UNIVERSIDAD DE GUADALAJARA

CENTRO UNIVERSITARIO DE CIENCIAS BIOLÓGICAS Y AGROPECUARIAS

DIVISIÓN DE CIENCIAS BIOLÓGICAS Y AMBIENTALES

MODULACIÓN DE LA RESPUESTA INMUNE CELULAR Y HUMORAL DE RATONES BALB/c CON LINFOMA MURINO L5178Y TRATADOS CON ACÍBAR DE ALOE VERA

ARTÍCULO CIENTÍFICO PUBLICADO

QUE PARA OBTENER EL TÍTULO DE

LICENCIADO EN BIOLOGÍA

P R E S E N T A

REFUGIO AZUCENA CHÁVEZ ANAYA

DIRECTOR: ARTURO OROZCO BAROCIO

LAS AGUJAS, ZAPOPAN, JALISCO. OCTUBRE DE 1999

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Manifestamos a Usted que con esta fecha ha sido aprobado su tema de titulación en la modalidad de ARTICULO PUBLICADO con el título **"MODULACION DE LA RESPUESTA INMUNE IN VIVO DE RATONES BALB/c CON LINFOMA MURINO L5178Y TRATADOS CON ACIBAR DE ALOE VERA (L).",** para obtener la Licenciatura en Biología.

Al mismo tiempo le informamos que ha sido aceptado como Director de dicho trabajo al **DR. ARTURO OROZCO BAROCIO**

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Por lo cual le extendemos una invitación a evaluar la presentación y defensa del Artículo, puesto que este fue aceptado y revisado por un Comité Editorial Científico.

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A Dios.
Por darme la sabiduría y prudencia
necesaria para realizar todo y por
revelaren mi persona, el saber que
todo lo que me proponga lo puedo
realizar en su nombre.

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Que con su amor, comprensión y apoyo
lograrón que yo alcanzara mi meta.
Por todo su cariño y dedicación que me
brindarón en todo momento.
Mi agradecimiento y admiración.

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que me apoyo en mi carrera, que ha sido
un respaldo para mí, y que me ha ayudado
a seguir adelante.

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Con todo mi Amor y Cariño.

A Mi Director de Tesis.
Por el esfuerzo y dedicación
en la elaboración del presente
trabajo.

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16.th November.1998

Dear Dr. Arturo Orozco-Barocio

On behalf of the Russian Journal of Immuniligy I have a pleasure to inform you, that your (with collaborants) article "Modulation of immune response in vivo of BALB/c mice with mirine lymphoma L5178Y treated with a bitter yellow juice (exudate) of Aloe vera" is accepted for publication.

Yours truly,

Anatoly N. Cheredeev,
Editor - in - Chief

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Модификация иммунного ответа in vivo у мышей BALB/c с лимфомой L5178Y под действием экстракта из Aloe vera (L)

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Aloe vera (L) - растение африканского происхождения, адаптировано в Мексике с XVI столетия. Это растение, наряду с другими видами *Aloe*, часто применяется в народной медицине при лечении артритов, ожогов, диабета, рубцевании ран и многочисленных нарушений иммунной системы.

Использованный в работе порошок горького желтого сока *Aloe vera* (L) (ГЖС) был получен из растений, коллекционируемых в Институте ботаники Гвадалахарского университета (Мексика). Этот препарат имеет высокое содержание антрахинов и их гликозидов.

В настоящей работе исследовано влияние ГЖС на иммунный ответ к эритроцитам барана (ЭБ), реакцию ГЗТ и фагоцитоз у мышей линии BALB/c. Использовали группы здоровых животных и мышей с иммунной системой, подавленной ростом лимфомы L5178Y.

Предварительный этап работы с ГЖС заключался в определении летальной дозы 50 (LD_{50}). Исследуя группы мышей, которые получили перорально разные количества ГЖС, разведенного в 0,1 мл стерильной воды, установили, что LD_{50} этого препарата является 3,2 г/кг веса.

