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Anti-tumor Activity of *Agaricus bisporus* Extracts and its Relation with IL-2 in Tumor-Bearing Mice

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Abstract :

Mushroom is widely used as a traditional medicinal fungus, and it has been known to exhibit immune-stimulatory and anti-cancer activity. However, the exact immune response in tumor-bearing mice after exposure to mushroom extract was unclear. The aim of this study was to investigate whether or not crude preparations extracted from Agaricus bisporus can promote type 1 immune response in tumor-bearing mice.

Watery and ethanolic extracts of mushroom were intraperitoneally administered into tumor-bearing mice for 28 alternative days, tumor volume was measured twice weekly, also IL-2 level was determined in the sera of mice one day before the beginning of treatment and after 2 and 4 weeks of treatment. The results showed that both extracts significantly inhibit the growth of tumor up to 72.2% at the end of experiment, while only the ethanolic extract significantly increased the level of IL-2 production from 11.2 and 16.9 pg/ml up to 26.4 and 42.5 pg/ml after 2 and 4 weeks of treatment respectively.

To confirm these results , further studies are required to investigate the ability of these extracts to promote the production of IL-12 & IFN-γ which are the major cytokines involved in immune response type 1 activation.

Key words:

Agaricus bisporus, Mushroom, Anti-tumor, Tumor-bearing mice, IL-2, Immune response type 1.

Introduction:

Mushroom is widely distributed and the number of different mushroom species on earth is estimated at 140000, of which may be only 1400 species that we know today, about 50% are considered to possess varying degree of edibility, more than 2000 are safe and about 700 species were known to possess significant pharmacological properties (1, 2). The gross composition of mushrooms is water (90%), and from the dry matter : protein (10 – 40%), fat (2-8%), carbohydrates (3-28%), fibers (3-32%) and ash (8-10%) which is mainly composed of salts and metals (3).

The therapeutic effects of mushrooms, such as anticancer activity, suppression of autoimmune diseases and allergy have been associated with their immunomodulating effects (4). The major immunomodulating effects of metabolites and extracts derived from mushrooms include mitogenicity and activation of immune cells (5), such as hematopoetic stem cells (6), lymphocytes (7, 8), macrophage (9, 10), and natural killer (NK) cells resulting in the production of cytokines (11 – 15).

Interleukin-2 (IL-2) was the first interleukin molecule to be discovered and purified to homogeneity by immuno-affinity chromatography by Kendall Smith and his team, but it was designated number 2 because Smith's data at the time indicated that IL-1, produced by macrophages, facilitates IL-2 production by T-lymphocytes (16, 17). IL-2 is necessary for the development of T-cell immunologic memory, one of the unique characteristic of the immune system and also necessary for the development of regulatory T-cells in the thymus (18). It has been found that IL-2 is able to facilitate production of immunoglobulins made by B-cells and induce the differentiation and proliferation of natural killer cells. Therefore, a recombinant form of IL-2 was manufactured for the treatment of cancers (malignant melanoma, renal cell carcinoma), and is in clinical trials for the treatment of chronic viral infections (19).

This study was designed to investigate whether or not the administration of extract derived from fruiting bodies of Agaricus bisporus mushroom has an effect on the production of IL-2 in tumor-bearing mice.

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MATERIALS AND METHODS:

Preparation of mushroom extracts

For watery extraction, 70 g of fresh fruiting bodies of Agaricus bisporus were homogenized with 200 ml of phosphate buffer saline (PBS) and left on a hot plate at 100 oc for 1h. The extract was filtered through Whatman No.1 under reduced pressure to remove debris. The resultant solution was centrifuged at 3500g for 30 min and the supernatant was lyophilized into a powder. One gram of dried powder was dissolved in 10 ml of PBS and sterilized by filtration through 0.22 µm filter to obtain a stock solution, from which a proper concentrations (30 mg/ml) can be prepared to be used in the therapeutic experiment (20).

For ethanolic extractions, 50g of air-dried mushroom of Agaricus bisporus was extracted with 250 ml of ethanol (70%) in Soxhlet apparatus at 60 oc for 8h. The extract was rotary evaporated at 60 oc into a powder. One gram of dried extract was dissolved in 10 ml of PBS (20).

