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INFLUENCE OF BIOACTIVE COMPOUNDS EXTRACTED FROM MUSHROOM *GANODERMA LUCIDUM* ON B AND T CELLS

ABSTRACT: *Ganoderma lucidum* (Leyss.: Fr.) Karst is one of the most often used mushrooms in traditional medicine of Far Eastern people. Because of its bitter taste and wooden build it is not suitable for nutrition, but the bioactive substances extracted from this mushroom possess very important medicinal characteristics. The aim of this experiment was to investigate the effects of different concentrations of isolated *Ganoderma lucidum* GL-I extract on the growth of JY (B) and Jurkat (T) cells. Obtained extracts were added to the cells in concentrations 1 mg/ml, 100 ng/ml, 10 ng/ml, 1 ng/ml and 100 pg/ml. JY and Jurkat cells were exposed to the action of bioactive compounds, b-glucans, during the incubation period of 72 h, at 37°C, in the atmosphere with 5% CO₂ and their number was counted. Among all tested concentrations of extract the most important influence showed concentration of 1 mg/ml, which reduced the number of B cells by 61.46%, while in the case of T cells their number was reduced by 57.14%.

KEY WORDS: b-glucans, bioactive polysaccharides, *Ganoderma lucidum*, Jurkat cells, JY cells

INTRODUCTION

Many mushrooms have long been valued as tasty, nutritious food by different societies worldwide. To the ancient Romans they were “the food of the Gods”; the Egyptians considered them as “the gift from the God Osiris”; the Chinese viewed them as “the elixir of life”. Mushrooms are popular and valuable functional food, low in calories and high in minerals, essential amino acids, vitamins and fibers. In the Orient, several thousand years ago, there was the recognition that many edible and certain non-edible mushrooms could have valuable health benefits (Bensky and Gamble, 1993, Hobbs, 1995).

Some of edible mushrooms demonstrate medicinal or functional properties, while others are known only for their medicinal properties, e.g. *Ganoderma lucidum*, commonly known as lacquered mushroom. It is a non-edible mushroom due to its coarse and hard texture and bitter taste. The historical evolution of usage of these essentially scarce, forest-obtained medicinal mushrooms certainly did not include whole mushrooms, but in the form of hot water extracts, concentrates, liquors or powders and used in health tonics, tinctures, teas, soups and herbal formulae (Smith et al., 2002). *Ganoderma lucidum* has been widely used in China (named Ling Zhi) and Japan (named Reishi, Manentake) for thousands of years for the treatment of various diseases, including cancers. The fact that this mushroom earned itself names like “Sky Plant” and “Mushroom of the Universe”, confirm its possibility of revitalization and curing of different illnesses. It acts antitumour, antiinflammatory, antiviral (e.g. anti-HIV), antibacterial, antiparasitic, immunomodulating and hepatoprotective, it has a role in blood pressure regulation, against cardiovascular disorders and chronic bronchitis, like kidney tonic and nerve tonic (Wasser and Weiss, 1999). By current techniques a numerous bioactive compounds were isolated from different parts of the mushroom, among which polysaccharides, b-D-glucans, peptidoglycans and bitter triterpens were the most important. Pharmacologically, a number of the water-soluble polysaccharides have demonstrated antitumour and immunostimulating activities.

The effects of bioactive compounds isolated from mushrooms could be observed on different cell lines, such as T cell and B cell lines, which are specifically transformed. T cells belong to a group of white blood cells known as lymphocytes and play a central role in cell-mediated immunity. They can be distinguished from other lymphocyte types, such as B cells and natural killer cells, by the presence of a special receptor on their cell surface called T cell receptors (TCR). B cells are lymphocytes that play a large role in the humoral immune response (as opposed to the cell-mediated immune response, which is governed by T cells). The principal functions of B cells are to make antibodies against antigens, perform the role of Antigen Presenting Cells (APCs) and eventually develop into memory B cells after activation by antigen interaction (Abbas et al., 2000).

MATERIALS AND METHODS

Dried carpophores of *Ganoderma lucidum* GL-I mushroom were used for this investigation. The samples were exposed to the hot water extraction of polysaccharides and alcohol precipitation, refined by dialyses and the obtained extracts were used for testing their influence on human B and T cells (Klaus, 2004).