В качестве иммуностимулирующего агента ГЖС использовали в низких концентрациях: его растворяли в 0,1 мл стерильной воды и вводили перорально в количестве 1 мг/кг веса в день в течение 10 дней.

Оценивали: 1) иммунный ответ к ЭБ по выработке АТ (гемагглютининов) через 7 дней после внутриперитонеального введения 1 мл 20% суспензии ЭБ, 2) кожную реакцию ГЗТ к динитрофлюоробензолу и 3) способность альвеолярных макрофагов фагоцитировать *Candida albicans*.

Введение ГЖС у здоровых животных способствовало статистически достоверной стимуляции иммунного ответа к ЭБ, реакции ГЗТ и фагоцитоза по сравнению со здоровыми мышами, не получавшими данный препарат. Иммунный ответ к ЭБ возрастал, примерно, в 3 раза, а ГЗТ и фагоцитоз - в 2 раза. Вероятно, этот эффект связан с наличием в ГЖС веществ, подобных ацеманнану, которые, действуя на макрофаги, стимулируют иммунный ответ в афферентную фазу представления и распознавания АГ.

В качестве биологической модели иммуносупрессии была выбрана лимфома L5178Y. На десятый день после инокуляции мышам 1 x 10^7 клеток лимфомы наблюдалось развитие опухоли, которое сопровождалось по сравнению с интактными животными угнетением иммунного ответа к ЭБ в 3 раза, а реакции ГЗТ - в 2 раза. Однако, показатели фагоцитоза оставались сходными у здоровых и больных животных.

В группах иммуносупрессированных мышей, которым на 10 день болезни начинали давать ГЖС, происходило значительное восстановление ряда параметров иммунитета: возрастала реакция ГЗТ до нормального уровня, усилнвался фагоцитоз, причем, его значения были столь же высоки как, и показатели у здоровых мышей, получавшнх ГЖС. Однако, используемый препарат не влиял на интенсивность иммунного ответа к ЭБ.

Предполагается, что разнообразие уже известных терапевтнческих эффектов препаратов из Aloe vera (L) объясняется активацней регуляторных клеток вследствие определенных клеточных событий, стимулнрующих защитные механизы, напрнмер, систему макрофагов. В связи с этим в дальнейшем планируется изучить действие горького желтого сока Aloe vera (L) на физиологию макрофагов и на другие типы АГ-представляющих клеток (В-лимфоциты, клетки Лангерганса).

Ключевые слова: *Aloe vera* (L), иммуномодуляция, мышиная лимфома L5178Y

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Modulation of Immune Response of BALB/Mice Bearing Lymphoma L5178Y Treated with Bitter Yellow Juice of Aloe vera (L) in vivo

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Aloe vera (L), a plant of African origin, has been introduced in Mexico since XVIth century. It has been used in the treatment of many diseases of immune system. In the present study we investigated a specific and non specific immune response of BALB/c mice, healthy and immunosuppressed with murine lymphoma L5178Y, treated with bitter yellow juice (extract) of *Aloe vera* (L). We observed that the immunosuppressed mice, treated with the whole extract of the bitter yellow juice achieved restoration of immunological parameters in cellular immune response and phagocytosis. On the other hand, the humoral immunity was not restored. Also, in the healthy rodents treated with the extract, it caused the stimulation of specific and non specific responses, the results had significant differences with the obtained ones in untreated mice.

INTRODUCTION

Aloe vera (L) has been used for many medical purposes since the ancient Egyptians [1]. Nowadays it is used with the other species of aloe in Mexico and in other countries for treatment of arthritis, skin burns, diabetes, acne, wound scarring, chronic bronchitis and other diseases [1, 2, 3]. The fresh leaves of *Aloe vera* (L) are used to obtain two components: a) bitter yellow juice (extract) or acibar, with high content of anthraquinones and their glycosides, which are used for their catharsis effect, and b) a mucilaginous gel. The aqueous, chloroform and ethanol extracts of *Aloe vera* (L) gel showed anti-inflammatory and immunomodulating effects [4, 5, 6]; also the centrifuged extracts of *Aloe vera* (L) has inhibited the growth of tumour cells [7]. However, these extracts promote the growth of normal cells [3]. Besides, certain carbohydrates such as acemannan, extracted from *Aloe vera* (L) pulp, stimulate macrophages as well as T lymphocytes [8]. We also observed that this mannose polysaccharide stimulates phagocytic and digestive activities *in vitro* of murine peritoneal macrophages against *Candida albicans* [9] as well as the production of a nitric oxide, on the part of chicken macrophages [10].

