


Evaluation of Some the Cytotoxic Effects of Edible Mushroom Extracts *Pleurotus ostreatus*

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Abstract

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Background: *Pleurotus* genus or oyster mushroom is the richest of all edible mushroom because of its medicinal and therapeutic value for human health, their medicinal importance due to their active compounds and enzymes that have been classified as biologically active, antitumor, anticancer, antihypersensitivity and antiaging, and in the treatment of HIV and high blood pressure.

Objective: To testing the cytotoxic effects of aqueous and alcoholic extracts of an Iraqi strain of edible mushroom *Pleurotus osteratus* in the presence of the positive control represented by the toxic drug methotrexate and measuring the percentage of free radical scavenging of these extracts.

Patients and Methods: The dried powder of fruiting bodies of Iraqi strain *Pleurotus ostreatus* was prepared aqueous (hot and cold) and ethanol 96% extracts at three different concentrations (100, 200, 300) µg/ml, the cytotoxic effects of these extracts was investigated using peripheral blood lymphocytes (PBLs) and (0.65) µg/ml of methotrexate (MTX) drug was counted as positive control. The slides were microscopically examined (10X), and at least 1000 cells were inspected to determine the Blastogenic Index BI, Mitotic Index MI and chromosomal aberration (TCA) and Antioxidant Test of the these extracts s were evaluated by DPPH radical-scavenging activity assay (2,2- diphenyl-1-picryl-hydrazylhydrate).

Results: the results of toxic effects of hot, cold aqueous and ethanol extracts on peripheral blood lymphocytes (PBLs) showed no significant differences in (TCA) % with negative control in comparison with positive control. While, There has been a noticeable change in (MI) % and (BI) % with negative control, likewise, Scavenging activity was the highest for aqueous and ethanol extracts with increasing the concentrations when compared with the control ascorbic acid.

Conclusion: Aqueous and ethanol extracts of *Pleurotus ostreatus* are free of any toxic effect on lymphocytes of the peripheral blood, which can be considered a mitogenic agent

Keywords: Aqueous and ethanol extracts; *Pleurotus ostreatus*; Total Chromosomal Abbreviation (TCA); (DPPH) assay

Introduction

The genus of *Pleurotus* or oyster mushroom is edible fleshy macrofungi that are classified in the family Pleurotaceae belonging to the order Agaricales, phylum Basidimycetes of Kingdom Fungi [1]. The

active components include pleuran, lectin, and PS-I and II (pure polysaccharides) [2]. In addition to rich in polysaccharides that have demonstrated high antioxidant and immunostimulating activities and narrow

antibacterial activity and widespread in temperate, subtropical and tropical regions and it appears the highest yield among the *Pleurotus spp* [3]. Mushrooms have also been described as a rich source of antioxidant components due to phenolic components and other polysaccharides. The phenolic compounds and other chemicals that can scavenge free radicals was associated to antioxidant activity [4]; [5]. Also, are enriched with polyphenols, ascorbic acid, tocopherols, lycopene, β -carotene, carboxylic acids, and various dietary fibers, which are the major bioactive component found in extracts isolates [6]. Then different concentrations 100 , 200, 300 were prepared from these extract. The main objective of this study was to investigate, cytotoxicity, and antioxidant effects of the cultivated Iraqi strain *P. ostreatus* polar extracts .

Patients and Methods

Preparation of Edible Mushroom *Pleurotus ostreatus* Extracts

The fruiting bodies dried of mushrooms Iraqi strain *Pleurotus ostreatus* was obtained from College of Agricultural Engineering Science -Baghdad university-Baghdad- Iraq , then crushed using a hand grinder and distributed to prepare extract (hot aqueous extracts and 96 % ethanol extract using Soxhlet system [7].in addition to cold aqueous extract was prepared according to [8].