Hot extraction of bioactive compounds

Powdered tissue was washed with 96% ethanol, filtered and dried in vacuum (at 40°C for 60 min.) until it turned into powder up to getting powder.

Dried filtercake was mixed with deionized water and glucans were extracted by autoclaving at 120°C for 20 min. Material was cooled down and centrifuged (10000rpm, at 4–9°C for 10min.). Supernatant was mixed with 2 vol. 96% ethanol and left at 4°C until precipitate was formed. After centrifuge, the collected pellets were dried in vacuum and the obtained powder was refined by dialysis.

Refining of bioactive compounds by dialysis

Bioactive compounds, polysaccharides, and b-glucans obtained by hot water extraction and alcohol precipitation from dry mushroom carpophore were purified by dialysis against MQ water. Suspensions were dialysed for 24 h at room temperature. Dialysis is necessary for refining because low-molecular weight molecules will pass through membrane in solution, while high-molecular weight molecules, b-glucan will stay inside the membrane. After the dialysing content was centrifuged, 2 vol. 96% ethanol was added to supernatant and left at 4°C for a couple of hours. To remove supernatant, the centrifugation was applied again and the pellets were dried in vacuum. Dried pellets were dissolved in phosphate saline buffer (PBS) and used for further examination on JY and Jurkat cells.

Jurkat and JY cell lines

Jurkat cell line is an immortalized line of human lymphocyte cells that are used to study acute T cell leukemia, T cell signaling and expression of various chemokine receptors susceptible to viral entry, particularly HIV. Jurkat cells are also useful in science because of their ability to produce interleukin 2. Their primary use, however, is to determine the mechanism of differential susceptibility of cancers to drugs and radiation (Abbas et al., 2000).

The JY cell line is a human B cell line transformed by Epstein-Barr virus (EBV); it does not produce EB virus but does produce Ig. They are a suspension cell line, though they are known to grow in clumps (Abbas et al., 2000).

Jurkat and JY cells were incubated in plastic 28 cm³ flasks filled by growing media RPMI 1640 with addition of 10% fetal calf serum (fcs), antibiotics (1% penicillin and 1% streptomycin) and 7.5% NaHCO₃ (buffer), at 37°C, in the 5% CO₂ atmosphere. After a seven day incubation, 10 ml of suspension were centrifuged for 10 min on 8000 rpm. 10 ml of fresh RPMI 1640 medium with 10% fcs, antibiotics and NaHCO₃ which were warmed up to 37°C were added to the obtained pellets. This suspension was used for making solution with 10⁵ CFU/ml.

Loading the wells

For all experiment, plates with 24 wells were used. First row was control and did not contain any extract of mushroom. In the 2nd row 1 µg extract per

well was added; in the 3rd row 100 ng extract per well were added; in the 4th row 10 ng extract per well were added; in the 5th row 1 ng extract per well was added and in the 6th row 100 pg extract per well were added. The rows 3, 4, 5, and 6, were serially diluted by pipetting 100 µl from row 2, into row 3, from 3, into 4, etc. The starting suspension added to the 2nd row was obtained by adding 100 µl of mushroom extract into the 900 µl sterile PBS. JY and Jurkat cells were exposed to the action of bioactive compounds, b-glucans, during the incubation period of 72 h, at 37°C, in the atmosphere with 5% CO₂ and their number was counted with Fuchs-Rosenthal chamber.

RESULTS AND DISCUSSION

From 9g powdered dried *Ganoderma lucidum* Gl-I carpophore 0.0055 g polysaccharides, b-glucans were obtained through the treatment of hot water extraction, alcohol precipitation and dialysis refining.

After the incubation period, in the presence of certain amount of extract, number of cells were counted with Fuchs-Rosenthal chamber, to establish the changes in the number of cells.

