Studies of Ethanolic Extract from *Lentinus edodes On Different Cell Lines And Lymphocytes Separated From Cancer Breast Patients*

Ahmed, W.A. *, Osman, M.E., Khattab, O.H., Hassan, F.R.H. ** , El-Henawy, H.E.

Department of Botany and Microbiology, Faculty of Science, Helwan University, Egypt.
* Department of Cancer Biology, National Cancer Institute, Cairo University, Egypt.
**Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.
drwafaaeg@hotmail.com

Abstract: *Lentinus edodes* a Chinese edible and medicinal mushroom was grown using submerged culture on different standard liquid media and different food wastes by products. Shiitake broth medium was the medium that showed the maximum biomass for both tested strains. The biomass obtained from cultivation on all tested media was extracted with ethanol to obtain crude extract that tested for its biological activity. Also, SM broth medium showed the highest crude extract yield for the two strains. Some physiological factors were studied to optimize the conditions for cultivation on SM broth medium for maximum biomass and crude extract yield. Although recent studies have demonstrated that *L. edodes* separate compounds can inhibit the proliferation of cancer cells *In vitro* and *in vivo*, crude extract of *L. edodes* mycelia was tested in this study *In vitro* and *in vivo*. The Invitro cytotoxic activity of the crude extracts of mycelia of the two strains were examined on human breast cancer (MCF7) cell line using trypan blue exclusion assay and MTT cytotoxicity assay. The results showed that the crude extract of *Lentinus edodes LC2141* inhibited the proliferation of MCF7 at high concentration with IC_{50} 178.8µg/ml while *Lentinus edodes LC202* extract inhibited the proliferation of MCF7 at high concentration with IC_{50} 94.025µg/ml. Also, The treatment of Hep-G2 cells with different concentrations of ethanolic extract of both *Lentinus edodes LC2141* and *Lentinus edodes LC202* dramatically inhibited the cell growth in a dose-dependent manner, with high IC_{50} values of 953.1µg/ml and 1868.3µg/ml, respectively. Immunomodulating activity of the tested extracts was determined on normal and breast cancer patient lymphocyte using MTT and trypan blue exclusion assays. The treatment of lymphocyte with different concentrations of the crude extract showed an increase in lymphocyte proliferation by increasing concentration (dose dependant).
[Ahmed, W.A., Osman, M.E., Khattab, O.H., Hassan, F.R.H. , El-Henawy, H.E.. Studies of Ethanolic Extract from Lentinus edodes On Different Cell Lines And Lymphocytes Separated From Cancer Breast Patients. Cancer Biology 2011;1(2):1-9]. (ISSN: 2150-1041). http://www.cancerbio.net.

Keywords: Lentinus edodes**,** Ethanolic crude extract**,** submerged culture, antitumor activity.

1. Introduction

Medicinal mushroom extracts have been considered as important remedies for the prevention and treatment of many diseases for thousands of years especially in the Orient (Israilides and Philippoussis, 2003; Kidd, 2000; Wasser and Weis, 1999). A plethora of medicinal effects has been demonstrated for mushrooms including antibacterial, antiviral, antifungal, antitumor and immuno-potentiating activities (Hobbs, 2003; Ooio and Liu, 1999). Among the various bioactive components which have been demonstrated to be most effective as antitumor and immunomodulatory agents are polysaccharides and polysaccharopeptides.

Nowadays macrofungi are distinguished as important natural resources of immunomodulating and anticancer agents and with regard to the increase in diseases involving immune dysfunction, cancer, autoimmune conditions in recent years, applying such immunomodulator agents especially with the natural original is vital. These compounds belong mainly to polysaccharides especially β-D-glucan derivates, glycopeptide/protein complexes (polysaccharide-peptide/protein complexes), proteoglycans, proteins and triterpenoids. Among polysaccharides, β (1 →3) - D-glucans and their peptide/protein derivates and among proteins, fungal immunomodulatory proteins (Fips) have more important role in immunomodulating and antitumor activities.