In ethnobotanical studies of bitter yellow juice of *Aloe vera* (L) **(BYJ)** is shown to be used in the treatment of diseases with disturbed immune system. The present study shows the immunostimulating effect of BYJ in immunosuppressed mice with murine lymphoma L5178Y.

MATERIALS AND METHODS

Preparation of Bitter Yellow Juice of Aloe vera (L)

Fresh *Aloe vera* (L) leaves were collected from the Botanic Garden of the Institute of Botanic of the University of Guadalajara, Mexico. BYJ was

Key words: *Aloe vera* (L), immunomodulation, murine lymphoma L5178Y

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obtained from the fresh leaves, cut in transverse form near a stem and it was deposited in a glass of precipitate. For its conservation and further use, it was dried in a freezer LabConco (model Freezone 4.5). The amount of BYJ collected from 500 g of *Aloe vera* (L) leaves consisted in 15 ml, which gave us 1.18 g of powder. A powder from BYJ was used as a whole extract.

Animals

Groups of 5 male BALB/c mice from 20 to 25 g of weight, from 6 to 8 weeks old, maintained at 20-25°C and 12 hours photoperiods, fed and watered ad libidum were used.

a) Group 0. Healthy mice treated with BYJ to determine a lethal dose 50 (LD_{50}).

b) Group I. Healthy mice treated with a solvent, 0.1 ml/day orally during 10 days.

c) Group II. Healthy mice treated with 1 mg/kg/day of complete extract (BYJ) dissolved in 0.1 ml of sterile water solution, orally, during 10 days.

d) Group III. Immunosuppressed mice infected with 1 x 10^7 cells of murine lymphoma L5178Y and treated with a solvent, 0.1 ml/day orally during 10 days.

e) Group IV. Immunosuppressed mice infected with 1 x 10^7 cells of murine lymphoma L5178Y, and treated with 1 mg/kg/day of a complete sterilized extract of BYJ dissolved in 0.1 ml of sterile water solution, orally during 10 days.

Determination of a LD_{50} and Sublethal Dose

LD_{50} of BYJ was determined in 3 groups of mice in each one, with a single dose of 3, 2 and 1 g/kg of weight orally, diluted in 0.1 ml of a sterile water solution.

The vital clinical functions, such as defecation, alteration of the moving activity, ocular irritability, weeping (epiphora), alteration of respiration, changed movements, raised fur, *etc.*, were under observation, as it is established in the studies of acute toxicity. This study consists in administration of the drug to the animals to estimate symptoms following the administration of the drug and its lethal grade [11].

Taking into consideration the principle of H.K.M. Wagner, that immunostimulating substance must be administered in a very low dose in order to obtain the effects of immune response modulation [12], we used the dose of 1 mg/kg/day of BYJ during 10 days as a therapeutical dose.

Determination of a Titre of Antibodies against Sheep Red Blood Cells in vivo

On the third day from the beginning of the treatment with BYJ and inoculating lymphoma, the mice were immunised intraperitoneally with 1 ml of 20% sheep red blood cells (**SRBC**). Seven days later blood was taken by cardiac puncture and the obtained plasma was inactivated at 56°C for 30 min. Immediately after that serial dilutions of 1:2 in 0.05 ml of 0.89% sodium chloride and 0.05 ml of 1% SRBC were made. After 4 hours of incubation in room temperature, the highest dilution of the assay which showed visible agglutination was expressed as a titre of hemagglutination [13, 14].