Test the Toxicity of Edible Mushroom *Pleurotus ostreatus* Extracts

The cytotoxic effects of aqueous and alcoholic extracts of *Pleurotus ostreatus* was investigated using peripheral blood lymphocytes (PBLs) according to the method mentioned in [9]. Blood samples (0.5 mL)

were drawn from health adult volunteers in heparinized tubes who are not subject to any chemotherapy and who do not suffer from any chronic disease or are exposed to any type of known pollutants and non-smokers, aged 25-35 years , then added to (4.5) ml of complete culture medium (RPMI-1640) supplemented with [10] μ l of t phytohemagglutinin (PHA) with four different concentrations of aqueous and alcoholic extracts of each strains of edible mushrooms at concentrations (0.0, 100, 200, 300) μ g/ml, and (0.65) μ g/ml of methotrexate (MTX) drug was counted as positive control.

The slides were microscopically examined (10X), and at least 1000 cells were inspected to determine the Blast Index (BI) , Mitotic Index MI and chromosomal aberration which were given as a percentage of total cell.

$$MI = \frac{\text{Mitotic cells}}{1000 \text{ cell}} \times 100\%$$

$$BI = \frac{\text{stimulated cells}}{1000 \text{ cell}} \times 100\%$$

Chromosomal aberrations= a minimum of 100 cells are counted.

Passing through the metaphase phase of cell division were selected randomly for each replicate.

Antioxidant Activity of Edible Mushroom *Pleurotus ostreatus* Extracts

Antioxidant test of the following extracts , alcoholic and aqueous extracts were evaluated by DPPH radical-scavenging activity assay (2,2- diphenyl-1-picrylhydrazylhydrate). The following solutions were prepared according to Brand-Williams [10].

Statistical Analysis

Data were analyzed statically using Statistical Package for the Social Sciences, SPSS version 16 software. The level of significance was measured by using the Analysis of Variance (ANOVA) test [11]. The level of significance was shown using the least significant difference (LSD) test.

Results

Test the Toxicity of Edible Mushroom *Pleurotus ostreatus* Extracts

The cytotoxic effects of aqueous and alcoholic extracts of *P. ostreatus* on Peripheral Blood lymphocytes (PBLs) were obtained from healthy donors in vitro as shown in Table -1- Different letters within the same column indicate significant differences at $P \leq 0.05$.

The results appeared no significant differences in Total Chromosomal Aberrations (TCA) % at three different concentration (100, 200, 300) $\mu\text{g/ml}$ of treated (PBLs) with hot, cold aqueous and ethanol extracts with negative control (-ve) at $P \leq 0.05$, in comparison with positive control (+ve) which represented treated (PBLs) with Methotrexate (MTX) at concentration 6.5 $\mu\text{g/ml}$ which was recorded

1.85%. Figure (1) and (2) showed some chromosomal aberration induced by Methotrexate and negative control. Mitotic index (MI) and blastogenic index (BI) are significant effects on (PBLs) treated with three concentrations of aqueous and ethanol extracts compared with negative control.

Antioxidant Activity of Edible Mushroom *pleurotus osteratus* Extracts

The free radical scavenging activities of fruiting bodies extracts *pleurotus osteratus* (hot aqueous, cold aqueous, and ethanol) and the standard ascorbic acid at different concentrations (100, 200, 300 $\mu\text{g/ml}$) were showed in table 2. The hot aqueous extract had the maximum DPPH radical scavenging efficacy (86.47%) at concentration 300 $\mu\text{g/ml}$. and the minimum DPPH radical scavenging efficacy was in cold aqueous extract (41.25%) at 300 $\mu\text{g/ml}$ and .The hot aqueous extract and ethanol at 200 $\mu\text{g/ml}$ were antioxidant activity (76.02, 79.88) % respectively that were higher than in ascorbic acid at the same concentration 70.79% DPPH is a stable free radical with good absorption at 515 nm, it is use to study radical scavenging activity of extracts.

