Arch Biol Sci. 2022;74(4):315-324 https://doi.org/10.2298/ABS220803030M

Anticancer and apoptotic effects of a polysaccharide extract isolated from *Lactarius chrysorrheus* Fr. in HepG2 and PANC-1 cell lines

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Received: August 3, 2022; **Revised**: September 4, 2022; **Accepted**: September 7, 2022; **Published online**: September 15, 2022

Abstract: Mushrooms are widely used in many cultures for nutritional and health benefits. The *Lactarius* species is found in the Aegean region of Turkey. *Lactarius chrysorrheus* Fr. is a wild mushroom that contains a milky juice. In this study, we investigated the *in vitro* cytotoxic potential and apoptotic effect of the polysaccharide extract prepared from *L. chrysorrheus* by water extraction and alcohol precipitation using the tetrazolium MTT dye assay, annexin V staining, wound healing and colony formation, and qRT-PCR. The molecular weights of three peaks observed in HPLC chromatograms were calculated as 1869.9, 3043.92 and 16821.47 Da. The extract exhibited cytotoxic activity at 72 h, with an IC_{50} value of 296.42 μg/mL in HepG2 and 444.43 μg/mL in PANC-1 cells; the extract that was tested on the normal HEK293 cell line exhibited no cytotoxicity. Further, *L. chrysorrheus* upregulated the expression of *CASPASE 3* and *CASPASE 9* while downregulating B-cell lymphoma 2 (*BCL-2)* and B-cell lymphoma-extra large (*Bcl-xL*) genes, and inhibited cell migration and colony formation in HepG2 and PANC-1 cells. This study provides new insight into the use of the polysaccharide from *L. chrysorrheus* in the development of novel anticancer agents.

Keywords: *Lactarius chrysorrheus*; mushroom; anticancer; cytotoxicity; apoptosis

INTRODUCTION

Mushrooms are known for their nutritional importance as they are high in protein and low in energy, and as functional foods [1-3]. Mushrooms, which have been eaten since ancient times, were considered God's food by the Romans, while in traditional Chinese medicine they were called the elixir of life and were used thousands of years ago [4]. They are commonly used in conventional medicine due to their therapeutic properties and have been the subject of extensive research [5]. They contain bioactive components such as secondary metabolites (terpenoids, acids, terpenes) and a large number of polysaccharides, primarily β-glucans and glycoproteins [6-8]. Due to their low fat and high fiber content, they have great potential in preventing cardiovascular disease and are also a leading source of natural antioxidants that are beneficial in reducing oxidative damage [9,10]. Edible mushrooms are used as a source of drugs with anticancer, antioxidant, and antibacterial properties due to the functional components they contain [11-13]. The polysaccharides found in mushrooms have different uses in the food and pharmaceutical industries.

Lactarius chrysorrheus belongs to the family of Russulaceae and is a member of the genus *Lactarius*, a genus of fungi with many members commonly known as yellow-staining milkcap [14,15]. The milk from this mushroom turns white quickly but then changes to a deep yellow color; the top of the cap is a rosy red with concentric rings on a background showing yellow tints. The mushroom is commonly found under broadleaved trees, such as oak [16,17]; it is widely distributed in Turkey, Russia, North America, Mexico and Colombia [18,19]. This mushroom has been listed as edible [20,21].

The present study describes the extraction and anticancer and apoptotic effects of the *L. chrysorrheus* polysaccharide. Our findings provide insight into the use of the polysaccharide of *L. chrysorrheus* in the development of novel anticancer agents.

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MATERIALS AND METHODS

Mushroom material

Fruiting bodies of *L. chrysorrheus* were collected from a *Pinus brutia* forest in Mentese, Mugla, Turkey in November 2018. The identity of the specimens was authenticated by Dr. Hakan Alli (Department of Biology, Mugla Sitki Kocman University, Turkey) and deposited in the Mugla Sitki Kocman University Herbarium with the voucher code A. 6881.

Polysaccharide extraction and purification

The collected mushroom *L. chrysorrheus* was dried using a fruit drier and then powdered. Powdered mushrooms were extracted with water at 80°C, then cooled and filtered to obtain the water extract. Three volumes of ethanol (95%) were added to the water extract, cooled and centrifuged for 5 min at 4000 ×g. The precipitated part was is referred to as HWEP (hot water extract precipitate), which may contain molecules that precipitate in 70% ethanol media [22]. The precipitated and separated HWEP was dried under nitrogen and kept in a refrigerator (4°C) until use.