Assay of Delayed Hypersensitivity to Dinitrofluorobenzen (DNFB)

To measure the cellular immune response of the mice treated with BYJ and suppressed by lymphoma L5178Y, an assay of delayed hypersensitivity to dinitrofluorobenzen (**DNFB**) was carried out [15, 16, 17].

On the fifth day since the beginning of the treatment with BYJ and evolution of lymphoma, the abdomens of mice were shaved on the 20 x 20 mm area, where 20 ml of 0.5% of DNFB solution, dissolved in a mixture of acetone and olive oil in proportion 4:1 was applied. The second sensitizing dose, the same as the first one, was applied in 24 hours.

Boosting dose of 10 ml of 0.2% DNFB in the same solvent was applied on a surface of a right external ear of each mouse. On the fifth day from the beginning of sensitizing the external ear volume was measured before application of boosting dose and 48 hours after it. It was measured by micrometer (Starrett Co., Athol, Mass. USA).

The biopsy (microscopic sections) of sensitized ears were made immediately to evaluate the intensity of a cellular hypersensitivity reaction. These microscopic sections were made immediately after measuring and were fixed in 10% formalin solution. Later the microscopic histological sections were stained by hematoxylin and eosin.

Non-Specific Immunity. Phagocytosis

Isolation of alveolar macrophages **(AM)**. Mice previously treated with BYJ during 10 days and immunosuppressed by lymphoma L517Y, were sacrificed by cervical dislocation and their thorax was opened. Lungs were washed with 0.5 ml of Hanks balanced salt solution **(HBSS)**, then massaged smoothly. The HBSS that contained the suspension of AM was placed directly on the cover glasses in order to accomplish the assay of phagocytosis.

Assay of Phagocytosis on AM

The method of adherence to glass [14, 18, 19, 20] was employed. The method consists of placing of 0.1 ml of the AM suspension on the fourth part of the cover glasses of 22 x 22 mm, previously cleared and adhered to rubber plugs. Then they were placed in a chamber with humidified atmosphere and incubated at 37°C during 30 min in order to allow their adherence to the glass. This time the cover glasses with HBSS were washed softly at 37°C in order to remove the cells that had not adhered to the glass. Immediately after that, without letting the cover glasses dry up, the adherent cells were covered with 0.5 ml of the suspension of *Candida albicans* that contained $1x10^6$ pathogens per ml of HBSS. Later they were incubated in the chamber at 37°C during 30 min. When the incubation was over, they were washed softly with HBSS in order to remove non-phagocyted *Candida albicans*. In the same way and without letting the cover glasses dry up they were covered with HBSS and incubated, as well as it was done previously, during 30 min, so that the AM could digest the phagocyted pathogens. The cover glasses were dried up on air and stained by Wright.

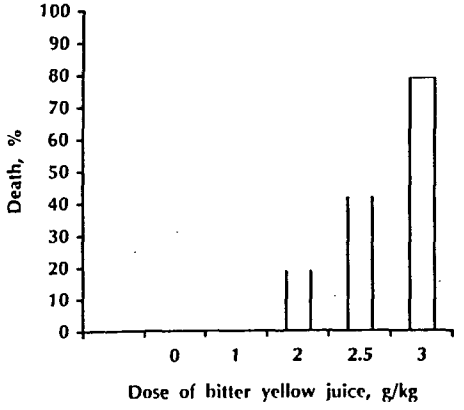
The estimation of phagocytosis was made by observing preparations under microscope with the 100X objective. 300 AM were counted in each cover glass, phagocytized and non-phagocytized cells were included in this number.

The phagocytized pathogens were observed as intracytoplasmic bodies of the intensive blue colour (non digested pathogens) inside of their respective phagosomes, as well as the vacuoles, empty optically (digested pathogens). Cunningham A.J. et al. describe this phenomenon as phantom [18]. In the same way phagosomes that contained remainders of pathogens were observed. Thus and so the non-digested pathogens were differentiated in the first case and the digested ones were differentiated in the last two cases.

