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STUDY OF CLASTOGENIC POTENTIAL OF GLYCINE, INTERFERON-BETA, AND TRANSFER FACTOR, IN VIVO

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ABSTRACT

Interferon-beta (IFN-B), transfer factor (TF), and glycine are agents that possess biomedical properties related to the modulation of the immune and the nervous systems, as well as to the inflammatory process. Therefore, safety evaluation of the compounds is necessary in regard to non genotoxic effect and safety use in human health. The present study evaluates their genotoxic and cytotoxic potential by determining the capacity of the compounds to induce sister chromatid exchanges (SCE), or to alter cellular proliferation kinetics (CPK) and the mitotic index (MI) in mouse bone marrow cells. Besides, it also determines their capacity to increase the rate of micronucleated polychromatic erythrocytes (MNPE) in peripheral mouse blood, and the relationship polychromatic erythrocytes /normochromatic erythrocytes (PE/NE) as an index of cytotoxicity. Two doses of each compound were tested. For the assays the mice received intraperitoneally (ip): $4x10^6$ UI/ kg and $8x10^6$ UI/kg of INF-B; 0.5 and 1 UI/kg of TF, 1 and 2 g/kg of glycine; 0.5 ml of distilled water (negative control group), and 5 mg/kg of cisplatin (positive control group) respectively. The results in regard to the agents showed no SCE increase induced by any of the tested doses, as well as no alteration in the CPK, or in the MI. With respect to the second assay, the results obtained with the tree agents were also negative for the MNPE and the PE/NE index along the daily evaluation made for four days. The results obtained establish that the studied agents have neither genotoxic nor cytotoxic effect on the model used.

1. INTRODUCTION

There is a great variety of factors that determine the toxic effects of substances in organisms, such as the chemical characteristics of xenobiotics, doses, administration routes, exposition time, and environmental factors[1]. These factors determine quantitatively and qualitatively the presence of toxic effects in genetic mutations and cellular alterations that could cause necrosis and cellular death[2].
In the research of new substances that modulate the immunological response, the interferon-beta (INF-β), the transfer factor (TF), and glycine, are drugs used indefinitely in degenerative chronic diseases, such as multiple sclerosis[3, 4].
Multiple sclerosis (MS) is an inflammatory demyelization disease of the central nervous system, probably immune mediated[5]. Among the major clinical manifestations of MS is the spasticity which produces a clinical disability by interfering with posture, motor capacity, nursing or daily living activities. When the muscle over activity is diffusely distributed, an adequate treatment should be implemented to prevent permanent musculoskeletal deformities or contractures. This treatment involves the use of drugs like glycine, GABA, glutamate among others; which are drugs that modulate neurotransmitters acting at cortical-spinal level[6].
Recombinant IFN-β has established efficacy for relapsing-remitting MS because it can mediate biological effects by receptor-mediated gene activation[7]. It has been demonstrated that intramuscular injections of IFN-β-1a up regulate IL-10 and IL-4 but not IFN-γ mRNA in peripheral blood mononuclear cells[8], and the effects IFNs (alpha and beta) in MS are mediated by immunoregulatory rather than anti-viral or antiproliferative mechanisms[9]. These found tend to inhibit the activity of IFN-gamma and to prevent disease activity. Therefore, the outcome of the use of INFs in the treatment of multiple sclerosis results in clinical important beneficial effects on disability progression in relapsing MS patients[4].
The other alternative treatment employed in MS patients is the administration of transfer factors[10]. Highly polar compounds, hydrophilic molecules of low molecular weight (approximately 5,000 Daltons), which are produced in small quantities by lymphoid cells and have potent biological activity and appear to be composed entirely of amino acids [8-10] or peptides that binds to the target cells[11]. The most consistent effects of the transfer factors on the immune system are the expression of delayed-type hypersensitivity and production of lymphokines such as macrophage migration inhibitory factor (MIF), which is probably identical to gamma-interferon response to exposure to antigen[12] and seems interact with the variable regions of the alpha and/or beta chain of T cell receptors to change their avidity and affinity for antigen in a way that otherwise would only occur after an encounter with antigen. Therefore, the MS patients treated with TF had a slower rate of progression of their illness[11].
Because of the indefinite use of these tree drugs in the treatment of MS it is necessary mutagenic assays *in vivo* to identify if they may induce DNA damage by the identification of micronuclei (MN) and sister chromatid exchange (SCE). Therefore, the objective of this work was to identify the genotoxic potential of INF-β, TF, and glycine in an experimental animal model using mice.