Tab. 1 — Influence of different concentration of *Ganoderma lucidum* Gl-I extract on JY cells number and standard deviation

treatment	extract concentration	number of JY cells (4 repeats)				average number	standard deviation
		1	2	3	4		
C	—	140	141	138	144	140.75	2.5
1	1 µg	47	57	59	54	54.25	5.25
2	100 ng	67	65	60	69	65.25	3.86
3	10 ng	76	78	75	76	76.25	1.25
4	1 ng	86	82	92	87	86.75	4.11
5	100 pg	88	86	93	92	89.75	3.30

It was established that average number of JY cells was changed in the presence of bioactive compounds extracted from karpophore *Ganoderma lucidum* Gl-I (Figure 1). In the presence of 1 mg/ml mushroom extract, the average number was reduced to 54.25 from 140.75, in comparison with the control, which didn't contain mushroom extract. The number of cells was reduced by 61.46% under the influence of 1 mg/ml mushroom extract. With the addition of 100 ng/ml mushroom extract, the average number of cells was reduced to 65.25, which represents a reduction of 53.64%. In the presence of 10 ng/ml of mushroom extract, the average number of cells was 76.25 and it was reduced by 45.82%. Addition of 1 ng/ml mushroom extract induced a reduction of cells number by 38.36%, and average number of cells was 86.75. The smallest change in the cells number appeared when 100 ng of mushroom extract were added; in that case average number of cells was reduced by 36.23% and it was 89.75 (Table 1).

On the basis of standard deviation and t-test, p-values were found and it was established that statistically significant difference existed between all treat-

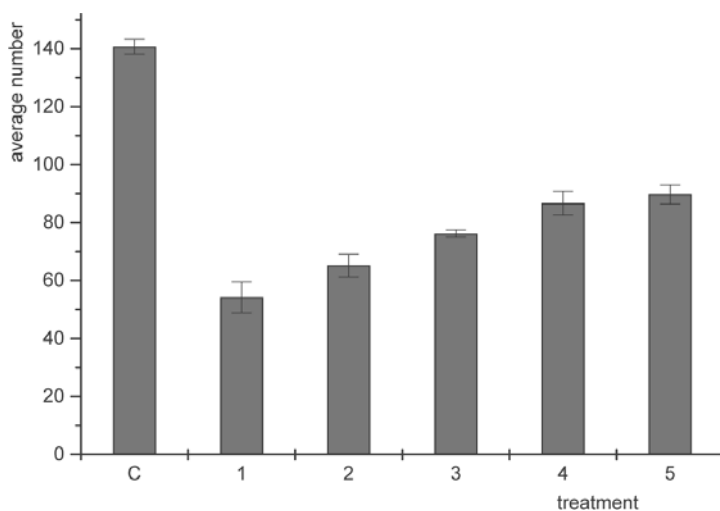


Fig. 1 — Changes of JY cells number in the presence of different concentration of *Ganoderma lucidum* Gl-I extract

ments, except the treatments 1—2. It means that differences in concentrations of added extract were important in all cases, except in the treatments 1—2. Compared to the control the biggest difference in the cells number was obtained in the treatment 1, when 1 mg/ml of mushroom extract was added to the suspension (Figure 1).

Tab. 2 — Influence of different concentration of *Ganoderma lucidum* Gl-I extract on Jurkat cells number and standard deviation

treatment	extract concentration	number of JY cells (4 repeats)				average number	standard deviation
		1	2	3	4		
C	—	110	107	105	112	108.5	3.109
1	1 µg	44	47	49	46	46.5	2.08
2	100 ng	82	93	89	87	87.75	4.57
3	10 ng	88	87	88	90	88.25	1.25
4	1 ng	85	93	82	87	86.75	4.64
5	100 pg	94	98	85	93	92.5	5.44

In the presence of bioactive compounds extracted from karpophore *Ganoderma lucidum* Gl-I, average number of Jurkat cells was changed (Figure 2). The most important change appeared with addition of 1 mg/ml of mushroom extract when average number of cells was reduced to 46.5 from 108.5, in comparison with the control which didn't contain mushroom extract. The number of cells was reduced by 57.14% under the influence of 1 mg/ml mushroom extract. With addition of 100 ng/ml of mushroom extract average cells number was reduced by 19.12% and it was 87.75. Similar result was obtained with the addition of 10 ng/ml of mushroom extract when average cells number was reduced by 18.66% and it was 88.25. In the presence of 1 ng/ml of mushroom extract average number of cells was 86.75 and it was reduced by 20.05%. Ad-