Preparation of tumor-bearing mice

Female mice (6-8 week age) were obtained from the animal house of Iraqi Center for Cancer and Medical Genetics Researches (ICCMGR). All animals were fed standard diet and water ad libitum. Implantaion of spontaneous murine mammary adenocarcinoma (AM3) in female mice has been established in this center according to Lee et al (2003)(21). A tumor-bearing female mouse (source) was anesthetized and needle gauge 18 was inserted into tumor mass to aspirate 3-6 ml of tumor content and transferred into a sterile beaker. Equal volume of a sterile PBS was added and the mixture was transferred into a sterile test tube to be centrifuged at 1000 rpm for 10 min at 20 oc. The supernatant was discarded and the tumor cells resuspended in adequate amount of PBS.

Tumor cell suspension at a concentration of 1o 10o cells/ml was injected into 6-8 weeks aged female mice by inserting needle (gauge 18) subcutaneously starting from inguinal region until reaching to the cervical region. After appearance of tumor mass (about 10-14 days), the mice become ready to be used in the therapeutic experiment.

Therapeutic Experiments

Tumor-bearing mice were divided into three major groups, group A (n = 9, control group) treated with PBS, group B was subdivided into three subgroups (n = 9 for each one) and treated with watery extract at doses of 120, 180, and 240 mg/kg body weight, group C also subdivided into three subgroups (n = 9 for each one) and treated with ethanolic extract at doses of 180, 360, and 540 mg/kg body weight. The manner of administration was intraperitoneally (i.p) in all groups and continued for 28- alternative days (administered every 48h).

The development of tumor volume was measured twice a week by using Vernier calipers and calculated from the following formula (22): TV= L . (w^2 /2), whereas, TV = tumor volume in mm^3, L= length of tumor in mm, and w^2= square width of tumor in mm^2.

The relative tumor volume (RTV) was calculated from the ratio between the average tumor volume of each group at x-day and its original volume at zero time before beginning the treatment. The percentage of tumor growth inhibition (TGI %) at the end of experiment was calculated from the following equation (23):

TGI% = (1 – B/A) x 100, whereas A is the RTV of the control group, and B is that of treated group.

Blood samples have been aspirated by heart puncture from three mice of each group just before the starting of treatment (zero time) and after 2 and 4 weeks of treatment. Sera were separated from blood samples and stored at -20 oc to be used later in the determination of IL-2.

Determination of IL-2

The levels of IL-2 in the collected sera were determined by using Eliza kit for mouse IL-2, 3441-1A purchased from MAB TECH, USA. The values of optical density (O.D) were measured at 405 nm in an Eliza reader and a standard curve was constructed to estimate the levels of IL-2 in the unknown samples in pg/ml.

Statistical Analysis

Crude data were analyzed by SPSS and values were expressed as mean ± S.E. The significance of differences between treated and control groups were analyzed by Duncan test and any p values <0.05 were considered as significant (24).

RESULTS:

The values of relative tumor volume (RTV) versus duration of exposure (in weeks) were illustrated in figure- 1 and figure- 2. The volume of non-treated group (control) after four weeks became 14.4 times more than its original volume (at zero time), while those treated with 120, 180, and 240 mg/kg of watery extract became 5, 4, and 14 times respectively (figure – 1). Similarlly, those treated with 180, 360, and 540 mg/kg of ethanolic extract RTV was increased up to 6, 4, and 15 times respectively (figure- 2).

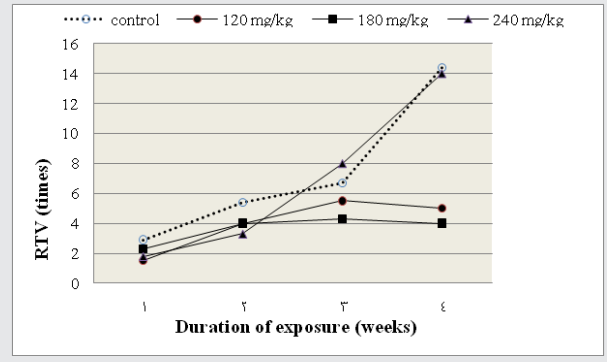

Figure-1: Effect of watery extract administration on the relative tumor volume (RTV) in tumor-bearing mice.

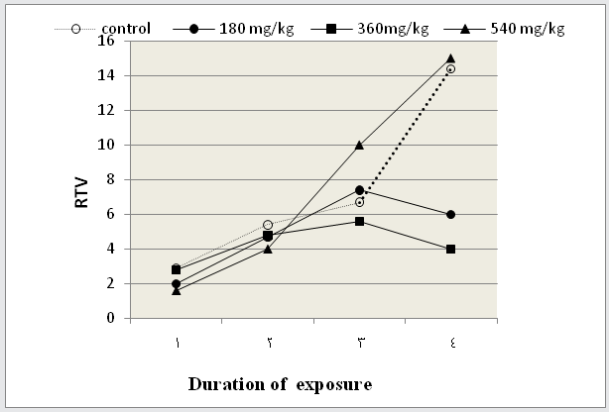

Figure-2: Effect of ethanolic extract administration on the relative tumor volume (RTV) in tumor-bearing mice.