2. Material and Methods

2.1. Chemicals and animals

Hoechst 33258, 5-bromodeoxyuridine (BrdU), colchicines, and cisplatin were purchased from Sigma Chemicals (St. Louis, MO, USA). Transfer factor was obtained from Laboratory of Immunological Specialties (Mexico City, Mexico); INF-β from Serono (Mexico City, Mexico), and glycine from Reproquifin (Mexico City, Mexico). Giemsa and potassium chloride were purchased from Baker, S.A (Mexico City, Mexico).
Eight-week-old male mice (NIH) with 25 g of weight were obtained from the National Institute of Rehabilitation; they were kept in metallic cages at a mean temperature of 23◦C and at a 12 hours dark-light cycle, and permitted to freely consume food (Purina) and water.

2.2. Micronuclei Assay

Eight groups with 5 individuals each per chemical were organized for this assay. The compounds were IP inoculated. The groups were a negative control group treated with distilled water, a positive control group administered with 5 mg/kg of cisplatin, and six more groups treated with $4x10^{(6)}$ UI/kg and $8 \times 10^{(6)}$UI/kg of INF-β; 0.5 UI/kg and 1 UI/kg of TF, and 1 and 2 g/kg of glycine. The results obtained in these animals were compared to the obtained results in the negative control group (distilled water) and the positive control group (cisplatin). Initially, we obtained two drops of blood from the tail of each mouse and smeared them on ethanol-cleaned slides; then, the cells were fixed in methanol for 10 minutes and stained for 15 minutes with Giemsa solution made in PBS (pH = 6.8) [13]. Afterward, the tested chemicals were administered to mice and their blood was obtained at 24, 48, 72, and 96 hours and then it was stained as indicated earlier. To determine the genotoxic potential of the chemicals, the rate of MNPE in 1000 polychromatic erythrocytes (PE) per mouse was quantified; to evaluate the probable cytotoxic damage, the number of PE and of normochromatic erythrocytes (NE) in 1000 cells per mouse was determined[14]. The statistical significance of the obtained data was determined with the ANOVA and the Student *t* tests.

2.3 Sister Chromatid Exchange Assay

Eight experimental groups (five mice per group) were used for these determinations. The compounds were intraperitoneally (IP) administered. The groups were as follows: a negative control group of animals administered with distilled water (0.4 mL/mouse), a positive control group treated with 5 mg/kg of cisplatin, and six groups administered with $4x10^{(6)}$ UI/kg and $8 \times 10^{(6)}$UI/kg of INF-β; 0.5 UI/kg and 1 UI/kg of TF, and 1 and 2 g/kg of glycine. To begin the procedure, a BrdU tablet of 50mg was partially coated with paraffin and subcutaneously implanted to each animal[15], 1 hour afterwards the substances were injected to mice. Twenty-one hours after the tablet implantation, 5 mg/kg of colchicine was IP injected to mice and permitted to act for 3 hours. Then, the femurs of each mouse were dissected and the bone marrow obtained in a solution of KCl (0.075 M) at 37◦C after which it was incubated for 30 minutes at

the same temperature.

The cell suspension was centrifuged for 10 minutes at 1500 rpm, the supernatant was discarded, and the cells were fixed in a solution of methanol-acetic acid (3:1). This fixation process was repeated at least twice. To carry out the staining process, two drops of each cell suspension were deposited onto ethanol-cleaned slides and stained with the Hoechst-Giemsa method to differentiate the sister chromatid[15].