Determination of average molecular weight

The average molecular weight of the polysaccharide was determined using a Shimadzu LC-20 AT (Shimadzu, Tokyo, Japan) model high-performance liquid chromatography (HPLC-RI) apparatus. The detector temperature was adjusted at 35°C. The method was based on the separation of macromolecules using an Ultrahydrogel 1000 (300 x 7.5 mm ID) gel column (Waters Co., USA), comparing the retention time (elution volume) with those of standard dextran [23]. The injection volume was 20 µL and 50.0 mM NaCl isocratic elution was used. The flow rate was adjusted to 0.50 mL/min. The column and the flow were calibrated using standard dextrans (2.5×10^3, 5.0×10^3, 12×10^3, 25×10^3, 5×10^4, 8×10^4, 15×10^4, 27×10^4, 41×10^4 and 67×10^4 Da). The natural logarithm of the molecular weights of each dextran was calculated and used with the elution volumes to obtain the calibration graph. The following equation obtained from the graph was used to calculate the average molecular weight (Supplementary Fig. S1).

$$\text{Elution volume (mL)} = -1.4035\times\log Mw + 15.777 \qquad R^2=0.9943$$

Cell culture and reagents

Human hepatocellular carcinoma (HepG2) (ECACC 85011430), pancreatic cancer (PANC-1) (ECACC 87092802) and embryonic kidney (HEK293) (ECACC 85120602) cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM, Sigma, Germany) supplemented with 10% fetal bovine serum (FBS, Capricorn, Germany) and 1% penicillin-streptomycin mix (Capricorn, Germany). Paclitaxel was purchased from Sigma-Aldrich (USA). Cells were cultured in a humidified atmosphere at 37°C with 5% CO_2 and passaged every 2-3 days when confluency reached 90%.

MTT assay

The viability of HepG2, PANC-1, and HEK293 cells was examined using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) reagent (BioVision, USA). A 100-µL cell suspension at a density of $2x10^3$ cells was pipetted into 96-well plates (Costar, Corning, USA). After 24 h, cells were treated with different concentrations of polysaccharide extract from 31.25 to 1000 µg/mL and incubated for 24, 48 and 72 h. The polysaccharide extract was solubilized in 0.9% saline solution. After treatments, the medium was removed gently and 10 µL of MTT (5 mg/mL) was added and incubated at 37°C for 3 h. The medium was removed, 50 µL of dimethyl sulfoxide (DMSO, Sigma, Germany) was added and the absorbances were measured at 590 nm using an Epoch microplate reader (BioTek, USA). The MTT assay was performed as previously described [24]. The percentage of cell viability was calculated using the following formula:

(%)=[100×(sample absorbance)/(control absorbance)].

The obtained data were used to estimate the IC_{50} values. Calculations were performed by AAT Bioquest calculator [25].

Wound healing assay

To assess the effect of the polysaccharide of *L. chrysorrheus* on cell migration, the wound-healing assay was performed on HepG2 and PANC-1 cells as follows:

3×10^4 cells/well were seeded on a 6-well plate (Jet Biofil, China) and allowed to grow in a CO_2 incubator until confluency. After 24 h, the cells were scraped vertically by a 200 µL tip. The cells were then washed with phosphate-buffered saline (PBS) carefully two times to remove the cell debris. Then the dose groups were treated with a dose of IC_{50}, and the culture medium was used for the control group. The scratch closure was imaged until the wound healed in the control group using an inverted microscope (Oxion Inverso, Euromex, Holland) at 10× magnification. The experiment was performed in triplicate in three independent experiments.

Colony formation assay

For the determination of colony formation ability, 1×10^3 cells were seeded on a 6-well plate. The cells were treated for 72 h and maintained for 9 days under culture conditions to form colonies. Complete medium was used for the control group. The cells were fixed with 100% methanol for 10 min and stained with 0.4% crystal violet for 15 min. After staining, the wells were washed with PBS two times and dried overnight. The colonies were captured and analyzed by ImageJ software 1.53e (USA).