Statistical Analysis

The obtained results were analyzed statistically on parameter analysis by Student's "t" test which allows us to compare experimental groups; non-parameter analysis of H of Krusckal-Wallis. Values of $p<0.05$ were considered significant.

Figure 1. Determination of the lethal dose 50 (LD_{50}) of *Aloe vera* (L) bitter yellow juice



RESULTS

The results of determination of a LD_{50} are shown on **Figure 1**. Symptoms, mentioned above, which the mice treated with *Aloe vera* (L) manifested, were presented at different time and with various intensity depending on administered single dose of BYJ.

Eighty percent of mice whom were administered 3 g/kg of weight diluted in 0.1 ml orally, died in 4 days, only one of them restored on the third day after taking BYJ. The mouse kept alive and recovered until sacrifice because of old age (12 weeks). 20% of animals from group II which were administered 2 g/kg, died. 60% of the rest of them recuperated from the symptoms in 72 hours after BYJ application. 20% of them recov-

Mouse	Agglutination titer			
	group 1 (healthy/ water)	group 2 (healthy/ BYJ)	group 3 (lymphoma/ water)	group 4 (lymphoma/ BYJ)
1	16	64	8	8
2	32	128	8	8
3	32	64	8	8
4	32	128	8	8
5	32	64	8	8
Mean	28.8	89.6	8	8

Table 1. *Humoral immune responce of BALB/c mice immunosuppressed with lymphoma L5178Y and treated with BYJ (1 mg/kg/10 days)*

Significant: 4,5, vs 1, 3 groups.
No significant: 4 vs 5 group.

ered from the clinical symptoms on the sixth day since the treatment was applied. Meanwhile, 100% of group III, which received 1 g/kg kept alive, the mice recovered from the clinical symptoms in 48 hours.

In **Table 1** the last dilutions of agglutination in the groups of mice treated with BYJ and immunosuppressed with murine lymphoma are presented, as well as the control groups. As it is shown in the table, in spite of the received treatment there is no difference among the immunosuppressed mice.

On the other hand, there is a significant difference in the non-suppressed group between the mice treated with BYJ and non-treated, $p<0.05$ (**Figure 2**).

In **Table 2** the assay of DNFB sensitising by measuring the inflammation in external ear where the mentioned assay was applied is shown. Delayed hypersensitivity of all the mice from all the groups was positive: both in immunosuppressed and non-immunosuppressed mice, with BYJ treatment and without it.

Intensity of the reaction to DNFB was measured considering the increase percentage (as an index between the obtained increase of the ear volume and its basic measure). When group I (healthy, without BYJ) was compared with groups II, III, V and VI, groups II (healthy, with BYJ) with groups III, V and VI, and group III (murine lymphoma, without BYJ) with group IV, statistically significant decrease ($p<0.05$) of inflammatory process

Figure 2. Humoral immune responce of BALB/c mice immunosuppressed with lymphoma L5178Y treated with BYJ (1 mg/kg/10 days)

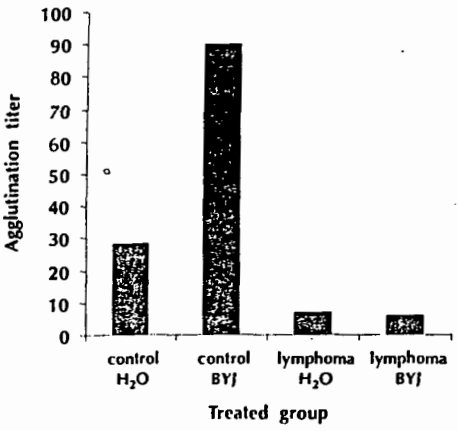
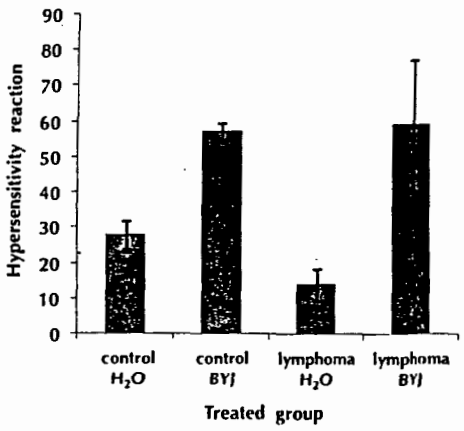