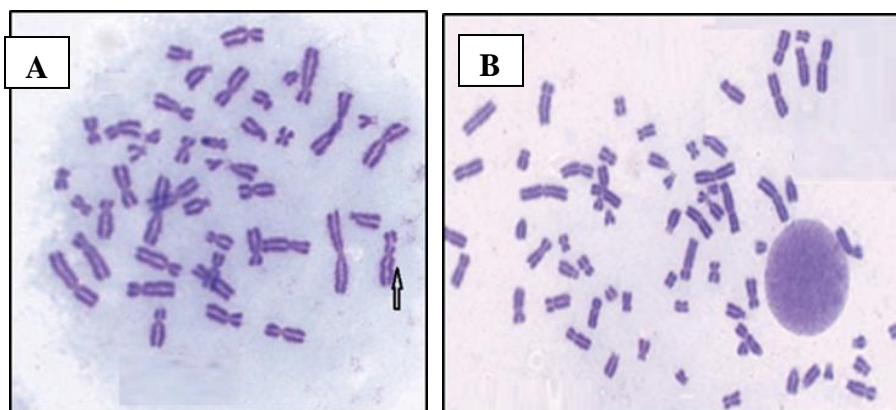


Figure (1): A- Chromosomal aberration Induced by Positive Control (MTX) Refer to Chromatids' GAP- B- Negative Control (view for the normal) metaphase 100 X

Table (1): Chromosomal Analyses in Peripheral Blood lymphocytes (PBLs) Treated with *P. osteratus* Extracts

Conc. µg/ml	Hot Aqueous Extract			Cold Aqueous Extract			Ethanol Extract		
	BI	MI	TCA	BI	MI	TCA	BI	MI	TCA
Control -ve	88.12c	1.77d	0.12 b	88.15c	2.31 a	0.12b	88.15c	1.77d	0.12b
100	88.82c	1.83c	0.11 b	88.07c	1.84 b	0.10b	91.23b	1.86 c	0.10 b
200	91.37b	1.91b	0.09 bc	90.85b	1.89ab	0.10 b	93.45	1.95b	0.00c
300	94.95a	2.21a	0.06 c	92.19a	2.31a	0.08b	94.24a	2.44a	0.00c
Control +ve	22.37d	0.16e	1.85a	22.37d	0.16 c	1.85a	22.37d	0.16 e	1.85a
LSD0.05	1.37	0.04	0.04	1.20	0.44	0.04	1.02	0.04	0.04

BI, blastogenic index, MI, mitotic index, TCA, total chromosomal aberrations.

Table (2): Antioxidant Activity% of *P. osteratus* Extracts

Type of Extracts	<i>Pleurotus osteratus</i>			Ascorbic Acid		
	Conc. µg/mL	Absorbance	Anti-oxidant activity %	Conc. µg/mL	Absorbance	Anti-oxidant activity%
Hot Aqueous Extract	0.0	0.880	0.0	0.0	0.880	0.0
	100	0.252	71.3	100	0.341	61.25
	200	0.211	76.02	200	0.257	70.79
	300	0.141	83.98	300	0.118	86.59
Ethanol Extracts	0.0	0.880	0.0			
	100	0.246	72.04			
	200	0.177	79.88			
	300	0.119	86.47			
Cold Aqueous Extract	0.0	0.880	0.0			
	100	0.517	41.25			
	200	0.324	63.18			
	300	0.229	73.97			

Discussion

From above results the aqueous and ethanol extracts *P. osteratus* induced MI and BI value, no significant differences in chromosomal aberration values compared with untreated PBLs. Which indicates that the three extracts are free of any toxic effect on lymphocytes of the peripheral blood, which can be considered a mitogenic agent that greatly affected the protein synthesis process inside these cells and contributed to increasing their size and content of compounds necessary for cell division

[12]. Among the studies, that found aqueous and alcoholic extracts of edible mushrooms contain high protein concentrations and sugar-related proteins (lectins) that work to strengthen immunity through a significant increase in the number of T lymphocytes responsible for cellular immunity necessary to fight tumors, viruses, and parasitic infections. And a significant increase in the concentration of antibodies after feeding or dosing with aqueous extracts of edible mushrooms, they play an important role in resisting infection and increasing the level of

immune response [13]. And compensation for damaged and damaged tissues by increasing the rate of mitosis for many types of somatic cells, not only lymphocytes of the peripheral blood, and it can be considered as a promising alternative therapies for immune modulation and strengthening the specific and specific immune response in the fight against tumors, and cardiovascular diseases [14]. It is used to study radical scavenging activity of extracts. When antioxidant donate proton to these radicals then absorption of samples decreases, radical scavenging activity is measured by decrease in absorption of samples. ethanol and hot aqueous extracts in fruiting bodies *P. ostreatus* tested at different concentrations in present study showed good radical scavenging activity. The antioxidant activity percentages increased with increasing concentrations for three extracts. This pattern is known as the dose dependent pattern, this action is most likely due to the presence of polar compounds in the extracts responsible for the mentioned activity [15] and [3].