Annexin V/PI staining

Apoptosis was evaluated using the Annexin V-FITC Apoptosis Kit (BioVision, CA, USA) as previously described [24]. Briefly, cells were cultured with the polysaccharide for 72 h, trypsinized with 0.05% Trypsin-EDTA (Sigma, Germany), centrifuged and washed with PBS (Capricorn, Germany). The cells were then resuspended with 1X binding buffer and incubated with annexin and propidium iodide (PI) for 15 min in a dark room. Lastly, the cells were analyzed by a fluorescence cell counter (NanoEnTek, USA); H_2O_2 (200 µM) served as a positive control. Cells were classified as live, apoptotic and dead and the percentage was determined from the total number of cells.

RNA isolation and quantitative real-time PCR (RT-qPCR)

Total RNA extraction from cells was performed using the innuPREP RNA Mini Kit 2.0 (Analytik Jena, Germany), and the OneScript® Plus cDNA Synthesis Kit (ABM, USA) was used for cDNA synthesis according to the manufacturer's protocol. RT-qPCR was performed using ABM KiloGreen 2X qPCR MasterMix (USA) in an Applied Biosystems™ StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific Inc., USA). The relative gene expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). For the determination of mRNA levels, the $2^{-\Delta\Delta Ct}$ method was used as described previously [26]. Primer sequences for all genes were acquired from the NCBI database (Supplementary Table S1) and were synthesized by Sentebiolab (Sentegen, Turkey). The data were analyzed using tools on the GeneGlobe Data Analysis Center (Qiagen).

Statistical analysis

GraphPad Prism 9.0 software was used for statistical analyses. The results are presented as the means±SD of at least three replicates. The statistical differences between two the groups were analyzed by Student's *t*-test. P-values <0.05 were statistically significant.

RESULTS

Molecular weight analysis

The HPLC chromatogram of standards and extracted polysaccharide is presented in Fig. 1. Standard dextrans ranging from 2.5 to 670 kDa were used to calculate molecular weights by plotting the calibration curve prepared from the dextrans under the same conditions. The HPLC (GPC) chromatogram of the polysaccharide extract (HWEP) of *L. chrysorrheus* has three peaks. The molecular weights of the peaks were

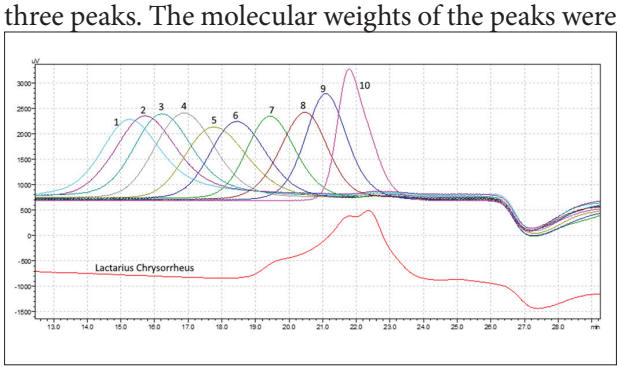

Fig.1. HPLC chromatograms of dextran standards and the *Lactarius chrysorrheus* extract.