Figure 3. Cellular immune responce of BALB/c mice immunosuppressed with lymphoma L5178Y treated with BYJ (1 mg/kg/10 days).



cellular one (Fig. 3). On the other hand, the non-specific response (phagocytosis) wasn't suppressed (Fig. 4 and Table 3).

As it was mentioned above, the hosts with tumour frequently present a multifactoral depression of the immune response, which is frequently attributed to the existence of suppressive factors in biological fluids of patients with tumour or in the supernatants of the tumour cell culture. The list of these factors includes cytokines derived from the tumour or from the host, such as IL3, IL4, GM-CSF, TGFα, IFNγ and prostaglandins [21, 22].

When 1 mg/kg of weight/day of the powder of BYJ diluted in 0.1 ml of sterile water solution was administered during 10 days per os, the specific immune response (humoral and cellular) (Fig. 2, 3 and Tables 1, 2) and non-specific immune response (phagocytosis) (Fig. 4) were stimulated in healthy mice.

This modulation is probably related to the action of substances, equal or similar to acemannane, which acts on macrophages, stimulating immune response in its afferent phase in presentation and recognition of the antigens [9]. Karaca K. et al. have determined that acemannane stimulates the production of a nitric oxide in macrophages of chicken, the stimulation depends on dose [10].

BYJ probably contains mentioned polysaccharide and it is able to move in blood and stimulate mannose receptors on macrophages. It is necessary to carry out further investigations in order to confirm this supposition.

Groups of immunosuppressed mice with $1x10^7$ cells of lymphoma L5178Y with 10 days development, treated with the same amount of BYJ powder, showed a notable recovery of the cellular immune response (Fig. 3), and in the percentage of phagocytosis (number of cells that phagocyte), however the humoral immune response doesn't present any restoration (Fig. 2).

Sell S. supposes that the immunity against tumours is related to the cells, responsible for delayed hypersensitivity. They recognise specific antigens in tissues and are activated to release mediators which attract and activate macrophages. The activated macrophages are able to phagocyte and destroy tumour cells. In some animal models this mechanism is highly efficient in destruction tumours *in vivo*, however this mechanism cannot be reproduced *in vitro* [23].

Perhaps owing to this synergistic phenomenon of activation by BYJ of such lymphocytes by DNFB and of macrophages by non-specific agents, immune cellular response was restored. It was observed that macrophages can be possible targets for action of immunostimulating substances of *Aloe vera* (L) because their effect was correlated with binding to mannose receptors [9]. Besides, the variety of curative phenomena (immunostimulating, bactericidal, antimitotic, antiviral and antitumour) that were presented by gel as well as by BYJ can be only explained by the activation of regulatory cells owing to a sequence of cellular events, which stimulate the defense system, such as macrophage.

It only remains to investigate the effects of BYJ on the physiology of macrophage and its immunological process in addition to investigation of the fact whether it also stimulates some other type of antigen presenting cell (APC) such as B lymphocytes and the Langerhance cells in the skin.

Observing these facts we came to the conclusion that BYJ stimulates immune response of healthy and immunosuppressed mice and that, in addition to modulation, also stimulates it in the normal state.

It is important to elucidate if BYJ contains the same compounds as gel does and to prove its immunobiological effects. These studies are in progress.

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REFERENCES

1. Huller J.S. A drug for all seasons: medical and pharmacological history of aloe. Bull. N.Y. Acad. Med. 1990. 66 (6). 647-657.
2. Linares E., Flores B., Bye R. Selection de plantas medicinales de Mexico. D.F., Ed. Limusa-Noriega. Mexico 1990.
3. Granados-Sanches D.Y., Castañeda-Perez A.D. Sabila, *Aloe barbadensis* Mill. Planta argoindustrial (medicinal) del desierto. Universidad autonoms de Chapingo. 1988.
4. Vazquez B., Avila G., Escalante B. Antiinflammatory activity of extracts from *Aloe vera* (L) gel. J. Ethnopharmacol. 1996. 55 (1) 69-75.