Cytogenetic analysis per mouse was made as follows: (a) the rate of SCE was determined in 60-second division metaphases, (b) the cellular proliferation kinetics (CPK) was determined in 100 metaphases, identifying the cells in first (M1), second (M2), and third (M3) cellular division. With these data, the average generation time (AGT) was obtained using the formula AGT = 21/ (M1+2M2+3M3) (100), and (c) the mitotic index (MI) was determined in 1000 cells. Statistical analysis of the obtained data was made with ANOVA test followed by the Student *t* test.

3. Results

The rate of micronuclei induced by these chemicals is shown in Table 1. The mean number of MNPE/1000 cells determined in the negative control group along the 96 hours was 1.1 ± 0.60; the mean values observed in the TF, INF-β and glycine treated groups were 1.6 ± 0.8, 1.4 ± 0.5, 1.5 ± 0.5, 1.2 ± 0.5, 1.5 ± 0.3, 1.3 ± 0.5, corresponding to the doses of $4x10^6$ UI/ kg and $8x10^6$ UI/kg of INF-β; 0.5 and 1 UI/kg of TF, 1 and 2 g/kg of glycine, respectively, clearly showing an absence of genotoxicity. On the contrary, the MNPE mean determined for cisplatin was high as expected, particularly at 72 hours (25.1 ± 0.7 MNPE/1000 cells). The innocuous capacity of TF, INF-β and glycine for micronuclei induction paralleled the absence of its cytotoxic potential shown by the frequency of polychromatic erythrocytes (Table 2). The table shows PE daily values similar to those determined in the negative control animals and contrary to those in cisplatin-treated mice, in which a significant PE rate decrease (74% at 72 hours) was found in comparison with the mean of the other groups.

Table. 1. Effect of interferon-β (INF-β), transfer factor (TF), and glycine on the frequency of micronuclei in polychromatic erythrocytes.

Agent	Doses	No. PCE	24 h X ±SD	48 h X ±SD	72 h X ±SD	96 h X±SD	Average X±SD
Distilled water	0.5 ml	1, 000	1.6 ± 0.51	2.1 ± 0.75	1.7 ± 0.42	1.1 ± 0.60	1.08± 0.57
Cisplatin	5 mg/kg	1, 000	*18.1 ± 1.1	*19.8 ± 0.9	*25.1 ± 0.7	*20.5± 1.0	*20.9 ±0.9
INF-β	$4x10^{(6)}$ U/kg	1, 000	1.6 ± 0.8	1.6 ± 0.8	1.6 ± 0.8	1.6 ± 0.8	1.6 ± 0.8
INF-β	$8x10^{(6)}$ U/kg	1, 000	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.5
TF	0.5 UI/kg	1, 000	1.6 ± 0.8	1.6 ± 0.8	1.6 ± 0.8	1.6 ± 0.7	1.5 ± 0.5
TF	1 UI/kg	1, 000	1.3 ± 0.5	1.4 ± 0.4	1.4 ± 0.5	1.3 ± 0.5	1.2 ± 0.5
Glycine	1 g/kg	1, 000	1.3 ± 0.4	1.4 ± 0.2	1.8 ± 0.3	1.5 ± 0.2	1.5 ± 0.3
Glycine	2 g/kg	1, 000	1.3 ± 0.1	1.3 ± 0.5	1.3 ± 0.5	1.4 ± 0.2	1.3 ± 0.5

*Statistically significant difference of cisplatin with respect to the negative control and the three substances values, ANOVA and t Student ($P<0.05$) (n = 5)

Table 2. Frequency of micronuclei in polychromatic erythrocytes induced by interferon-β (INF-β), transfer factor (TF), and glycine