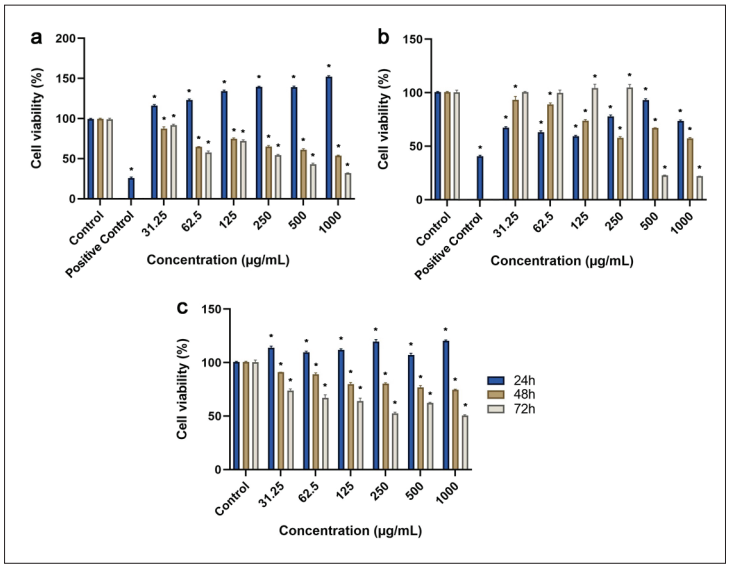

Fig.2. Cytotoxicity of the polysaccharide extract from *L. chrysorrheus* on HepG2 (**a**), PANC-1 (**b**) HEK293 cells (**c**) for 24, 48 and 72 h as determined by the MTT assay. Positive control – paclitaxel (10 µM). Each value is the mean±SE of three experiments. *P<0.05 treatment vs. control group.

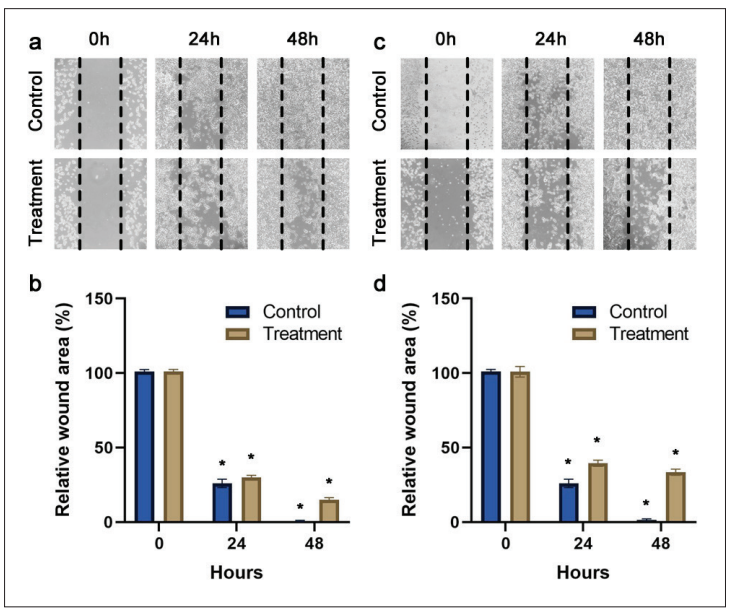

Fig.3. *In vitro* wound-healing assay at 0, 24 and 48 h. Images of wound-healing treatment with the extract and the percentage of wound closure areas: control (**a**), *L. chrysorrheus* extract (**b**) HepG2 (**c**), PANC-1 cells (**d**). Results are expressed as % vs. control group that was set to 100%. The error bars represent the mean±SE (n=3), P<0.05 treatment vs. control.

calculated as 1869.9, 3043.92 and 16821.47 Da, respectively (Supplementary Table S2).

Cytotoxic activity of the polysaccharide extract of *L. chrysorrheus*

Results of the MTT assay of the polysaccharide extract of *L. chrysorrheus* are presented in Fig. 2. The extract after 72 h of treatment had an inhibitory potential on the viability of two tested human cancer cell lines, HepG2 and PANC-1, and one human embryonic kidney (HEK293) cell line. The results of the cytotoxic activity assay suggested that the polysaccharide extract of *L. chrysorrheus* induced inhibition of cell viability in all cancer cell lines in accordance with the determined IC_{50} values (296.42 µg/mL for HepG2 and 444.43 µg/mL for PANC-1; Fig. 2). The extract exhibited selective toxicity in cancer and normal cells; the selectivity was evidenced by the fact that the viability at the highest dose (1000 µg/mL) did not decrease below 50% after 72 h in HEK293 cells in comparison to the IC_{50} values obtained in cancer cell lines.

Wound healing (scratch assay)

HepG2 and PANC-1 cells were used in the wound-healing assay. Fig. 3 shows the relative wound area (in percentages) in different groups at 0, 24 and 48 h. At 48 h, the experiment was terminated when the wound gap was closed in the control group. The treatment with the extract inhibited closure of the scratch area in a time-dependent manner. These results show that the polysaccharide extract of *L. chrysorrheus* inhibited cell migration.

Inhibition of colony-forming ability

The colony formation assay was performed by seeding 10^3 cells onto a 6-well plate and treating with the polysaccharide extract for 72 h. Colonies were stained with 0.4% crystal violet and counted by ImageJ software.