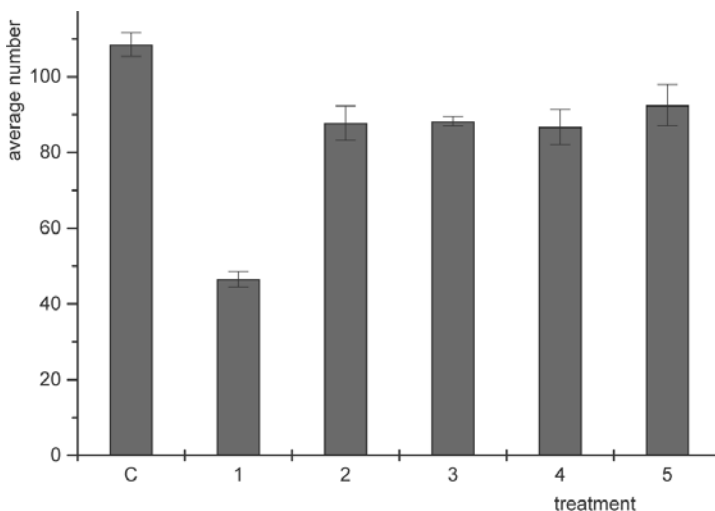


Fig. 2 — Changes of Jurkat cells number in the presence of different concentration of *Ganoderma lucidum* Gl-I extract

dition of 100 $\mu\text{g}/\text{ml}$ of mushroom extract induced a reduction of cells number by 14.75% and average number of cells was 92.5 (Table 2).

On the basis of standard deviation and t-test, p-values were found and it was established that statistically significant difference existed between all treatments except treatments 2—3, 2—4, 2—5, 3—4 and 3—5. It means that differences in concentrations of added extract were important in all cases, except in treatments 2—3, 2—4, 2—5, 3—4 and 3—5. When compared to the control the biggest difference in the cells number obtained in the treatment 1 was when 1 mg/ml of mushroom extract was added to the suspension (Figure 2).

CONCLUSION

Ganoderma lucidum Gl-I is one strain of this species which could be found in the woods, but it is also suitable for artificial growing under controlled conditions. This kind of mushroom belongs to the very important medicinal mushroom thanks to its bioactive substances content, which has been confirmed for over thousands of years now; it is not edible because of its bitter taste and wooden build. This investigation showed that bioactive substances, polysaccharides, β -glucans, obtained through the processes of hot water extraction, alcohol precipitation and dialyses refining had influence on the reduction of B cells (a human B cell line transformed by Epstein-Barr virus) and T cells (an immortalized line of human T lymphocyte cells). Among all tested concentrations of mushroom extract, the most intensive influence showed concentration of 1 mg/ml , which reduced the number of B cells by 61.46%, while in the case of T cells their number were reduced by 57.14%.

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УТИЦАЈ БИОАКТИВНИХ КОМПОНЕНАТА ЕКСТРАКОВАНИХ ИЗ ГЉИВЕ *GANODERMA LUCIDUM* НА Б И Т ЋЕЛИЈЕ

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Резиме

Ganoderma lucidum (Leyss.: Fr.) Karst је једна од најчешће коришћених гљива у традиционалној медицини народа Далеког истока. Горког је укуса и дрвенасте грађе, па није погодна за исхрану, али биоактивне компоненте екстраковане из ове гљиве показују врло важне медицинске карактеристике. Циљ овог рада био је испитивање дејства различитих концентрација издвојеног екстракта гљиве *Ganoderma lucidum* GL-I на раст ЈУ (Б) и Jurkat (Т) ћелија. Издвојени екстракт је додат ћелијама у концентрацијама 1 mg/ml, 100 ng/ml, 10 ng/ml, 1 ng/ml и 100 pg/ml. ЈУ и Jurkat ћелије су инкубиране 72 h на 37°C у атмосфери са 5% CO₂ у присуству биоактивних компонената, полисахарида, б-глюкана, а затим је утврђен њихов број. Од свих примењених концентрација екстракта највећи утицај је показала концентрација 1 mg/ml, која је у случају дејства на Б ћелије довела до смањења њиховог броја за 61.46%, а у случају дејства на Т ћелије број је смањен за 57.14%.