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Cytotoxicity Assay of Agaricus Bisporus Extract , Oxaliplatin and Combination of both on Melanoma-B16 and Vero-101 Cell line:An in Vitro study

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***Abstract*— The present study included extracted Agaricus bispours and estimated proteins and carbohydrates amount in extract then assessment activity of methanolic crude extract of medicinal mushroom A. bisporus as in vitro anticancer by using melanoma-B16 cell line ,and compared with chemotherapy Oxaliplatin and approved reduction toxic effect of chemotherapy on normal cells when exposed to combination between chemotherapy and mushroom extract on normal vero-101 cell line. Proteins and carbohydrate estimation results referred to there are about 219.33 and 412.98 µg/ml respectively. The results showed that inhibition rate of melanoma-B16 cell line was about 82.15% according to cytotoxicity assay test by crystal violate when treated these cells by methanol crude extract of A. bisporus at 1000 mg/ml .But the cytotoxicity assay of using chemotherapy oxaliplatin on melanoma-B16 at 100 mg/ml results appeared that the inhibition rate was 64.52%. The cytotoxicity assay of combination between methanolic crude extract of A.bisporus 1000 mg/ml and oxaliplatin 100 mg/ml results the inhibition rate was 70.04% on melanoma B-16 cell line.Crystal violate cytotoxicity assay of treatments on normal vero-101 cell line results were revealed that non significantly inhibition of A.bisporus extract on normal cells only 5.37%.While chemotherapy oxaliplatin have inhibition rate on vero-101 normal cell line about 65% ,While the combination treatment cytotoxicity assay on vero-101 normal cell line showed that inhibition rate was reduced to 20.35% comparing with cancer cell which was about 65.3%.**

Keywords— Agaricus bisporus, Oxaliplatin , melanoma-B16 , vero-101, cell line, cytotoxicity, anticancer.

I. INTRODUCTION

The button mushroom, Agaricus bisporus is the most critical developed mushroom on the world (Masoumi et al., 2015). Be that as it may, thinks about into the anticancer properties of A. bisporus are clearly restricted, when contrasted with other imperative developed mushrooms. Especially, exceptionally restricted examinations have been attempted to distinguish anticancer compounds from this mushroom. Up until now, extremely restricted research articles have laid out accomplishments on anticancer activities detailed in a few species of edible mushrooms, including A. bisporus (Patel and Goyal, 2012; Xu et al., 2012). However, recent developments in the research into anticancer properties of A. bisporus have not been reviewed. Furthermore, the anticancer potential of wild populations of A. bisporus has not yet been evaluated. Many researches have investigated anticancer properties of crude extracts of A. bisporus in vitro.That is because the cancer is a major cause of death all over the world, promoting long lasting effects throughout the lifetime of the patient and chemotherapy using for treatment it. Be that as it may, late improvements in the research into anticancer properties of A. bisporus have not been inspected. Moreover, the anticancer capability of wild populaces of A. bisporus has not yet been assessed. Numerous looks into have researched anticancer properties of A. bisporus crude extracts in vitro. That is on the grounds that the cancer is a noteworthy reason for death everywhere throughout the world, advancing durable impacts all through the lifetime of the patient and chemotherapy utilizing for treatment it. So ,Chemotherapy is best at killing cells that are quickly division. Unfortunately chemotherapy does not know the contrast between the carcinogenic cells and the normal cells. The "normal" cells will develop back and be healthy however meanwhile, side effect happen. The "normal" cells most normally influenced by chemotherapy are the blood components, the cells in the mouth, stomach and gut, and the hair follicles; bringing about low blood count, mouth sores, nausea, diarrhea, and additionally hair loss. Distinctive medications may influence diverse pieces of the body(Woynarowski et al., 2000; Di Francesco et al., 2002)

In recent years, research has observed into different sorts of mushroom and mushroom extracts or compounds. Studies have looked at whether mushrooms can stop cancer, stop the growth of cancer cells, decrease cancer treatment

side effects, or help people with advanced cancer to live longer (Zhou et al., 2005) so, along with these line our study aimed to using crude extract of Agaricus bisborus as in vitro anti melanoma cancer cell line and approved less toxic effect of chemotherapy Oxaliplatin on normal cell line when combined with it.

II. MATERIAL AND METHODS

1- Prepared of fungal extracts:

The fruiting bodies were granulate utilizing a blender . This crushed biomass (100 g) was suspended in 400 ml of absolute methanol and incubation at 200 rpm for 48 hrs. and, 37°C. The suspension was separated with paper Whatman No. 2 to expel the biomass, this strategy was repeated twice. The supernatant was concentrated in a rotational evaporator at 40°C under decreased pressure. The subsequent dried biomass was dissolved in distilled water to make stocks 50 mg/ml and put away at 4°C. according to (Jedinak and Sliva, 2008) with modification.

2- Prepare Liquid –Liquid extract

Equal volume of deionized distilled water and chloroform were added to above dried extracts and shaking at 250 rpm for 1 hour then lifted until two layers, upper water layer was gained for the following study (Berk, 2013).