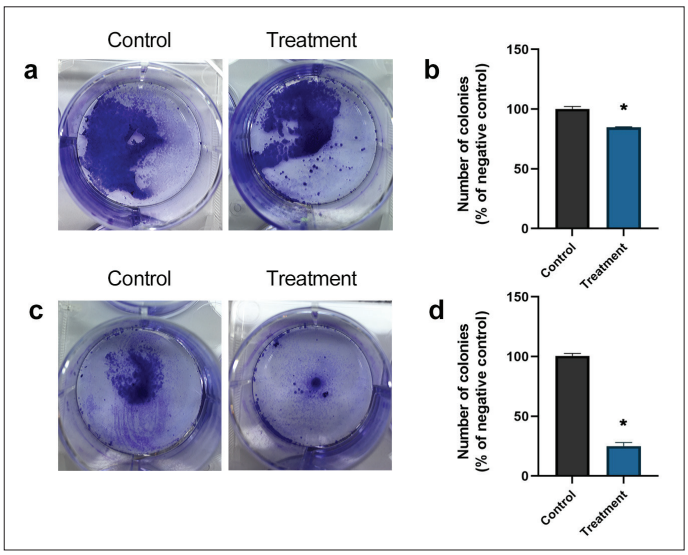

Fig.4. The effects of *L. chrysorrheus* extract on colony formation ability. Representative images showing colony formation in HepG2 (**a**), PANC-1 cells (**c**). Bar graphs showing the number of colonies (%) in HepG2 (**b**), PANC-1 cells (**d**). *Significantly different from the control. Each value is the mean±SE of three experiments, (P<0.05).

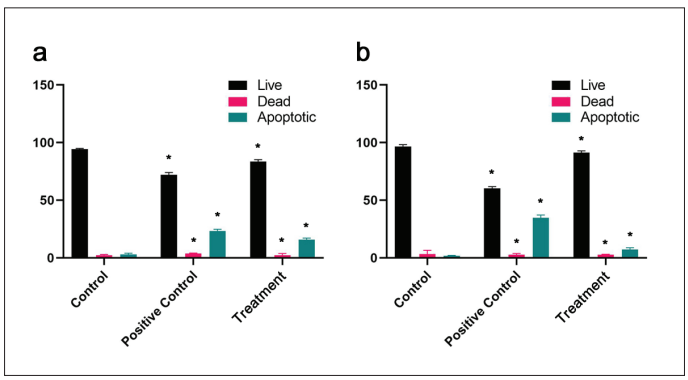

Fig.5. Percentage of live, dead, apoptotic HepG2 (**a**) and PANC-1 cells (**b**) after 72 h of treatment with the *L. chrysorrheus* extract. Data are representative of three independent experiments. H_2O_2 (200 µM) was used as a positive control. *Significantly different from the control (*P<0.05).

The polysaccharide extract was not significantly different from the control group in HepG2 cells, slightly reducing colony formation (11%). On the other hand, a marked reduction in colony formation was observed in PANC-1 cells by 73% (P<0.05) (Fig. 4).

Detection of apoptotic cells

The annexin V-FITC/PI Apoptosis Kit (K101, BioVision) was used to detect apoptosis. As shown in Fig. 5, the percentages of apoptotic cells were 14% and 7% in HepG2 and PANC-1 cells, respectively, compared with 23-37% in the positive control group (P < 0.05). This result suggests that the polysaccharides of *L. chrysorrheus* could promote apoptosis to a greater degree in the HepG2 cell line as compared to PANC-1 cells.

Table 1. The mRNA level changes of expression in apoptosis-related genes.

	HepG2		PANC-1	
Gene	**Fold change**	**P-value**	**Fold change**	**P-value**
BID	-1.66	0.000057	-3.12	0.000415
BAX	4.17	0.000372	-1.06	0.703316
P53	3.41	0.003321	1.31	0.155425
TNF alpha	-1.05	0.227695	1.21	0.247269
APAF-1	1.75	0.001003	1.43	0.082043
CYCLIN D1	1.09	0.147350	-4.03	0.001361
Bcl-2	-2.58	0.003371	-3.42	0.000928
Bcl-xL	-1.35	0.027364	-2.80	0.010841
CASP3	1.02	0.938848	1.15	0.051034
CASP8	66.26	0.358729	-1.28	0.063604
CASP9	2.74	0.003405	3.17	0.001676

