and immunized (group I) for 30 days, shows mild hyperplasia in white pulp (400XH&E).

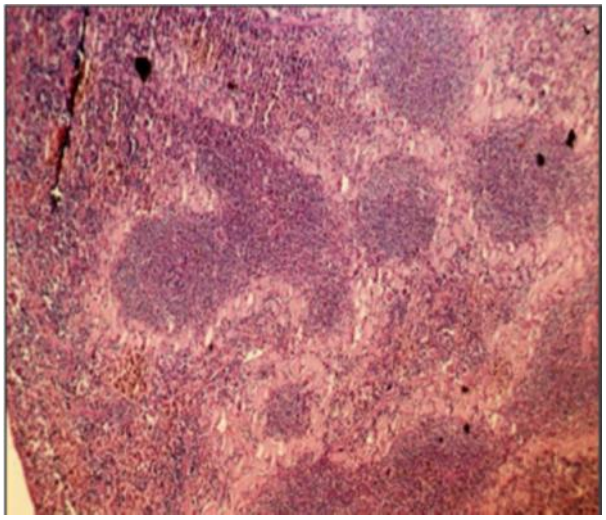

FIGURE 2: Histological section in spleen of tumor-bearing female mice (group II) treated with 200mg/kg B.W S/C of ethanolic extract of *Portulaca oleracea* for 30 days and immunized, shows extensive hyperplasia in white pulp and amyloid deposition surrounding the white pulp (40X H&E).

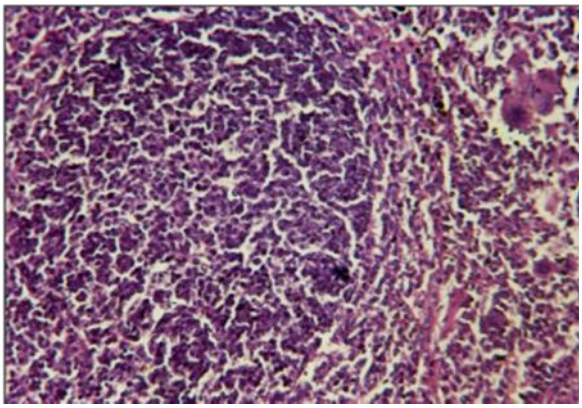

FIGURE 3: Histological section in spleen of healthy female mice treated with 200 mg/kg B.W S/C of P.O for 30 days and immunized (group III) shows extensive hyperplasia in white pulp and proliferation of megakaryocytes (200XH&E).

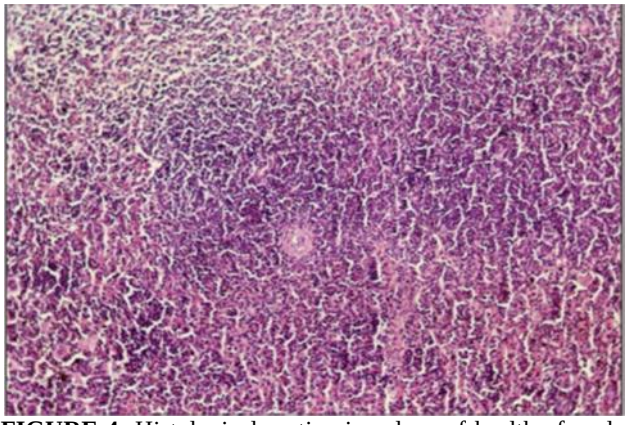

FIGURE 4: Histological section in spleen of healthy female mice treated with distills water for 30 days and immunized (group IV), shows hyperplasia of white pulp (200XH&E).

Pathology of liver

1. Liver of tumor-bearing mice treated with distill water and immunized (control group) (group I). Showed vacuolar degeneration in hepatocytes (Figure 5).
2. Liver of tumor-bearing mice treated with P.O and immunized (group II) showed highly infiltration of inflammatory cells (mononuclear cells) around portal area, B .V and early granuloma formation in liver parenchyma (Figure 6, 7).
3. Liver of healthy mice treated with P.O and immunized (group III): showed aggregation of perivascular mononuclear cells and forming early granuloma in liver parenchyma (Figure 8).
4. Liver of healthy mice treated with distilled water and immunized (group IV) showed no significant lesion detected.