Group	Treatment	Hypersensitivity reaction	Standard error	Infiltration (histology)
1	healthy/water	29.05	3.23	+++
2	healthy/BYJ	56.00	2.45	++++
3	lymphoma/water	14.67	2.66	+
4	lymphoma/BYJ	57.78	20.96	++++

Table 2. Cellular immune response of BALB/c mice immunosuppressed with lymphoma L5178Y and treated with BYJ

Significant: 1vs 2, 3; 2 vs 1, 3; 3 vs 1, 2 and 4 groups.
No significant: 1 vs 4, 2 vs 5 group.

was observed. This decrease was more notable in groups III, V and VI (**Figure 3**).

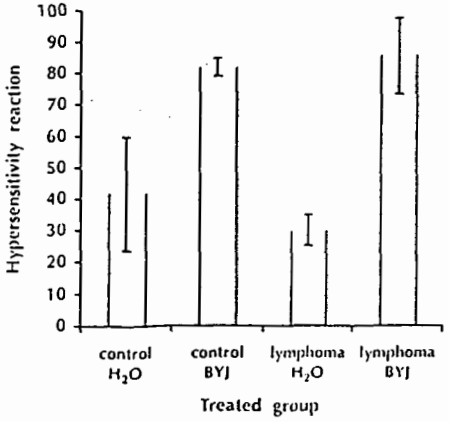
On the other hand, in group I, II (healthy, with BYJ and without it) and VI (lymphoma with BYJ) there were no any significant differences there.

Microscopic analysis of histological sections has confirmed the manifestation of delayed hypersensitivity to DNFB (**Table 2**).

For this purpose the mark from 1 to 4 crosses was chosen, depending on the amount of cellular elements that participated in the inflammatory reaction of external ear of mice, in which the developing assay of sensitivity to DNFB was applied.

The highest inflammation (4 crosses) was observed in groups II and IV. There was a lower inflammatory reaction, equivalent to 3 crosses in groups I, and group III had a suppression of inflammatory reaction which reached only 1 cross (Table 2).

Figure 4. Phagocytosis of alveolar macrophages of BALB/c mice immunosuppressed with lymphoma L5178Y treated with BYJ (1 mg/kg/10 days).



These results correlates with the obtained ones of measuring the inflammation of external ear of mice and the difference was statistically significant ($p<0.05$) by the Kruskal-Wallis assay.

The phagocytic activity of the AM of the healthy and with lymphoma treated mice without BYJ (groups I and III) wasn't disturbed; moreover, the groups of healthy and with lymphoma treated animals with BYJ (groups II and IV) had an increase of number of phagocytic cells, they haven't shown difference (**Table 3, Figure 4**).

DISCUSSION

In the present study we investigated the influence of BYJ on immune system of BALB/c mice. Murine lymphoma L517Y, a biological model, was used as a suppressive agent of the immune response.

Mentioned lymphoma provokes the immunosuppression state of BALB/c mice at 10 days of development, according to Daneri-Navarro.

The obtained results of the specific immune response evolution of BALB/c mice, inoculated with $1x10^7$ lymphoma cells and 10 days of tumour development, show us that the suppression is general for humoral response (Table 1) as well as for

Table 3. Phagocytosis of alveolar macrophages of BALB/c mice immunosuppressed with lymphoma L5178Y treated with BYJ (1 mg/kg/10 days)

Group	Treatment	Phagocytosis %	DS
1	healthy/water	42.25	15.54
2	healthy BYJ	81.33	2.08
3	lymphoma/water	30.67	4.62
4	lymphoma/BYJ	86.67	8.5

Significant: 1vs 2, 4; 3 vs 2, 4 groups.
No significant: 1 vs 3; 2 vs 4 group.