Gene expression analysis

To determine the alteration of apoptosis pathways by the polysaccharide extract of *L. chrysorrheus*, a PCR array was used to evaluate the expression levels of the BH3 interacting-domain death agonist, or *BID*, gene, apoptosis regulator *BAX* (bcl-2-like protein 4) gene, the protein 53 *(P53)* gene, tumor necrosis factor alpha (*TNF-α*), apoptotic protease activating factor 1 (*APAF1*), *CYCLIN D1, Bcl-2, Bcl-xL, CASPASE 3, 8* and *9* genes. For this purpose, the effects of *L. chrysorrheus* on the mRNA expression of these genes were determined in HepG2 and PANC-1 cell lines by qRT-PCR (Table 1). After 72 h of treatment, *BAX, P53, CASPASE 3, 8* and *9* mRNA levels were increased 4.17-, 3.41-, 1.02-, 66.26- and 2.74-fold in HepG2 cells (P<0.05), respectively. In contrast, extract treatment caused 1.66-, 2.58- and 1.35-fold decreases in *BID, Bcl-2,* and *Bcl-xL* mRNA levels in the HepG2 cell line when compared to the control group (P<0.05), respectively. *P53, TNF-α, APAF1, CASPASE 3* and *9* mRNA levels were increased 1.31-, 1.21-, 1.43-, 1.15- and 3.17-fold in PANC-1 cells, respectively. Extract treatment caused 4.03-, 3.12-,

3.42- and 2.80-fold decreases in *Cyclin D1, BID, Bcl-2* and *Bcl-xL* mRNA levels in the PANC-1 cell line ($P<0.05$), respectively. Our results showed that the polysaccharide extract displayed potent apoptotic action as revealed by the reduction in the expression of *Bcl-2* and *Bcl-xL* in all cell lines ($P<0.05$).

DISCUSSION

Chemotherapy treatments produce serious side effects that limit the usefulness of different agents in a variety of cancers, and researchers are continuously studying novel potential drugs that specifically target and kill cancer cells without affecting normal cells for cancer therapy. Natural therapies even at high doses may be less harmful than synthetic agents. Alternative and complementary therapies, such as traditional herbal medicine, are becoming increasingly important in the development of new cancer treatments [27,28]. While mushrooms have been used in traditional medicine for a long time, research in this area is still growing and achievements in studies of the biological activity of potential therapeutics are increasing [29-31]. Mushrooms are nutritious foods used for their culinary value and health benefits [32,33]. Extracts from mushrooms have been found to have an anticancer effect, and many researchers have investigated their activity against different types of cancers and in a variety of experimental models [34-37]. More than 100 medicinal properties have been linked to mushrooms and many molecules that are synthesized by mushrooms are known to be biologically active. These bioactive compounds include proteins, fats, polysaccharides, flavonoids, terpenoids, minerals and organic acids [38]. The available literature suggests that mushroom extracts and polysaccharides may have anticancer properties, however, little is known of their mechanisms of action [39,40].

Polysaccharides are complex molecules that are commonly found in foods. The molecular weights and composition of monosaccharides in polysaccharides could be used in quality control of polysaccharides in edible and medicinal mushrooms. Many studies have reported the average molecular weights of polysaccharides from different mushrooms [41]. In previous publications, the water-soluble polysaccharides of *Suillellus luridus* and *Boletus violaceofuscus* were reported as around 1000 Da, and the average molecular weight of *Phellinus igniarius* is 1500 Da [42]; moreover, the molecular weights of polysaccharides of *Agaricus bisporus*, and *Ganoderma lucidum* are 1900 Da [43] and 8000 Da [44], respectively. The results given in this study are in agreement with the molecular weights reported in the literature.