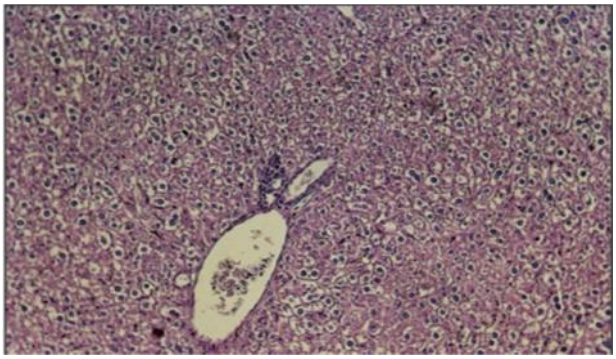

FIGURE 5 : Histological section in liver of tumor-bearing female mice treated with distill water for 30 days and immunized (group I), shows vacuolar degeneration in hepatocyte. (100 X H&E).

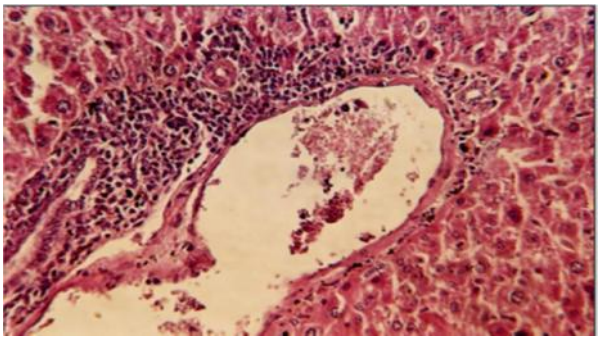

FIGURE 6 : Histological section in liver of tumor-bearing female mice treated with 200mg/kg. B.W S/C of P.O for 30 days and immunized (group II) shows highly infiltration of inflammatory cells (mononuclear cells) around portal area, B .V (200XH&E).

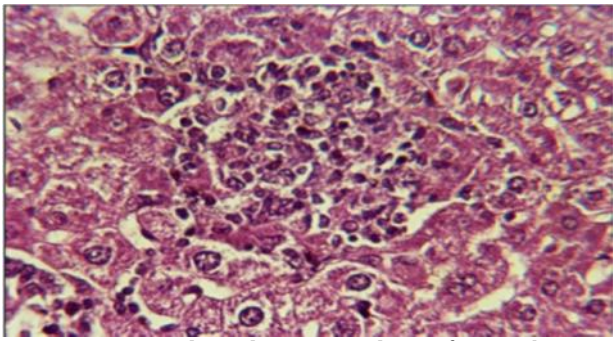

FIGURE 7: Histological section in liver of tumor-bearing female mice treated with 200mg/kg. B.W S/C of P.O for 30 days and immunized (group II), shows early granuloma formation in liver parenchyma (400XH&E).

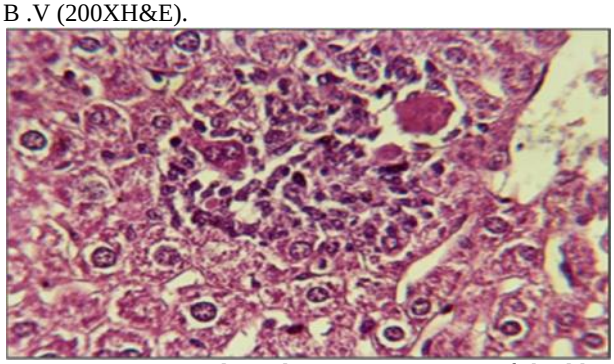

FIGURE 8 : Histological section in Liver of healthy female mice treated with 200 mg/kg .B.W S/C of P.O for 30 days and immunized (group III) shows early granuloma formation in liver parenchyma (400XH&E).

Pathology of kidney

1. Kidney of tumor-bearing mice treated with distill water and immunized (control group) (group I) showed distention of Bowman's capsule with hydropic degeneration of convoluted tubules.
2. Kidney of tumor-bearing mice treated with P.O and immunized (group II) showed infiltration of mononuclear cells (lymphocyte and macrophage) between glomeruli, renal tubule and adjacent B.V (Figure 9).
3. Kidney of healthy mice treated with P.O and immunized (group III): showed infiltration of mononuclear cells (lymphocyte and macrophage) between glomaruli, renal tubule and adjacent B.V. (Figure 10)
4. Kidney of healthy mice treated with distill water and immunized (group IV) showed no significant lesion detected.