Agent	Dose	24 h X ±SD	48 h X ±SD	PE/NE 72 h X ±SD	96 h X±SD
Distilled water	0.5 ml	19.5 ± 0.12	20.33 ± 0.21	20.16 ± 0.29	21.4 ± 0.18
Cisplatin	5 mg/kg	*9.1± 0.37	*7.33± 0.61	*5.33± 0.33	*6.36± 0.42
INF-β	$4x10^{(6)}$ U/kg	19.8 ± 0.46	18.8 ± 0.17	17.0 ± 0.42	19.5 ± 0.28
INF-β	$8x10^{(6)}$ U/kg	20.3 ± 0.43	19.16 ± 0.11	19.7 ± 0.52	20.0 ± 0.48
TF	0.5 UI/kg	20.0 ± 0.19	19.5 ± 0.44	19.83 ± 0.61	21.0 ± 0.12
TF	1 UI/kg	22.0 ± 0.43	20.3 ± 0.19	20.14 ± 0.05	21.3 ± 0.57
Glycine	1 g/kg	16.33 ± 0.38	19.66 ± 0.18	20.6 ± 0.19	21.8 ± 0.49
Glycine	2 g/kg	14.9 ± 0.61	21.16 ± 0.37	20.36 ± 0.10	22.9 ± 0.35

*Statistically significant difference of cisplatin with respect to the negative control and the three sustances values, ANOVA and t Student ($P<0.05$) (n = 5)

The result of the SCE, AGT, and the MI are indicated in Table 3. With respect to the rate of SCE, we found a similar level (with the two tested doses for each compound) to the one observed in the negative control group; however, the positive control agent (cisplatin) revealed an increase of about three times that level. The same table shows a homogeneous AGT value of the two tested doses of TF, INF-β and glycine, with a variability of no more than 30 minutes among them; these values are very close to those obtained in the controls. A similar effect, that is, no modification by TF, INF-β and glycine were also determined with respect to the cell division rate, as shown by the similar MI values observed in all groups.

Table 3. Sister chromatid exchange (SCE), average generation time (AGT), proliferation kinetic (CPK), and mitotic index (MI) induced by interferon-β (INF-β), transfer factor (TF), and glycine in bone marrow cells of treated mice. M1,M2,M3 = cells in first, second, and third cellular division. AGT = 21(M1+2M2+3M3) (100).

AGENT	DOSE	SCE X ± SD	M 1	CPK M2	M 3	AGT	MI
Distilled water	0.5 ml	1.9 ± 0.82	34	51	15	12.45 ± 0.23	5.94 ± 0.37
Cisplatin	5 mg/kg	*6.02 ± 1.32	34	49	17	12.63 ± 0.17	7.13 ± 0.39
INF-β	$4X10^6$ UI/kg	1.97 ± 0.83	35	51	14	12.36 ± 0.11	5.97 ± 0.28
INF-β	$8X10^6$ UI/kg	1.78 ± 0.82	37	50	13	12.46 ± 0.34	5.80 ± 0.39
TF	0.5 UI/kg	1.70 ±0.77	39	50	11	12.47 ± 0.23	6.16 ± 0.25
TF	1.0 UI/kg	1.50 ±0.67	34	51	15	12.25 ± 0.18	6.70 ± 0.19
Glycine	1 g/kg	1.53 ± 0.62	38	52	10	12.89 ± 0.22	6.00 ± 0.25
Glycine	2 g/kg	1.63 ± 0.70	33	53	14	12.76 ± 0.13	6.21 ± 0.24

*Statistically significant difference of cisplatin with respect to the control group and the three sustances values, ANOVA and t Student ($P<0.05$)

4. **Discussion**

In the genotoxic procedures used in this investigation, we observed that the doses of INF-β, TF and glycine administered to mice caused neither genotoxic nor cytotoxic effects.