Lentinus edodes is the source of many therapeutic polysaccharide macromolecules among which the ones with proven pharmacological effects are lentinan, LEM and KS-2. Lentinan is a high molecular weight (about one million) homopolysaccharide in a triple helix structure, with linear chains consisting of (1 -3)- β - D-glucopyranosyl (Gl*cp*) residues with two β-(1 - 6)-linked Gl*cp* branchings for every five β -(1 - 3)-Gl*cp* residues (Aoki, 1984). LEM is a mycelial extract

preparation of *L. edodes* harvested before the cap and stem grow. It is a heteroglycan–protein conjugate containing 24.6% protein and 44% sugars, comprising mostly pentoses as well as glucose and smaller amounts of galactose, mannose and fructose (Lizuka, 1986; Sugano *et al.*, 1982). It also contains nucleic acid derivatives, B complex vitamins, ergosterol, eritadenine (an anticholesteremic amino acid), and water- soluble lignins (Sugano *et al.*, 1985). KS-2 is a peptide –polysaccharide complex. Besides its anti-tumour activity, it has been demonstrated to increase the host resistance to bacterial and viral infections (Jong and Birmingham, 1993). Lenthionine, a sulphur-containing compound, has antibacterial and antifungal activity (Yasumoto *et al.*, 1971; Morita and Kobayashi, 1967), and bis [(methylsulfonyl) methyl] disulphide, a derivative of lenthionine, has strong inhibitory effects against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (Takazawa *et al.*,1982). The chloroform and ethyl acetate extracts of the dried mushroom have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Hirasawa *et al.*, 1999). Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity (Jong and Birmingham, 1993). Several fractions of LEM (an aqueous extract of the *L. edodes* mycelium and its solid culture medium) have immunoactive properties such as the induction of interferon in vitro (Hibino *et al.*, 1994) and in vivo (Suzuki *et al.*, 1979), inhibition of the infectivity and cytopathic effect of human immunodeficiency virus (Suzuki *et al.*, 1989; Tochikura, 1988) and blockade of the release of herpes simplex virus type 1 from tissue culture cells (Sarkar *et al.*, 1993).

Since many of the compounds, which are found in *L. edodes* have been shown to act synergistically (Yamasaki *et al.*, 1989), it is worth testing the biological activity of the whole mushroom and mycelium extract rather than its individual components. This principle (synergy) is compatible with similar natural biological products like the essential oils, which allow the achievement of strong effects when used as whole products, while quenching or nullifying potential unwanted side effects by the presence of individual components.

The aim of this work is study the antitumor activity (*In vitro*) and immumomodulating activity of ethanolic extract from mycelia of two different strains of *Lentinus edodes.*

2. Material and Methods

Fungal Strains:

The two edible fungal strains *Lentinus edodes LC2141* and *Lentinus edodes LC202* were kindly obtained from Fujian Agriculture Univ., China. The culture was maintained on Potato Dextrose Agar (PDA) medium and stored in refrigerator at 5 -7 °C after growth as recommended by Stamets, (1993) for routine culture and storage purposes.

Culture Media:

Medium for Submerged Culture of Shiitake(SM) (Mizuno, 1995):

Mycelia were grown in a submerged liquid culture in 250ml conical flasks. The medium composition for strain *Lentinus edodes LC2141* was: Thiamine -Hcl, 1.0 mg; KH_2PO_4 , 1.0g; $Cacl_2 .2H_2O$, 0.5g; $MgSO_4 . 7H_2O$, 0.5g; $FeSO_4. 7H_2O$, 10mg; $MnSO_4 .6H_2O$, 7.2mg; $ZnCl_2$, 4mg; $CuSO_4$, 1.0mg; Starch, 70g; corn steep liquor, 10g; Fructose, 15g; $NaNO_3$, 2g and distilled water, 1 L; Initial pH= 7). While the medium composition for strain *Lentinus edodes LC202* was: Thiamine -Hcl, 1.0 mg; KH_2PO_4 , 1.0g; $Cacl_2 .2H_2O$, 0.5g; $MgSO_4 . 7H_2O$, 0.5g; $FeSO_4. 7H_2O$, 10mg; $MnSO_4 .6H_2O$, 9mg; $ZnCl_2$, 4mg; $CuSO_4$, 0.8mg; Starch, 70g; corn steep liquor, 10g; Fructose, 10g; yeast extract,5g and distilled water, 1 L; Initial pH= 7). Each flask was inoculated with 25 agar plugs 0.7cm covered by the mycelium obtained from a 15 days old plate culture for 15 days incubation period in case of strain *Lentinus edodes LC2141* and 13 days in case of strain *Lentinus edodes LC202*.

Preparation of Crude Ethanol Extract (Turkoglu et al., 2007):

The mycelia of each strain were dried at 40°C before analysis. These dried mycelia were pulverized and 20.0g each of the powdered samples were soaked separately in 200 ml of 95% ethanol in an Erlenmeyer flask. The sample was extracted by stirring at 30°C at 150 rpm for 24 h. The mixture was filtered through Whatman's filter paper no 4. The residue was then extracted with two additional 200ml of ethanol as described above. The combined ethanolic extracts were concentrated in a rotary evaporator at 40°C. The ethanol was recovered and the extract was collected and dried and stored at 4°C for further use.