5. Hutter J.A., Salman M., Stavinoha W.B., Satsangi N., Williams R.F., Streeper R.T., Weintraub S.T. Antiinflammatory C-glucosyl chromone from *Aloe barbadensis*. J. Nat. Prod. 1996, 59 (5), 541-543.
6. Arion V.Y., Sanina I.V., Breusov Yu.N. Chemistry and biology of immunoregulators. Zinatne, Riga 1985, 39-52.
7. Peng S.Y., Norman J., Curtin G., Mc.Daniel H.R., Busbee D. Decreased mortality of Norman murine sarcoma in mice treated with the immunomodulator, acemannan. Mol. Biother. 1991, 3, 79-87.
8. Womble D., Helderman J.H. Enhancement of alloresponsiveness of human lymphocytes by acemannan. Int. J. Immunopharmacol. 1988, 10, 967-974.
9. Stuart R.W., Lefkowitz D.L., Lincoln J.A., Howard K., Gelderman M.P., Lefkowitz S.S. Upregulation of phagocytosis and candidicidal activity of macrophages exposed to the immunostimulant acemannan. Int. J. Immunopharmacol. 1997, 19 (2), 75-82.
10. Karaca K., Sharma J.M., Nordgren R. Nitric oxide production by chicken macrophages activated by acemannan, a complex carbohydrate extracted from *Aloe vera*. Int. J. Immunopharmacol. 1995, 17 (3), 183-188.
11. Loomis T.D. Functions de toxicologia. Ed. Zaragoza, Esp. edit. Acribia. 1982.
12. Wagner H.K.M. In: Phytochemistry of medicinal plants. Armason J.T., Mats R., Romeo J.T. (eds.). Plenum Press. 1995, 29, 1-17.
13. Ivanovska N., Philipov S., Istatkova N., Georgieva P. Antimicrobial and immunobiological activity of ethanol extracts and fractions from isopyrum thalictroides. J. Etnthopharmacol. 1966, 54143-54151.
14. Agarwal A.K., Singh M., Gupta N., Saxena R., Puri A. et al. Management of giardiasis by and immunomodulatory herbal drug *Pippali rassyana*. J. Ethnopharmacol. 1994, 44, 143-146.
15. Mori M., Fuchigami M., Inoue N., Nagai H., Koda A. et al. Principle of the bark of *Phellodendron amurense* to suppress the cellular immune response. Effect of phellodendrine on cellular and humoral immune responses. Planta Med. 1995, 61, 45-49.
16. Gad S.C. The mouse ear swelling test (MEST) in the 1990th. Toxicology 1993, 1004, 33-46.
17. Streilein W.P., Galen T.T., Toews M.D., James N.G., Bergnstresser P.R. The role of Langerhance cell sensity within epidermis. J. Invest. Dermatol. 1980, 74, 319-322.
18. Cunningham A.J., Szenberg A. Futher improvements in the plaque technique for detecting single antibody-forming cells. Cell 1968, 14, 599-600.
19. Courreges M.C., Benencia F., Massouth E.J., Coulombie F.C. *In vitro* antiphagocytic effect of *Melia azedarach* leaf extracts on mouse peritoneal exudate cells. J. Ethnopharmacol. 1994, 43, 135-140.
20. Ingolfdottir K., Jurcic K., Fischer B., Wagner H. Immunologically active polysaccharide from *Cetraria islandica*. Panta Med. 1994, 60, 527-531.
21. Weinberg R.A. How cancer arises. Sci. American. 1966, 275 (3), 32-42.
22. Daneri-Navarro A., Del Toro-Arreola A., Garcia-Velazco Del Toro-Arreola S., Orbach-Arbouys S., Bravo-Cuellar A. L5178Y lymphoma associated immunosupression in BALB/c mice. Biomed. Pharmacother. 1995, 49, 39-44.
23. Sell S. Immunology, Immunopathology and Immunity. Fifth Ed. Appleton & Lange 1996.