Review in the literature about genetic toxicity of certain cytokines such as INFα, β and γ, TNF-α, epidermal growth factor and interleukins, have reported that these cytokines may exhibit genotoxic properties in human peripheral blood lymphocyte cultures[16]; in KB cell line the IFN-α showed a slightly increase the incidence of SCE following IFN treatment but without dose-effect relationship[17] and it has also determined the inhibitory effect of both IFN-α and β on human erytrhroid colony-forming units despite binding to the same receptor[18]. However, it have been found a new biological functions of interferon, namely, their capacity to protect human cells from the action of some physical and chemical mutagens[19], their capacity to alter the DNA repair[20], and that do not produce neither genotoxic/clastogenic nor embryotoxic effects in high doses (10^3-10^5 UI) *in vitro*[21]. Our results demonstrated that the two doses of INF-β (higher doses that those employed by Mertens et. al., did not have genotoxic effects *in vivo*; therefore, our results complement those made *in vitro* and represent an important outcome for the long time treatment of demyelization disease as sclerosis multiple among others.

Finally, the exit of the use of TF as a therapy is due to its clinical effectiveness in treating many infectious (viruses, fungi and bacteria) and immunodeficiency diseases[22], as well as its down regulatory effect on HIV-1 gene expression[23] and it capacity to interrupt the progress of diabetes in streptozozin treated mice by means of diminishing the inflammation in the pancreatic tissue[24]. Our results have demonstrated that TF did not cause genetic nor cellular toxicity *in vivo* which represents an important finding because of use of TF wide world; although, there is an experimental evidence that the TF present in injected newborn human serum produce significant embryo toxic and teratogenic effects, as well as changes in coordination of motor activity in newborn rats[25] , however, this results were made utilizing the complete serum, so they can not ensure that this toxic effects were due only to the TF present in the complete serum.

There is a substantial experimental evidence that the glycine may have a role in protecting tissues against insults such as ischemia, hypoxia, reperfusion and nephrotoxicants[26] , glycine can also modulates pro and anti-inflammatory cytokine levels[27], improves survival of rats given endotoxin, most likely by inactivation of Kupffer cells, since tumor necrosis factor-α production was decreased[28], has a cytoprotective effect and it diminishes the apoptosis caused by cisplatin in renal tubules in kidney[29].

However, when the nitrite group of glycine reacts with Cl^- at acidic pH, it give rise to genotoxic compounds. Although, it is not full understood the nature and the mechanism of genetic lesion induced by the ultimate genotoxicant arising from the nitrosidation of glycine, the influence of Cl^- in the genotoxic activity of glycine may have a possible involvement in the etiology of gastric cancer[30]. Our results show that the two doses of glycine administered to the mice did produce neither genotoxic nor cytotoxic effects; therefore, the utilization of glycine for a long period of time in degenerative diseases may not represent any additional danger for patients. We conclude that the three inmunomodulators did not show neither genotoxic nor cytotoxic effect compared with cisplatin, and suggest that these drugs can be employed for long periods of time in those chronic diseases.

Declaration: studies involving humans or experimental animals were conducted in accordance with national and institutional guidelines for the *protection of human subjects and animal welfare*. The experiments used in this study, comply with the current laws of the country in which the experiments were performed.

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References

[1] Hiroshi Suzuki, Hironao Takasawa, Kazuo Kobayashi, Yukari Terashima, Yasushi Shimada, Izumi Ogawa, Jin Tanaka, Tadashi Imamura, Atsushi Miyazaki and Makoto Hayashi. Evaluation of a liver micronucleus assay with 12 chemicals using young rats (II): a study by the Collaborative Study Group for the Micronucleus Test/Japanese Environmental Mutagen Society-Mammalian Mutagenicity Study Group *Mutagenesis (2009) 24 (1): 9-16*

[2] Curtis D. Klaassen, Steven Gilbert. *Casarett & Doull's Toxicology* . Louis J. *Casarett & John Doull first edited, Toxicology:* The Basic Science of Poisons, in 1975. In 2013 the 8th edition was edited by, published by McGraw-Hill, (2013)

[3] A Bertolotto, A Sala, S Malucchi, F Marnetto, M Caldano, A Di Sapio, M Capobianco. (*2004)* Biological activity of interferon betas in patients with multiple sclerosis is affected by treatment regimen and neutralising antibodies. F Gilli *J Neurol Neurosurg Psychiatry;75:1294-1299*