Cytotoxic, antioxidant, antibacterial, antiviral and antifungal studies have been previously reported in the literature on extracts of fungi belonging to the genus *Lactarius* [45-48]. The antigenotoxic effects of the *Lactarius vellereus* methanol extract and its cytotoxicity on the HepG2 cell line have been described [49], including the antifungal and cytotoxic effects of *L. deliciosus* extract [50]. In a study of basidiocarp extracts isolated from *L. indigo*, antibacterial and cytotoxic effects on human lung (A549) and breast (MCF-7) cancer cell lines were reported [51]. The current study showed that the polysaccharides of *L. chrysorrheus* have a cytotoxic effect on HepG2 and PANC-1 cell lines. The extract from *L. chrysorrheus* was cytotoxic after 72 h at concentrations above 250 μg/mL, decreasing the viability of HepG2 cells to about 32% at the highest concentration (1000 μg/mL). Similarly, the extract was cytotoxic for PANC-1 cells, decreasing cell viability by 22%. These results confirm the effects observed in HepG2 cells, where *L. chrysorrheus* was more cytotoxic under the same conditions (1000 μg/mL and 72 h of incubation). While the polysaccharide extract was capable of significantly decreasing HepG2 and PANC-1 cell viability, HEK293 viability at the highest concentration (1000 μg/mL) did not decrease below 50%.

Cell migration and proliferation ability play an important role in cancer progression and metastasis [52]. In this study, the colony formation assay was used to test cell growth, and the wound-healing assay was used to evaluate cell migration. Similar effects were observed in both cell lines, the polysaccharide extract effectively decreased the wound-healing ability. In the colony formation assay, we found that *L. chrysorrheus* significantly inhibited the colony formation ability for PANC-1 cells compared to HepG2 cells.

Deregulated proliferation and apoptosis are major players in cancer development, and they present important targets for therapeutic interventions [53]. Annexin/PI double staining confirmed apoptosis initiation by *L. chrysorrheus*. Fragmentation of DNA

following apoptosis is detectable by positive staining with PI because PI can bind to DNA molecules; also, annexin-V binds to phosphatidylserine (PS) on the surface of apoptotic cells, thus, the increase in PS on the cell surface is an indicator of apoptosis in cells. The rise in fluorescence in cells that is seen when they are double-stained (with annexin-V and PI) reflects the presence of apoptosis [54]. Our results showed that the *L. chrysorrheus* polysaccharide extract-promoted loss of viability of cells could be correlated to apoptosis. After 72 h of treatment with *L. chrysorrheus*, 14% of the HepG2 cell population was apoptotic, 85% was viable, and 1% of cells were dead. For PANC-1 cells, the matching values were 7% apoptotic, 91% live and 2% dead cells. The percentage of apoptotic cells after extract treatment was nearly 4-fold higher than that of the control for HepG2 and 3-fold higher for PANC-1 cells.

In order to determine the effects of polysaccharide extract on apoptosis, changes in *BID, BAX, P53, TNF alpha, APAF1, CYCLIN D1, Bcl-2, Bcl-xL, CASPASE 3, 8*, and *9* gene expression were assessed by qRT-PCR. The results showed that the polysaccharide extract stimulated apoptosis by affecting the expression levels of *BAX, Bcl-2, CASPASE 3, 9, APAF1*, and *P53*. It is well established that apoptosis is controlled by different genes that are involved in two different apoptotic pathways, extrinsic and intrinsic. The extrinsic pathway is mainly mediated by the mitochondria and the Bcl-2 family of genes [55]. The balance between antiapoptotic proteins such as *Bcl-2* and *Bcl-xL*) and proapoptotic proteins such as *BAX* and *BIM* are important for the activation of caspases in apoptosis [56,57]. The observed levels of *Bcl-2, BAX* and caspase genes in this study showed that the extract caused apoptosis and had a cytotoxic effect.

A previous study on *L. chrysorrheus* from India reported that this species showed cytotoxicity (>100 μg/mL) on L1210 and 3LL cell lines [58]; however, no scientific data exist concerning the biological properties of polysaccharides extracted from *L. chrysorrheus*. The results of the present study showed that the polysaccharide extract triggered inhibition of cell viability, inhibited the ability to form colonies and wound-healing capability of HepG2 and PANC-1 cells, and induced apoptosis.

Our study is the first examination of the anticancer properties of the polysaccharide extract of *L. chrysorrheus*. Our results demonstrate that the extract from *L. chrysorrheus* exhibited potent cytotoxic effects against hepatic and pancreatic cancer cell lines. Further research is needed to understand the significance of *L. chrysorrheus* polysaccharide extract for use in cancer treatment regimens. Future studies may help to classify different mushrooms as nutraceuticals or to create dietary supplements that may be used in cancer therapy.