Tumor cell lines:

Ehrlich ascites carcinoma (EAC) cells (mouse tumor):

Ehrlich ascites carcinoma (EAC) cells were used in *In vitro* and *in vivo* experiments. The parent cell line was kindly supplied by National Cancer Institute, Cairo University, Egypt. The tumor cell line was maintained in female mice Swiss albino through serial intraperitoneal transplantation of 1x 10^6 viable tumor cells in 0.2 ml of saline. The tumor is characterized by

moderately rapid growth, which kill mice in 16 to 18 days due to accumulation of ascetic fluid and seldom shows distal metastasis or spontaneous regression. Ehrlich ascites carcinoma (EAC) cells were obtained by needle aspiration of ascetic fluid from the preinoculated mice under aseptic conditions using ultraviolet laminar air flow system. The cells in the ascetic fluid were tested for viability and contamination by staining 0.1 ml of this fluid by 0.1 ml of trypan blue dye which stains only the dead cells (Lazarus *et al*., 1966). Preliminary test for Invitro antitumor activity of the crude extracts was done by using Ehrlich ascites carcinoma (EAC) cells by trypan blue exclusion test (Sheldon and Preskorn, 1996). EAC cells were incubated with RPMI medium in a tissue culture plate, then the extract concentrations were added that content of each well was (0.8 ml medium + 0.1 ml cells + 0.1 ml extracts). The final concentrations of extract were as follows (25, 50, 100 and 200 mg/ml) dissolved in PBS. After 24 hrs incubation of cells with extract, the cells were stained with trypan blue dye and percent survival of cells was determined by counting dead and viable cells using haemocytometer. Control treatment in which EAC cells were cultured without extracts was evaluated and The dose response curve of viable cells was determined.

*Percent survival of the cells **= T/C x 100**
Where:
T …. Number of viable cells in unit volume of the test drug well.
C …. Number of viable cells in unit volume of the control well.

Human tumor cell lines:

Human carcinoma cell lines were used in this study, were MCF7 (Breast carcinoma cell line) and Hep-G2 (Liver carcinoma cell line). It was obtained frozen in liquid nitrogen (-180^{o}C) from the American Type Culture Collection. The tumor cell line was maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. For the assessment of the cytotoxic and cytostatic activities of *L. edodes* extracts cells were seeded in 96- well flat-bottomed microtiter plates at a density of approximately ($0.5X10^5$ cells/well), in complete RPMI-1640 Medium. After 24 h to ensure cell attachment, serial dilutions of the extracts in physiological saline were prepared. 100 µl of different concentrations of each tested extracts were added for 24 h at 37^{o}C, in a humidified 5 % CO_2 atmosphere. Cytotoxicity was determined using the MTT assay (Hansen *et al.*,1989). After incubation, 10 µl MTT reagent solution/well was added and incubated for an additional 4 h. MTT crystals were solubilized by added 100 µl of MTT detergent/well then the plate was shacked at room temperature. It was followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader (Meter tech. Σ 960, USA) after development of violet color. Control cells were treated with vehicle alone. For each compound concentration, 3 wells were used (triplicate wells were prepared for each individual dose). The average was calculated. Data was expressed as the percentage of relative viability compared with the untreated cells. The cytotoxicity dose was calculated as a dose induced ≈100% relative non viability.

Calculation:

Percentage of relative viability was calculated using the following equation:
[Absorbance of treated cells /
Absorbance of control cells] X 100

Then the half maximal inhibitory concentration IC_{50} was calculated by the trend line equation.

Immunomodulating activity of the two tested ethanolic extracts:

Collection of blood:

Venous blood (5ml) was drawn from healthy volunteers and breast cancer patient volunteers. Human peripheral blood was collected in sterile heparin tube and lymphocyte separated according to the method described by (Boyum, 1976). Lymphocyte proliferation was determined by MTT assay and trypan blue exclusion assay for both control and breast cancer human lymphocytes.

Statistical analysis:

Statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by homogenous subsets (Duncun[a]) at confidence level of 5% (0.05) using the Statistical Package for the Social Science (SPSS) version8. Duncan's multiple range tests were used to compare between means of treatments according to Walter and Duncan (1969) at probability 5%.

3. Results and Discussion

In vitro "cell culture" experiments

The effect of mycelia ethanolic extract on EAC viability by Trypan blue exclusion test:

As shown in data that have been summarized in the table **(1)** and figures (1 and 2), viability was measured and expressed as the survival fraction compared with untreated control cells. Ehrlich cells were treated with concentrations (400, 200, 100, 50 and 25µg/ml) of the ethanolic extracts of Lentinus edodes LC2141 and Lentinus edodes

LC202 for 24 h. percent of dead cells increased by increasing concentration. At concentration 200µg/ml, the viability percentage in comparison with control was the lowest.