[4] Rudick, R. A., Goodkin, D. E., Jacobs, L. D., Cookfair, D. L., Herndon, R. M., Rochert, J. R., Salazar, A. M., Fischer, J. S., Granger, C. V., Simon, J. H., Alam, J. J., Simonian, N. A., Campion, M. K., Bartoszak, D. M., Bourdette, D. N., Braiman, J., Brownscheidle, C. M., Coats, M. E., Cohan, S. L., Dougherty, D. S., Kinkel, R. P., Mass, M. K., Munschauer, F. E., Priore, R. L., & Whitham, R. H. 1997. Impact of interferon beta-1a on neurologic disability in relapsing multiple sclerosis. The Multiple sclerosis Collaborative Research Group (MSCRG), *Neurology* 49, 358-363.

[5] Abbruzzese, G. 2002. The medical management of spasticity. *European Journal of Neurology, 9 Suppl 1*, 30-61.

[6] Gonzalo E Yevenes and Hanns Ulrich Zeilhofer (2011) Allosteric modulation of glycine receptors. *Br J Pharmacol*. 164(2): 224–236.

[7] Paty, D. W., & Li, D. K. 1993. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI study group and the INFB Multiple sclerosis study group. *Neurology*, 43, 662-667.

[8] Christoph Kamm, Uwe K. Zettl (2012) Autoimmune disorders affecting both the central and peripheral nervous system. *Autoimmunity Reviews*, Volume 11, 3:196–202

[9] Beck RW, Chandler DL, Cole SR, Simon JH, Jacobs LD, Kinkel RP, Selhorst JB, Rose JW, Cooper JA, Rice G, Murray TJ, Sandrock AW. (2002) Interferon beta-1a for early multiple sclerosis: CHAMPS trial subgroup analyses. *Ann Neurol*. 51(4):481-90.

[10] Beck RW, Chandler DL, Cole SR, Simon JH, Jacobs LD, Kinkel RP, Selhorst JB, Rose JW, Cooper JA, Rice G, Murray TJ, Sandrock AW. (2002) Interferon beta-1a for early multiple sclerosis: CHAMPS trial subgroup analyses. *Ann Neurol*. 51(4):481-90.

[11] Frith, J. A., McLeod, J. G., Basten, A., Pollard, J. D., Hammond, S. R., Williams, D. B., & Crossie, P. A. 1986. Transfer factor as a therapy for multiple sclerosis: a follow-up study. *Clinical and Experimental Neurology*, 22, 149-154.

[12] Kirkpatrick, C. H. 2000. Transfer factors: identification of conserved sequences in transfer factor molecules. *Molecular Medicine*, 6, 332-341.

[13] Rogelio Paniagua-Pérez, Eduardo Madrigal-Bujaidar, Dolores Molina-Jasso, Susana Reyes-Cadena, Isela Álvarez-González, Laura Sánchez-Chapul and Javier Pérez-Gallaga (2009) Antigenotoxic, antioxidant and lymphocyte induction effects produced by pteropodine *Basic & Clinical Pharmacology & Toxicology* 104 , 222–227

[14] Paniagua-Pérez, R., Madrigal-Bujaidar, E., Reyes-Cadena, S., Alvarez-González, I., Sánchez-Chapul, L., Pérez-Gallaga, J., Velasco,-Mora O. (2008). Cell protection induced by beta-sitosterol: inhibition of genotoxic damage, stimulation of lymphocyte production, and determination of its antioxidant capacity. *Archives of toxicology*, *82*(9), 615-622.

[15] Goto, K., Akematsu, T., Shimazu, H., & Sugiyama, T. (1975). Simple differential Giemsa staining of sister chromatid's after treatment with photosensitive dyes and exposure to light and the mechanism of staining. *Chromosoma*, 53, 223-230.

[16] Lazutka, J. R. 1996. Genetic toxicity of cytokines. *Mutation Research*, 361, 95-105.