3- Chemical Analysis of A.agaricus crude extract:

A- Determination of Protein Concentration:
Protein concentration was determined according to Bradford (Bradford, 1976) , the Standard solutions 20, 40, 60, 80 and 100 µg/ml of bovin serum albumin were prepared from the stock solution 1 mg/ml, in order to plot the standard curve.

B-Carbohydrate Determination:
According to Doboie et al.., (Dubois *et al.*, 1956) , the phenol-sulphuric acid method was used to determine carbohydrate concentration ,carbohydrate concentration was estimated from the standard curve of glucose stock solution(1mg/ml) using curve fitting equation.

C- Prepration of anticancer drug:
100 mg ampole of oxaliplatin were prepared by filtrated it through 0.2µm Nalgin filter them used it.

D- Anticancer activity assay:
Melanoma-B16 human skin cancer cells and monkey normal vero-101 fibroblasts cells were prepared and cultured in RPM1-1640 medium , According to Freshney (Freshney, 1994), the cytotoxicity assays were applied for determination of the effect of fungal crude extract on B16-melanom cell line culture with 1000µg\ml of fungus extract and compared with anticancer drug oxaliplatin with 100mg\ml concentration and test of combination between crude extract and anticancer drug for defined duration.
When the growth of Melanoma-B16 and Vero-101 cell line culture in the flask became as monolayer before it reached the exponential phase, the cell monolayer were harvested and re-suspended with a growth medium in a concentration of 5×10^5 cell / ml and seeded in a 96 well microtiter plate. Since the cell growth reaches 80%, Then all plate was covered with self plastic lid and incubated for 24 hrs at $37c^0$. After the end of the exposure the wells washed with 200 µl of a sterile PBS. The effect on cell line growth was assessed by Crystal Violate assay (Freshney, 1994).

III. RESULTS AND DISCUSSION

1-Chemical Analysis of A.bisporus crude extract:
Proteins and carbohydrate approximation results mentioned to there are near 219.33 and 412.98 µg/ml respectively (figure 1). That's settled with (Boda *et al.*, 2012) who mentioned that carbohydrate content was 4.85 g/ml while protein content was 1.80g of dehydrated weight of A.bisporus . From nutritious perspective, mushrooms are exceptionally esteemed because of high proteins and additional nutrients than most of the plants (Chang, 1980).The carbohydrate was found at highest concentration in extract. This is like the discoveries of (Blumenthal, 1976) The chemical structure of mushrooms decides their nutritive rate and this nutritive rate differs depends upon the nature of substrate, atmospheric settings, stage of progress of the mushrooms and part of fruiting body used further the situations of packing later harvest [Manzi *et al.*, 2001; Adejumo and Awesanya, 2005).

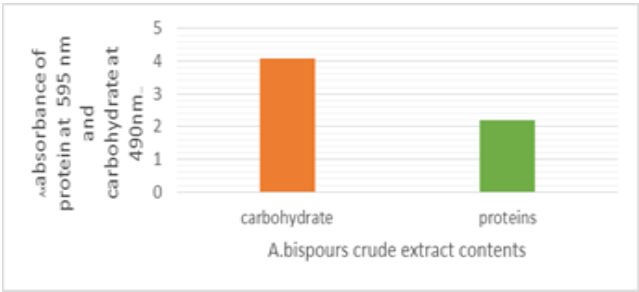


Fig.1. Estimation of carbohydrates and proteins in A.bispours crude extract

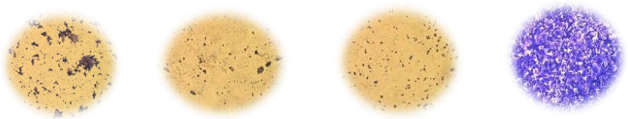
Anticancer activity assay of Agaricus bisporus crude extract ,chemotherapy oxaliplatin and combination between them on melanoma B-16 cell line : The results indicated that inhibition rate of melanoma-B16 cell line was about 82.15% giving to cytotoxicity assay test by crystal violate when treated these cells by methanol crude extract of Agaricus bisporus at 1000 mg/ml (table1 and figure2).
Same results gained by Yu(Yu *et al.*, 1993) who referred to the White button mushroom (WB)- determined lectin repressed the development of human colon cancer cells. Also the high temp water concentrate of WB restrained aromatase movement and the development breast cancer cells that permitted by Grube (Grube *et al.*, 2001).And the 20% methanol-water fraction from WB reserved prostate cancer cell growth founded by Adam (Adams *et al.*, 2008). While the high temp water concentrate of WB incited apoptosis in breast cancer cells gained by Martin(Martin.*et al.*, 2009).

These effects of cancer cell inhibition may be related to few compounds of A. bisporus have been identified to exert anti-proliferative or cytotoxicity; including lectin, unsaturated fatty acids such as linoleic, linoleic conjugate, and linolenic, and polysaccharides, meriting further study to detect further bioactive combinations in this important viable mushroom. Finding and description of anticancer myco-compounds existing in A. bisporus are necessary to better know their type of action and enable future studies by distilled compounds. Besides, wild populations of A. bisporus may offer a great source of nutritional and medicinal bioactive combinations.The cytotoxicity prove of using chemotherapy oxaliplatin on melanoma-B16 at 100 mg/ml results looked that the inhibition rate was 64.52% (table 1 and figure 2).