Table (1): The effect of *Lentinus edodes LC2141* and *Lentinus edodes LC202* mycelia ethanolic extract on EAC viability at 24 hrs of exposure determined by Trypan blue exclusion test.

Concentration (µg/ml)	*Lentinus edodes LC2141*		*Lentinus edodes LC202*	
	Viability (control %) (24 hrs exposure)	% of dead cells (24 hrs exposure)*	Viability (control %)(24 hrs exposure)	% of dead cells (24 hrs exposure)*
25	94.60	5.40^{e}	96.80	3.20^{e}
50	72.00	28.00^{d}	64.50	35.50^{d}
100	36.60	63.40^{c}	38.70	61.30^{c}
200	15.20	84.80^{a}	20.50	79.50^{a}
400	22.60	77.40^{b}	26.90	73.10^{b}

*: Means in the same column with different letters have significant differences between each other.

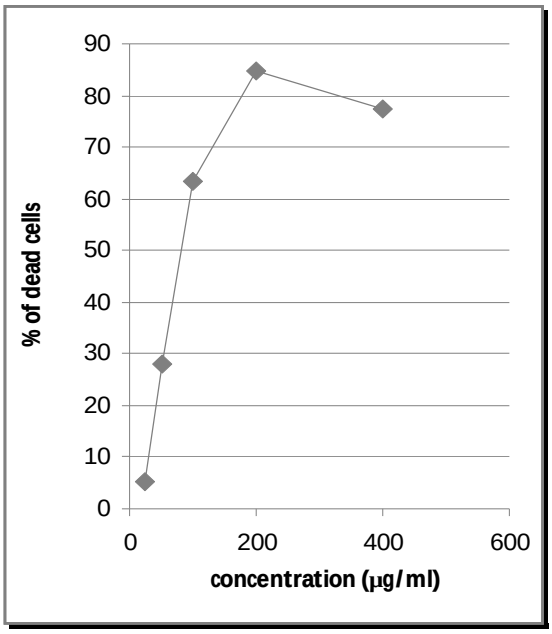

Fig. 1: Dose response curve on the effect of *Lentinus edodes LC2141* ethanolic extract on EAC viability at 24 hrs of exposure determined by trypan blue exclusion test.

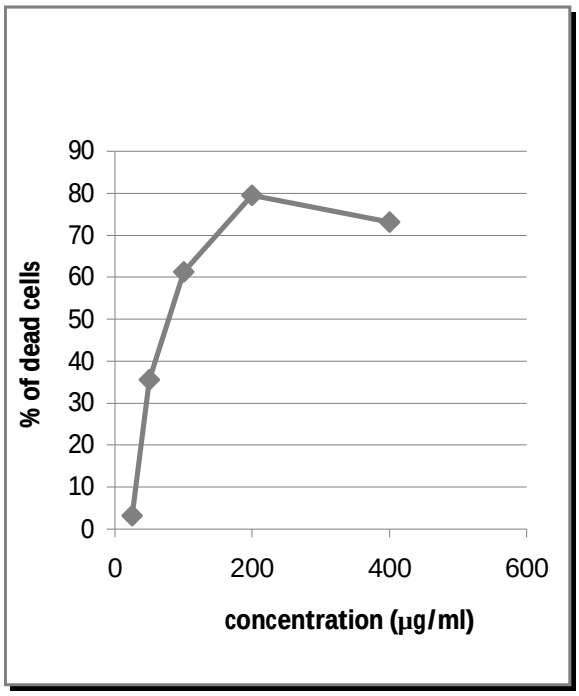

Fig. 2: Dose response curve on the effect of *Lentinus edodes LC202* ethanolic extract on EAC viability at 24 hrs of exposure determined by trypan blue exclusion test.