Funding: This research was financially supported by the Pamukkale University Scientific Research Projects Coordination Department (Project Number 2021FEBE023).

Acknowledgments: The authors are thankful to the Dr. Hakan Alli (Department of Botany, Mugla Sitki Kocman University) for access to the herbarium specimen and confirmation of mushroom identification.

Author contributions: All authors contributed to the study concept and design. Material preparation, data collection and analysis were performed by Dogukan Mutlu, Cansel Cakir, Mehmet Ozturk and Sevki Arslan. The first draft of the manuscript was written by Dogukan Mutlu, and all authors commented on the previous versions of the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of interest disclosure: The authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Data availability: All data underlying the reported findings have been provided as part of the submitted article and are available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Mutlu%20et%20al_7967_Data%20Report.pdf

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Supplementary material

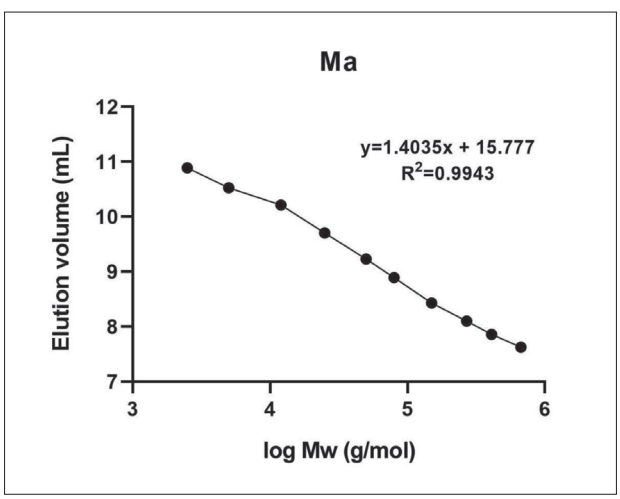
Supplementary Table S1. Sequences of primers.

Gene	GenBank Accession code	Primer sequence
GAPDH	NM_002046	**F:** GTCTCCTCTGACTTCAACAGCG **R:** ACCACCCTGTTGCTGTAGCCAA
BAX	NM_004324	**F:** TCAGGATGCGTCCACCAAGAAG **R:** TGTGTCCACGGCGGCAATCATC
BCL-2	NM_000633	**F:** ATCGCCCTGTGGATGACTGAGT **R:** GCCAGGAGAAATCAAACAGAGGC
BCL-XL	NM_138578	**F:** GCCACTTACCTGAATGACCACC **R:** AACCAGCGGTTGAAGCGTTCCT
CASP3	NM_004346	**F:** GGAAGCGAATCAATGGACTCTGG **R:** GCATCGACATCTGTACCAGACC
CASP8	NM_001080125	**F:** AGAAGAGGGTCATCCTGGGAGA **R:** TCAGGACTTCCTTCAAGGCTGC
CASP9	NM_001229	**F:** GTTTGAGGACCTTCGACCAGCT **R:** CAACGTACCAGGAGCCACTCTT
P53	NM_000546	**F:** CCTCAGCATCTTATCCGAGTGG **R:** TGGATGGTGGTACAGTCAGAGC
BID	NM_197966	**F:** TGGGACACTGTGAACCAGGAGT **R:** GAGGAAGCCAAACACCAGTAGG
TNF alpha	NM_000594	**F:** CTCTTCTGCCTGCTGCACTTTG **R:** ATGGGCTACAGGCTTGTCACTC
APAF1	NM_181861	**F:** GCCAAGCAGGAGGTCGATAATG **R:** GACCATCCTCAGAAAAGCAGGC
CYCLIN D1	NM_053056	**F:** TCTACACCGACAACTCCATCCG **R:** TCTGGCATTTTGGAGAGGAAGTG

F – forward, **R** – reverse.

Supplementary Table S2. Molecular weight of the polysaccharide.

Mushroom name	Molecular weight (Da)		
Lactarius chrysorrheus	1869.91 Da	3043.92 Da	16821.47 Da



Supplementary Fig. 1S. Calibration curve for the determination of the molecular weight of the polysaccharide from *L. chrysorrheus*.