When the TGI% at the end of experiment was calculated, only the two doses of watery extract (120, and 180 mg/kg) and ethanolic extract (180, and 360 mg/kg) showed a significant TGI% (65.3%, 72.2%) and (58.3%, 72.2%) respectively, while those treated with the high doses of watery extract (240 mg/kg) and ethanolic extract (540 mg/kg) showed a non-significant TGI% (2.8% and 0%) respectively (Figure – 3).

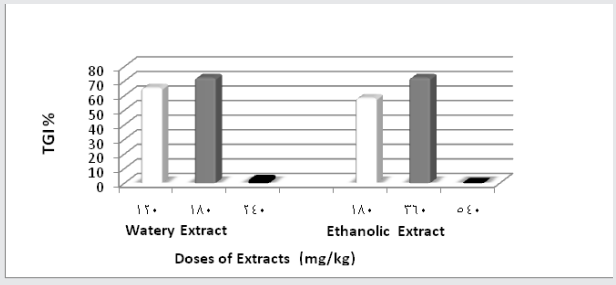

Figure-3: The percentage of tumor growth inhibition (TGI %) after four weeks of watery and ethanolic extracts administration in tumor-bearing mice.

On the other hand, the level of IL-2 at zero time was about 26.7 ± 3.1 pg/ml and then significantly dropped to (11.2 ± 0.2, and 16.9 ± 0.9 pg/ml) in control group after two and four weeks of PBS administration. This significant reduction in IL-2 levels also seen in those treated with 120, 180, and 240 mg/kg of watery extract whether after two weeks (10.9 ± 1.9, 6.9 ± 2, and 9.5 ± 0.5 pg/ml respectively) or even after four weeks of treatment (15.2 ± 2.9, 11.1 ± 0.7, and 16.3 ± 0.7 pg/ml respectively) (Figure – 4). However, no significant reduction has been shown in the IL-2 level after two weeks of treatment with 180, 360, and 540 mg/kg of ethanolic extract (26.4 ± 1.3, 23 ± 1.2, and 21.2 ± 2.6 pg/ml respectively), in contrast, they are significantly elevated up to 42.5 ± 5.9, 35.5 ± 3.6, and 32.8 ± 3.7 pg/ml respectively after four weeks of treatment (Figure-5).

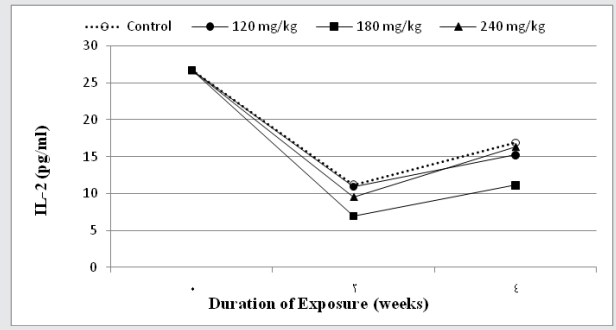

Figure-4: Effect of watery extracts administration on the serum level of IL-2 in tumor-bearing mice.

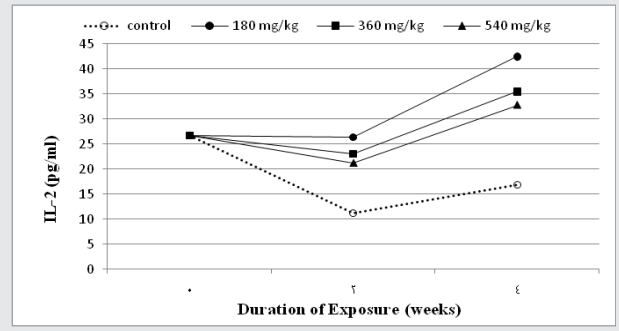

Figure-5: Effect of ethanolic extract administration on the serum level of IL-2 in tumor-bearing mice.