Anti-tumor activity against human breast cancer cell line (MCF7) and human hepatocellular carcinoma cell line (Hep-G2):

cytotoxicity was measured and expressed as the survival fraction compared with untreated control cells. The possible anti-proliferative effect of ethanolic extract of both Lentinus edodes LC2141 and Lentinus edodes LC202 was studied on the growth of MCF7 cell line after incubation for 24 h as shown in Fig. (3 and 4). The treatment of MCF7 cells with different concentrations of ethanolic extract of both Lentinus edodes LC2141 and Lentinus edodes LC202 dramatically inhibited the cell growth in a dose-dependent manner, with high IC_{50} values of 132.05µg /ml and 153.59 µg/ml, respectively. Also, the possible anti-proliferative effect of ethanolic extract of both Lentinus edodes LC2141 and Lentinus edodes LC202 was studied on the growth of Hep-G2 cell line after incubation for 24 h as summarized in Fig. (5 and 6). The treatment of Hep-G2 cells with different concentrations of ethanolic extract of both Lentinus edodes LC2141 and Lentinus edodes LC202 dramatically inhibited the cell growth in a dose-dependent manner, with high IC_{50} values of 953.1µg/ml and 1868.3µg/ml, respectively. Similar results were reported by Israilides et al., 2007 who found that Aqueous extracts of Lentinus edodes can significantly suppress the proliferation of cancer cell line MCF-7 in vitro. This is reflected by the comparative low IC_{50} values and the simultaneous higher IC_{50} values on normal cells. L. edodes mushroom water extracts are more cytotoxic than mycelial aqueous extracts. Methanolic extracts of either mushroom or mycelia of L. edodes do not exhibit any inhibitory (cytostatic) effect on MCF-7 cancer cell line supports the direct cytostatic/cytotoxic action of the L. edodes extracts on cancer cells, which is in parallel action with its host-mediated antitumor activity.

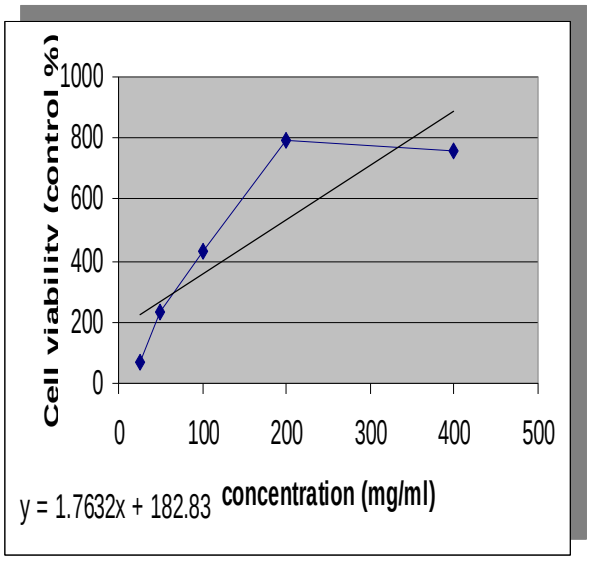

Fig. 3: Cell viability of breast carcinoma cell line MCF7 with ethanolic extract of both *Lentinus edodes LC2141* at concentration range from 400 to 25 µg/ml.

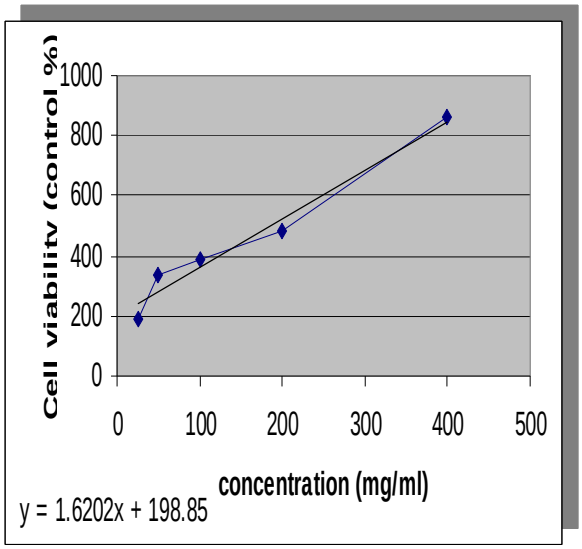

Fig. 4: Cell viability of breast carcinoma cell line MCF7 with ethanolic extract of both *Lentinus edodes LC202*at concentration range from 400 to 25 µg/ml.

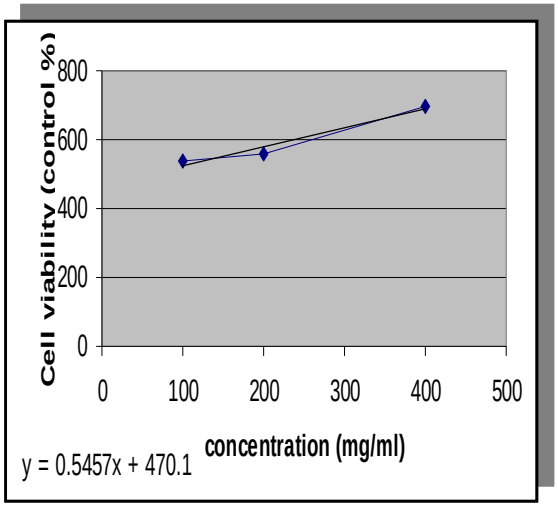

Fig. 5: Cell viability of liver carcinoma cell line Hep-G2 with ethanolic extract of both *Lentinus edodes LC2141*at concentration range from 400 to 100 µg/ml.