The inhibition action of oxaliplatin connected to mechanism of action of this medicine that involved prevents the production of deoxyribonucleic acid (DNA). The guanine and cytosine content associates with the unit of Oxaliplatin-induced cross-linking. At in height concentrations of the medicine, cellular RNA and protein synthesis are also blocked (Alcindor and Beauger, 2011). The cytotoxicity prove of mixture between methanolic crude extract of A. bispours (1000 mg/ml) and oxaliplatin (100 mg/ml) results the inhibition rate was 70.04% on melanoma B-16 cell line (table 1and figure 2). This effect of mixture is recognized indifferent effect in which one of composition no decrease action additional one .so, the action of fungus extract and oxaliplatin continuous as achievement alone.

TABLE.1. Cytotoxicity assay of Agaricus bisporus crude extract, chemotherapy oxaliplatin and combination between them on melanoma B-16 cell line:

Treatments	Absorption rate±sd	Inhibition rate%
Agaricus bisporus crude extract	0.085±0.018	82.15
Oxaliplatin	0.169±0.066	64.52
Combination	0.142±0.008	70.04
Control	0.476±0.09	0



A B C D

Fig.2.Micrograph of inverted phase contrast microscope after 24 h exposure of melanomaB-16 cells to: (A): Oxaliplatin.(B): A.bisporus extract.(C): combination (A+B).(D) control without treatment.

2- Cytotoxicity assay of Agaricus bisporus crude extract , chemotherapy oxaliplatin and combination between them on vero-101 cell line:

Crystal violate cytotoxicity evaluate of behaviors on normal vero-101 cell line effects were shown that non expressively inhibition of A.bisporus extract on normal cells only 5.37% (table 2 and figure 3) ,these result lined with Hamzaa (Hamzaa, 2011) ,who mentioned to no cytotoxic effect of A.bisborus extract on rat embryo fibroblasts (REF) normal cell line.That's connected to the selectivity might be as such as of particular of the metabolic characteristics that the cancer cells have , and not extant in the normal cells, such as the metabolic characteristic to yield new blood vessels, also the form and nature of the receptors existing on the cancer cells' surface and the probability of their binding with different combinations(Moteki *et al.*, 2002).

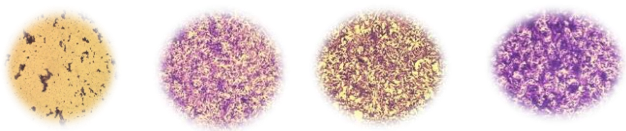
Chemotherapy oxaliplatin have inhibition rate on vero-101 normal cell line 65.3% , (table 2 and figure 3) that's since of Conservative mono-therapeutic techniques non-selectively target vigorously proliferating cells, which eventually indications to the construction of both healthy and cancerous cells. Chemotherapy can be poisonous to the patient with many side effects and dangers, and can too strongly decrease their immune system by affecting bone marrow cells and increasing predisposition to host diseases (Partridge *et al.*, 2001; LeBaron *et al.*,1988)

Whereas the mixture treatment cytotoxicity prove on vero-101 normal cell line indicated that embarrassment rate was reduced to 20.35% matching with cancer cell which was about 70.04% (table 2 and figure 3).

Though mixture therapy can be poisonous if one of the agents used is chemotherapeutic, the toxicity is considerably less because unlike pathways will be targeted. Eventually, this works in a synergistic or preservative way, and then a minor therapeutic dose of every individual medicine is necessary (Albain *et al.*, 2008; Mokhtari *et al.*, 2013). Moreover, mixture therapy may be able to stop the poisonous effects on normal cells whereas concurrently producing cytotoxic effects on cancer cells. This may happen if one medicine in the mixture regime is antagonistic, in terms of cytotoxicity, to additional medicine in normal cells, fundamentally protective normal cells from cytotoxic effects (Blagosklonny, 2005). To achieve this, the mixture regimen takes benefit of the unimportant changes between cancer cells and normal cells, such as the deficiency of a target (lack of p53), or by the existence of a target (surface marker) (Blagosklonny, 2008).

TABLE.2.Cytotoxicity assay of Agaricus bisporus crude extract, chemotherapy oxaliplatin and combination between them on vero-101 cell line

	Treatments	Absorption rate±sd	Inhibition rate%
1	Agaricus bisporus crude extract	0.193±0.006	5.37
2	Oxaliplatin	0.071±0.016	65.3
3	Combination	0.163±0.058	20.35
4	Control	0.204±0.014	0



A B C D

Fig.3.Micrograph of inverted phase contrast microscope after 24 h exposure of vero-101 cells to: (A): Oxaliplatin.(B): A.bisporus extract.(C): combination (A+B).(D) control without treatment.

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