DISCUSSION:

The results showed that ethanolic extract can induce type 1 immune response in tumor-bearing mice based on its ability to increase the production of IL-2 (Figure 5). These findings confirmed the results obtained by other studies in which intraperitoneal administration of polysaccharide derived from Grifola frondosa mushroom in the tumor-bearing BALB/c mice promote type 1 response through inducing IFN-γ production and inhibiting IL-4 synthesis (29). However, the failure of watery extract in the present study to enhance IL-2 production (Figure-4) may be due to the high temperature (100˚c) used in the preparation of this extract, because one study indicated that the active components of watery extracts were susceptible to heat and both the NK cell activity and IFN-γ production decreased with the increasing extraction temperature above 80˚c (13).

With regard to the highest dose of watery extract (240 mg/kg) and that of ethanolic extract (540 mg/kg), both watery and ethanolic extracts exhibited moderate anti-tumor activity at the end of the experiment (63.3% and 72.2%) and (58.3% and 72.2%) respectively (Figure-3). However, only ethanolic extract caused a significant enhancement in IL-2 production (Figure-5). Several mechanisms have been suggested for interpretation of the anti-tumor activity of mushroom which include: inhibition of tumor-induced neo-vascularization and anti-metastatic effect (25, 26), induction of apoptosis in the tumor cells (27, 28), direct destruction of tumor cells through their anti-oxidant activity (14), as well as inhibition of immune suppression caused by tumors (5, 14, 25).

In respect to the last mechanism, the production of two distinct cytokine patterns was originally recognized in subset of helper T cells (Th): the set designated as Th1 is characterized by IL-2, IFN-γ, and IL-12 production that promote cell-mediated immunity through activation of Tc cells, NK cells, and macrophages. The other set designated as Th2, is characterized IL-4,5,6,10, and 13 synthesis and promote humoral immunity (7). It would have been of interest to determine whether mushroom extract is able to induce the response toward type 1 or type 2 patterns.

Interestingly, results demonstrated that anti-tumor activity was completely abrogated by administration of high doses of both watery and ethanolic extracts (Figure-3). These results suggested that watery and ethanolic extracts may have effective components that contribute in chronic toxicity because many mushroom species have the ability to accumulate relatively high concentration of toxic metals such as arsenic, lead, cadmium, and mercury (30), in addition to the naturally presence of " agaritine" component which is a well-known carcinogenic and toxic substance in animals (31). Also, it was reported that continuous production of large amount of cytokines such as IL-12, IL-18, and IFN-γ might be toxic and induce hepatotoxicity (32).

In summary, the findings of this study markedly demonstrate that i.p administration of each of water and ethanolic preparations extracted from the fruiting bodies of Agaricus bisporus mushroom may exhibit anti-tumor activity by inducing type 1 immune response in tumor-bearing mice. Further studies are required to improve their ability to induce IL-12 and IFN-γ production, as well as to identify their chronic toxicity.

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الفعالية المضادة للورم لمستخلصات عرهون *Agaricus bisporus* وعلاقتها بمستوى IL–2 في مصل الفئران الحاملة للورم

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الخلاصة:

العرهون كفطر طبي تقليدي واسع الاستخدام معروف بقابليته على التحفيز المناعي و فعاليته المضادة للسرطان. لكن نوع الاستجابة التي يسببها في الفئران الحاملة للورم غير واضحة بشكل مضبوط . ان الهدف من هذه الدراسة هو للتحري عما اذا كانت المستحضرات الخام المستخلصة من عرهون Agaricus bisporus لها القابلية على تنشيط الاستجابة المناعية من النوع الاول في الفئران الحاملة للورم.

تم اعطاء الفئران الحاملة للورم كل من المستخلص المائي و الكحولي للعرهون عن طريق البريتون و لمدة 28 يوم ، ثم قيس حجم الورم مرتين كل اسبوع، كما تم تقدير مستوى IL–2 في مصول الفئران قبل يوم واحد من بدء المعاملة و كذلك بعد 2 و 4 اسابيع من المعاملة. اوضحت النتائج بان كلا المستخلصين المائي و الكحولي ثبطا نمو الورم بنسبة وصلت اعلاها الى حوالي 72.2 % عند نهاية التجربة، بينما استطاع المستخلص الكحولي فقط من زيادة مستوى انتاج IL–2 و بشكل معنوي من 11.2 و 16.9 بيكوغرام /مل وصولا الى 26.4 و 42.5 بيكوغرام/مل بعد 2 و 4 اسابيع من المعاملة على التوالي .

و لغرض تأكيد هذه النتائج فانه يتطلب دراسات اكثر على قابلية هذه المستخلصات في تنشيط انتاج كل من IL–12 و IFN–γ اللذان يعتبران من السايتوكينات الرئيسية المتضمنة في تنشيط الاستجابة المناعية من النوع الاول.