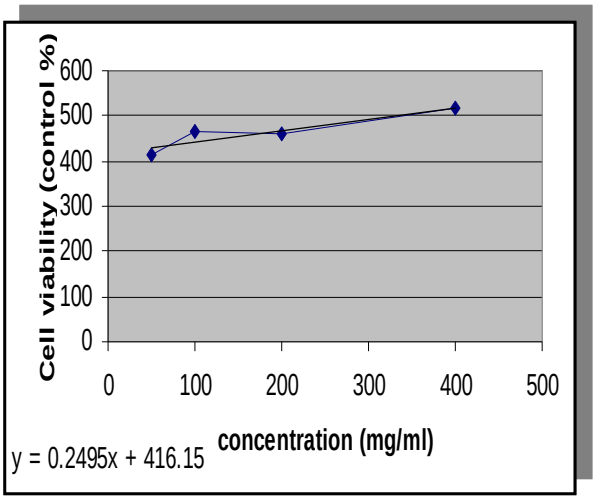

Fig. 6: Cell viability of liver carcinoma cell line Hep-G2 with ethanolic extract of both *Lentinus edodes LC202*at concentration range from 400 to 50 µg/ml.

The effect of the ethanolic extract from the two different strains of Lentinus edodes on lymphocyte proliferation:

Lymphocyte viability and proliferation were determined by trypan blue exclusion assay and MTT assay. Normal and breast cancer patient human lymphocyte was assayed. Proliferation was measured and expressed as count and absorbance compared with untreated control cells. The possible proliferative effect of ethanolic extract of both Lentinus edodes LC2141 and Lentinus edodes LC202 was studied on the growth of lymphocyte after incubation for 24 h. The results represented in Fig. (7 and 8) showed that the treatment of control human lymphocyte cells with different concentrations of ethanolic extract of Lentinus edodes LC2141 and Lentinus edodes LC202 resulted in dramatically increase in the lymphocyte proliferation in a dose-dependent manner. The highest count and absorbance was observed at concentration 400mg/ml. Also, treatment of lymphocyte cells of control human with the combination of the two ethanolic extract of strain Lentinus edodes LC2141 and strain Lentinus edodes LC202 showed significant increase in cell number and lymphocyte proliferation in a dose-dependent manner. The results represented in Fig. (48 and 49) showed that the treatment of lymphocyte of breast cancer patient with different concentration of ethanolic extracts of Lentinus edodes LC2141 and Lentinus edodes LC202 mycelia showed a significant increase in cell number and cell proliferation in a dose-dependent manner. Treatment with concentration 400µg/ml showed the highest increase in lymphocyte proliferation in comparison with control. Israilides et al., 2007 who found that L. edodes extracts supports the direct cytostatic/cytotoxic action of on cancer cells, which is in parallel action with its host-mediated antitumor activity. Furthermore, it was demonstrated that L. edodes can act as an immunomodulator to augment the proliferative response of rat thymocytes to T mitogens in vitro, indicating another mechanism for immunostimulatory activity. Overall there seems to be a therapeutic advantage in using L. edodes extracts orally administered instead of a single substance like Lentinan given intravenously. In addition, Nitha et al., 2007 reported that the ethanolic extract of M. esculenta mycelium is also found to possess significant antitumor activity against both ascites and solid tumour. The results indicate that the extract possessed both curative and preventive properties against solid tumour in a dose-dependent manner. The extract is also significantly effective against ascites tumour. These results suggest that M. esculenta mycelia contain compounds that may modulate tumourigenesis at different stages or may act at the same stage. Polysaccharide isolated from the fruiting bodies of M. esculenta has been reported to exhibit immunostimulatory activity (Duncan et al., 2001). Similarly, Lobanok et al., 2003 showed that the submerged mycelium and fruit bodies of L. edodes contain significant amounts of biologically active substances and exhibit immunomodulatory activity.

Table (2): The effect of *Lentinus edodes* ethanolic extracts on normal human lymphocyte proliferation "control group" at 24 hrs of exposure determined by Trypan blue exclusion test and MTT assay.