Conclusions

The fruiting bodies aqueous and ethanol extracts of *P. ostreatus* have low toxic effects on Peripheral Blood lymphocytes (PBLs) also these extracts revealed a good radical scavenging activity were evaluated by DPPH radical-scavenging activity assay 2,2-diphenyl-1-picryl-hydrazylhydrate

Recommendations

Carry out several studies should be done on other genera of Iraqi wild mushrooms that have medical important and test further cytotoxicity effects such as DNA damage in addition to more biological activities around genus *Pleurotus* extracts.

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Ethical clearance: The Ethics Committee of the College of science Mustansiriyah university approved the current study.

Conflict of interest: Nil

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تقييم بعض التأثيرات السمية الخلوية لمستخلصات العرهون القابل للاكل

Pleurotus ostreatus

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الملخص

خلفية الدراسة: جنس الفطر Pleurotus أو الفطر المحاري هو أغنى أنواع الفطر الصالح للأكل نظرًا لقيمتها الطبية والعلاجية لصحة الإنسان ، وتعود أهميته الطبية الى وجود مركبات نشطة بايولوجيا بالاضافة الى الإنزيمات من خلال فعاليتها المضادة للأورام ، والمضادة للسرطان ، والمضادة للحساسية ، والمضادة للشبخوخة ، و في علاج فيروس نقص المناعة البشرية وارتفاع ضغط الدم.

اهداف الدراسة: لاختبار التأثيرات السمية الخلوية لمستخلصات المائية والكحولية لسلاله عراقية من العرهون القابل للاكل بوجود السيطرة الموجبة المتمثلة بعقار الميثوتركسيت ذو التأثير السمي وقياس نسبة المئوية لكسح الجذور الحرة لهذه المستخلصات

المرضى والطرائق: تم تحضير المسحوق المجفف لأجسام الثمرية للسلالة العراقية Pleurotus ostreatus المائي (ساختن وبارد) وخلاصة الإيثانول 96% بتركيزات مختلفة (100 ، 200 ، 300) ميكروغرام / مل ، تم فحص التأثيرات السمية الخلوية لهذه المستخلصات باستخدام الخلايا الليمفاوية في الدم المحيطي وبوجود السيطرة الموجبة المتمثلة بعقار الميثوتركسيت بتركيز (0.65) ميكروغرام / مل. تم فحص الشرائح مجهرًا على قوة تكبير (x10) ، وتم فحص ما لا يقل عن 1000 خلية لتحديد مؤشر معامل الانقسام الخلوي (MI) Mitotic Index ومعامل الارومي (BI) Blastogenic وانحراف الكروموسومات (TCA Total Chromosomal Abberation) تم تطبيق أنشطة الكسح الجذري ومضادات الأكسدة الكلية على المستخلص المائي والكحولي بواسطة مقياس (DPPH-2,2) diphenyl-1-picrylhydrazyl .

النتائج: لم تظهر نتائج التأثيرات السامة لمستخلصات المائية الساخنة والباردة ومستخلص الإيثانول على الخلايا الليمفاوية في الدم المحيطي (PBLs) اي فروق معنوية في مؤشر الانحراف الكروموسومات (TCA) مقارنة مع معاملة السيطرة السالبة والسيطرة الموجبة. لكن لوح تغير ايجابي واضح في مؤشري (MI) و (BI) مقارنة مع السيطرة السالبة ، ومن جهة اخرى كان نشاط كسح الجذور الحرة هو الأعلى بالنسبة للمستخلصات المائية والإيثانولية مع زيادة التركيز عند مقارنتها بحمض الأسكوربيك الذي يمثل معاملة السيطرة .

الاستنتاجات: المستخلصات المائية والإيثانولية للفطر P.ostreatus خالية من أي تأثير سام على الخلايا الليمفاوية في الدم المحيطي ، والتي يمكن اعتبارها عاملاً مسبباً للانقسام.

الكلمات المفتاحية: المستخلصات المائية والإيثانولية- Pleurotus osteratus - الانحراف الكروموسومات TCA - تقنية ال (DPPH)

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