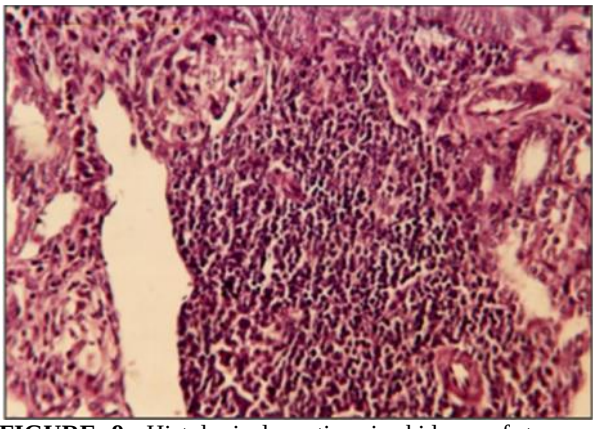

FIGURE 9: Histological section in kidney of tumor-bearing mice treated with 200mg/kg .B.W S/C of P.O for 30 days and immunized (group II), shows highly mononuclear cells infiltration in kidney parenchyma (200XH&E).

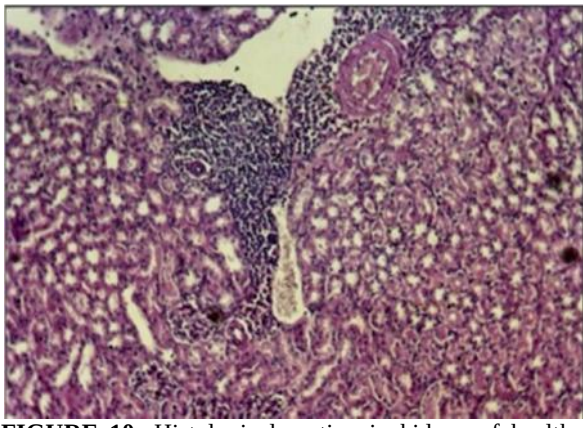

FIGURE 10: Histological section in kidney of healthy female mice treated with 200 mg/kg. B.W S/C of P.O for 30 days and immunized (group III) shows highly mononuclear infiltration in kidney parenchyma (100XH&E).

The 2nd lymphoid organs comprise the spleen, lymph nodes and Liver had been considered the largest reservoirs of CD^+ 4 memory T cells in mice after the effectors phase of an immune reaction [15,16]. Non treated tumor bearing female mice (group I), histological section showed mild reactive hyperplasia of spleen and vacuolar degeneration of hepatocyte. In contrast, to early granuloma formation in the liver observed in treated tumor bearing female mice with plant extract (group II) and more in healthy female mice treated with P.O (group III), compared with tumor bearing female mice (group I) and healthy female mice treated only with distill water (group IV). Our suggestion like in immunological study, tumor cells causing suppression of T cells by tumor derived factors e.g. (TGF-B .IL10 and VEGF)[17,18,19,20]. All these factors can induced a state of immune unresponsiveness that allow progressive tumor growth[7], and may induced reversible cell injury (cellular swelling) due to failure of energy dependent ion pump in the plasma membrane and nutritional deficiency remain a major cause of cell injury[1]. While group II, III and health mice treated with P.O. only showed extensive hyperplasia of white pulp, may be due to increased mitotic index of splenocyte during immune response [21]. *Portulaca oleracea* have active compound like antioxidant, flavonoid, catechine and alkaloids which may act as immune stimulant and increased splenocyte proliferation[22,23] Showed that spleen follicular hyperplasia due to catechines of *Camellia sinensis* plant extract. There was deposition of amyloid in spleen in group II, III. Deposition of amyloid fibril protein (Amyloid light chain) type is associated with some form of monoclonal B-cell proliferation[1], and proliferation of megakaryocytes in their spleen as a result of response to multiple cytokines[24], which secreting immune mediators to enhance immune response. *Urtica dioca* plant extract also observed this phenomenon in the mice as an immunological and immune complex[25,26]. The histopathological in kidney of non treated tumor bearing mice (group I) showed hydropic degeneration and extended of Bowman's capsule, it may be due to reversible injury caused by tumor secreting mediators, while the treated group of tumor bearing mice with plant extract (group II), healthy mice treated with P.O (group III) received 200 mg/kg. B.W S/C of P.O only for 30 days, showed high mononuclear cell infiltration between renal tubule as a result of high immune complex compared with healthy mice treated only with distill water (group IV) that have no significant lesion detected [1].

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