Concentration (µg/ml)	Ethanolic extract of *Lentinus edodes LC2141*mycelia		Ethanolic extract of *Lentinus edodes LC202* mycelia		Combination of the two ethanolic extract of strain *Lentinus edodes LC2141* and strain *Lentinus edodes LC202* mycelia	
	Count*	Absorbance *	Count *	Absorbance *	Count*	Absorbance *
Control	**5050^{c}**	**0.55^{c}**	**5050^{d}**	**0.55^{d}**	**5050^{d}**	**0.55^{d}**
100	**4166^{d}**	**0.53^{d}**	**6633^{c}**	**0.65^{c}**	**5425^{c}**	**0.83^{c}**
200	**6516^{b}**	**0.62^{b}**	**8410^{b}**	**0.89^{b}**	**6966^{b}**	**0.88^{b}**
400	**7523^{a}**	**0.74^{a}**	**16466^{a}**	**0.97^{a}**	**8600^{a}**	**0.95^{a}**

*****: Means in the same column with different letters have significant differences between each other.

Table (3): The effect of *Lentinus edodes* ethanolic extracts on breast cancer patient lymphocyte proliferation at 24 hrs of exposure determined by Trypan blue exclusion test and MTT assay.

Concentration (µg/ml)	Ethanolic extract of *Lentinus edodes LC2141*mycelia		Ethanolic extract of *Lentinus edodes LC202* mycelia	
	Count*	Absorbance *	Count*	Absorbance *
Control	8500^{e}	0.02^{e}	8500^{e}	0.02^{e}
50	9900^{d}	0.31^{d}	9250^{d}	0.16^{d}
100	10450^{c}	0.43^{c}	10120^{c}	0.21^{c}
200	14750^{b}	0.50^{b}	12650^{b}	0.23^{b}
400	22275^{a}	0.59^{a}	16850^{a}	0.26^{a}

*****: Means in the same column with different letters have significant differences between each other.

References

1. Aoki, T. (1984): Lentinan. In: Fenichel, R.L., Chigris, M.A. (Eds.), Immune Modulation Agents and Their Mechanism. Marcel Dekker, Inc., New York and Basel, pp. 63–77.
2. Boyum, A. (1976): Separation of white blood cells. Nature 204: 793-794.
3. Chihara, G.; Hamuro, J.; Maeda, Y.Y.; Arai, Y. and Fukuoka, F. (1970): Fractionation and purification of the polysaccharides with marked antitumor activity, especially Lentinan, from Lentinus edodes (Berk.) Sing. (an edible mushroom). Cancer research 30: 2776-2781.
4. Duncan, C.J.; Pugh, N.; Pasco, D.S. and Ross, S.A. (2001): Isolation of a galactomannan that enhances macrophage activation from the edible fungus Morchella esculenta. J. Agric. Food Chem.(50): 5681–5685.
5. Hansen, M.B; Nielsen, S.E. and Berg, K. (1989): Method. J. Immunol.119: 203-210.
6. Hibino, Y.; Konishi, Y.; Koike, J.; Tabata, T.; Ohashi, Y. and Sugano, N. (1994): Productions of interferon-g and nitrite are induced in mouse splenic cells by a heteroglycan-protein fraction from culture medium of Lentinus edodes mycelia. Immunopharmacol. 28:77–85.