[17] Georgian, L., Ghyka, G., & Savi I. 1990. The effect of human alpha leukocyte interferon (IFN) on the frequency of sister chromatid exchanges (SCE) in the KB cell line. *Morphologie embryologie*, 36, 13-17.

[18] Means, R.T., & Krantz, S.B. 1996. Inhibition of human erythroid colony-forming units by interferons alpha and beta: differing mechanisms despite shared receptor. *Experimental Hematology*, 24, 204-208.

[19] Shafik, H. M., Nokta, M. A., & Pollard, R. B. 1991. Recombinant interferon-beta ser protects against zidovudine-induced genetic damage in AIDS patients. *Antiviral Research*, 16, 205-212.

[20] Makedonov, G.P. Chekova, V.V. Yakubovskaya, E.L. & Zasukhina G.D.. 1990. Modification of DNA repair by human interferons. *Acta Biologica Hungarica*, 1,187-197.

[21] Mertens, R., Severi, K., & Habedank, M. 1993. Effect of human and recombinant IFN-alpha and IFN-beta on the sister chromatid exchange (SCE) frequency in amniotic fluid cells in vitro. *Mutation Research*, 300, 195-200.

[22] Prasad, U., bin Jalaludin, M. A., Rajadurai, P., Pizza, G., De Vinvi, C., Viza, D., & Levine, P. H. 1996. Transfer factor with anti-EBV activity as an adjuvant therapy for nasopharyngeal carcinoma: a pilot study. *Biotherapy*, 9, 109-115.

[23] Ojeda, M. O., Fernández-Ortega, C., & Rosainz, M. J. 2000. Dialyzable leukocyte extract suppresses the activity of essential transcription factors for HIV-1 gene expression in unstimulated MT-4 cells. *Biochemical and Biophysical Research Communications*, 273, 1099-1103.

[24] Borkowsky, W., Pilson, R., & Lawrence, H. S. 1996. Dialyzable lymphoid extract (DLE from mice resistant to STZ-induced diabetogenesis can interrupt the progress of diabetes in STZ-treated CD-1 mice. *Biotherapy*, 9, 149-157.

[25] Abdalaze, N. S., Avaliani, T. V., Chebotar, N. A., Ataeva, O. V., & Bogdanov, O. V. 1994. The embryotoxic effect in rats exposed to the blood serum of newborn infants tested for the presence of transfer factor. *Ontogenez*, 25, 38-44.

[26] Miller, G. W., Lock, E. A., & Schnellmann, R. G. 1994. Strychnine and glycine protect renal proximal tubules from various nephrotoxicants and act in the late phase of necrotic cell injury. *Toxicology and Applied Pharmacology*, 125, 192-197.

[27] Bruck, R., Wardi, J., Aeed, H., Avni, Y., Shirin, H., Avinoach, I., Shahmurov, M., & Hershkoviz, R. 2003. Glycine modulates cytokine secretion, inhibits hepatic damage and improves survival in a model of endotoxemia in mice. *Liver Internacional*, 23, 276-282.

[28] Wheeler, M. D., Rose, M. L., Yamashima, S., Enomoto, N., Seabra, V., Madren, J., & Thurman, R. G. 2000. Dietary glycine blunts lung inflammatory cell influx following acute endotoxin. *Am. J. Physiol. Lung Cell. Mol. Physiology*, 279, L390-398.

[29] Heyman, S. N., Rosen, S., Silva, P., Spokes, K., Egorin, M. J., & Epstein, F. H. 1991. Protective action of glycine in cisplatin nephrotoxicity. *Kidney Inernational*, 40, 271-279.

[30] Gaspar, J., Laires, A., Va, S., Pereira, S., Mariano, A., Quina, M., & Rueff, J. 1996. Mutagenic activity of glycine upon nitrosation in the presence of chloride and human gastric juice: a possible role in gastric carcinogenesis. *Teratogenesis, Carcinogenesis, and Mutagenesis*, 16, 275-286.