7. Hirasawa, M.; Shouji, N.; Neta, T.; Fukushima, K. and Takada, K. (1999): Three kinds of antibacterial substances from Lentinus edodes (Berk.) Sing. (Shiitake, an edible mushroom). Int. J. Antimicrob. Agents 11:151–157.
8. Hobbs, Ch. (2003). In: Miovic, Michael (Ed.), Medicinal Mushrooms: An Exploration Of Tradition, Healing and Culture. Botanica Press, Williams, OR.
9. Ikekawa,T.; Uehara, N.; Maeda, Y.Y.; Nakanishi, M. and Fukuoka, F. (1969): Antitumor activity of aqueous extracts of edible mushrooms. Cancer research 29: 734-735.
10. Israilides, C. and Philippoussis, A.(2003): Bio-technologies of recycling agro-industrial wastes for the production of commercially important fungal polysaccharides and mushrooms. Biotechnol. Genet. Eng. Rev. 20: 247–259.
11. Israilides, C.; Kletsas, D.; Arapoglou, D.; Philippoussis, A.; Pratsinis, A.; Ebringerova, H.; Hrˇı'balova, V. and Harding, S.E. (2007): In vitro cytostatic and immunomodulatory properties of the medicinal mushroom Lentinula edodes. Phymed. 1-8.
12. Ivankovic, S.; Hirsl, N.; Jakopovic, I. and Jurin, M. (2004): the influence of medicinal mushroom preparations on mouse tumors. International journal of medicinal mushrooms 6: 107-116.
13. Jong, S.C. and Birmingham, J. M. (1993): Medicinal and therapeutic value of the Shiitake mushroom. Adv. Appl. Microbiol. 39:153-184.
14. Kidd, P.M. (2000): The use of mushroom glucans and proteoglycans in cancer treatment. Altern. Med. Rev. 5: 4–27.
15. Lazarus, H.; Tegeler, M.; Mazzone, H.; Leroy, J.; Boone, B. and Foley, G. (1966): Determination of sensitivity of individual Biopsy specimens to potential inhibitory agents. Evaluation of some explants cultures as assay systems. Cancer chemother. Rep. 50: 543-554.
16. Lizuka, C. (1986): Antiviral Substance and the Manufacturing Method Thereof. United States Patent 4: 627-629.
17. Lobanok, A.G.; Babitskaya, V.G.; Plenina, L.V.; Puchkova, T.A. and Osadchaya, O.V. (2003): Composition and biological activity of submerged mycelium of the xylotrophic Basidiomycete Lentinus edodes. Appl. Biochem. Microbiol. 39: 60–64.
18. Mizuno, T. (1995): Shiitake, Lentinus edodes: functional properties for medicinal and food purposes. Food Rev. Int. 11: 7–21.
19. Mizuno, T. (1999): The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan. International Journal of Medicinal Mushrooms 1: 9-29.
20. Morita, K. and Kobayashi, S. (1967): Isolation, structure, and synthesis of lenthionine and its analogs. Chem. Pharm. Bull 15:988–93.
21. Nitha, B.; Meere, C.R. and Janardhanan, K.K. (2007): Anti-inflammatory and antitumor activities of cultured mycelium of morel mushroom, Morchella esculenta. Current science 92(2): 235-239.
22. Ooio, V.E. and Liu, F. (1999): A review of pharmacology activities of mushroom polysaccharides. Int. J. Med. Mushrooms 1: 195–206.
23. Sheldon, H. and Preskorn, M.D. (1996): Why Did Terry Fall Off the Dose-Response Curve? Journal of Practical Psychiatry and Behavioral Health; 39-43.
24. Stamets, P. (1993): Growing gourmet and Medicinal mushrooms. Ten Speed Press, Berkeley, CA 94707.
25. Sugano, N.; Choji, Y.; Hibino, Y.; Yasumura, S. and Maeda, H. (1985): Anticarcinogenic actions of alcohol-insoluble fractions (LAP) from culture medium of Lentinus edodes mycelia. Cancer Lett., 1–6.
26. Suzuki, F.; Suzuki, C.; Shimomura, E.; Maeda, H.; Fujii, T. and Ishida N. (1979): Antiviral and interferon-inducing activities of a new peptidomannan, KS-2, extracted from culture mycelia of Lentinus edodes. J. Antibiot. 32:1336–45.
27. Suzuki, H.; Okubo, A.; Yamazaki, S.; Suzuki, K.; Mitsuya, H. and Toda, S. (1989): Inhibition of the infectivity and cytopathic effect of human immunodeficiency virus by water-soluble lignin in an extract of the culture medium of Lentinus edodes mycelia (LEM). Biochem. Biophys. Res. Commun. 160:367–73.
28. Takazawa, H.; Tajima, F. and Miyashita, C. (1982): An antifungal compound from Shiitake (Lentinus edodes). Yakugaku Zasshi 102:489–91 in Japanese.
29. Tochikura, T.S. (1988): Inhibition (in vitro) of replication and of the cytopathic effect of human immunodeficiency virus by an extract of the culture medium of Lentinus edodes mycelia. Med. Microbiol. Immunol. 177: 235–244.
30. Turkoglu, A.; Duru, M.E. and Mercan, N. (2007): Antioxidant and antimicrobial activity of Russula delica Fr. An edible wild mushroom.

Eurasian journal of analytical chemistry 2(1): 54-67.
31. Walter, A. and Duncan, D.B. (1969): Multiple ranges and multiple tests. Biometries, 11:1-24.
32. Wasser, S. and Weis, A. (1999): Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (review). Int. J. Med. Mushrooms 1: 31–62.
33. Yamasaki, K.I.; Sone, S.; Yamashita, T. and Ogura, T. (1989): Synergistic induction of lymphokine (IL-2)-activated killer activity by IL-2 and the polysaccharide Lentinan, and therapy of spontaneous pulmonary metastases. Cancer Immunol. Immun. 29: 7–92.
34. Yasumoto, K.; Iwami, K. and Mitsuda, H. (1971): A new sulphur-containing peptide from Lentinus edodes acting as precursor for lenthionine. Agric. Biol. Chem. 35:2059–69.

5/23/2011