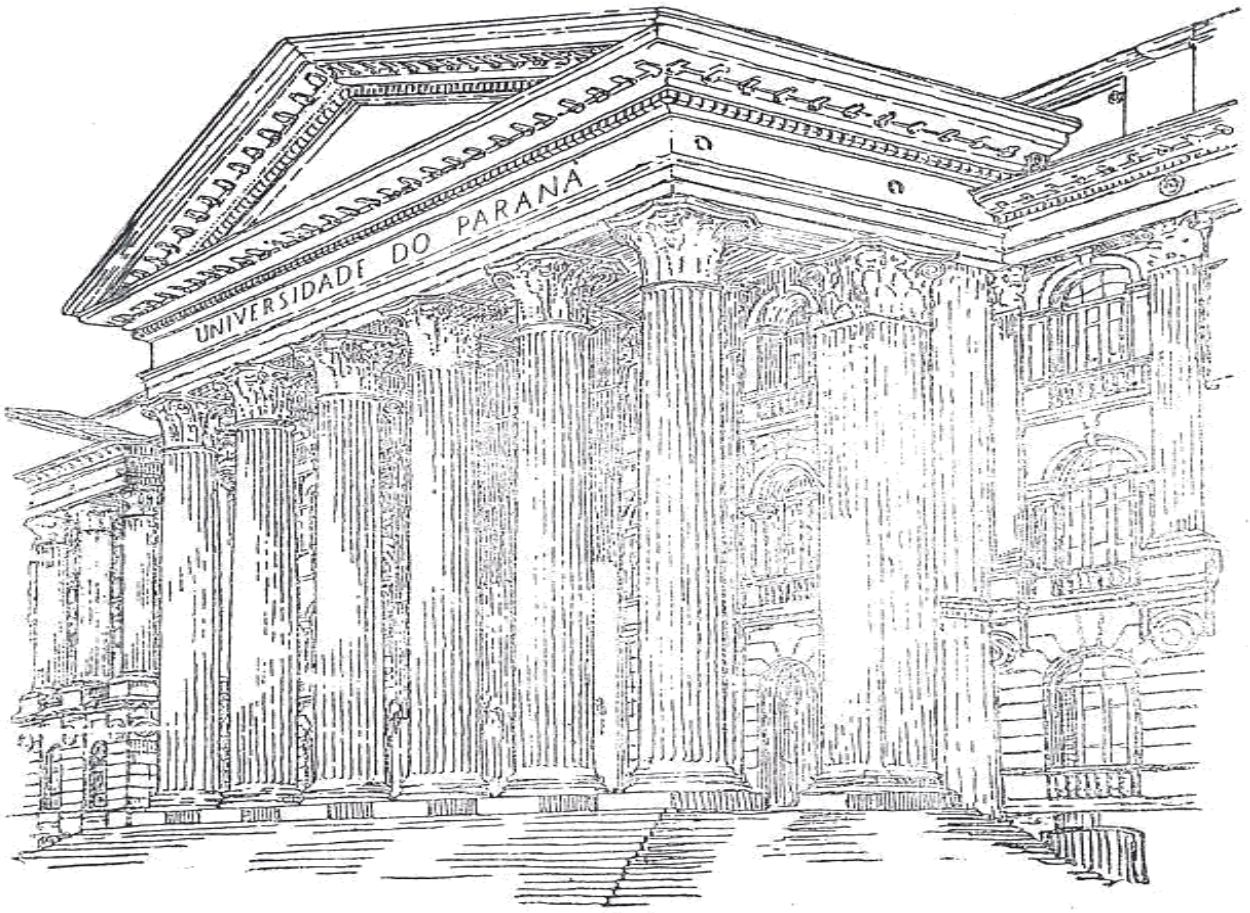

Randomized Placebo-Controlled Clinical Trial to appraise the effectiveness and safety of Immunomodulator Canova® in the therapeutics of patients who have HIV/AIDS on anti-retroviral use.

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"Sad times, since it is easier to do the fission of an atom that of a prejudice."

Albert Einstein

1 - INTRODUCTION

AIDS is an immunodeficiency caused by the retrovirus HIV, which uses several "escape" mechanisms to the processes of organic defense. One of the object mechanisms of study in the world is the frequent mutation suffered by its genetic material, fooling this way, partially, the cellular and humoral defense. Human Immunodeficiency Virus (HIV), isolated from individuals that received highly active antiretroviral therapy (HAART), based on the Association of Inhibitors of Transcriptase Reverse Pharmaco (ITRNN) and of the inhibitors of protease (IP), evidenced resistance to multiple drugs, causing therapeutic failure.[1] This fact has been forcing the researchers to revise the therapeutic strategies, trying to block the entrance of the virus into the cells and to increase the immunity of the host. It is also known that the unbalance in the cytokines production, mainly the pro-inflammatory ones, is directly linked to the immunopathogeny of AIDS. Among them, it is the Tumor Necrosis Factor α (TNFα), which can induce the expression of HIV directly through the activation of the Necrosis Factor - K β, increasing its replication.[2] Immunomodulator CANOVA® is a medicament compound produced from homoeopathic tinctures that are listed in the world pharmacopoeia and of wide application: *Aconitum napellus + associations.* It doesn't present toxicity and it is indicated to the diseases where the immunological system is depressed, like cancer and HIV/AIDS. The medicament CANOVA® presents immunomodulator effect, acting directly on the macrophages, cells that influence direct and indirectly the Immune System (Piemonte and Buchi) showed that, under the action of CANOVA®, there was a decrease in the production and liberation of TNFα by the macrophages,[3] justifying the positive answer partly evidenced at the clinic.

Individuals who have HIV/AIDS, mainly the ones that, due to the resistance or intolerance to the conventional medication, are out of therapeutic possibility, have used this medicament. With the objective of proving the real effectiveness or not of this medicament in patients who have HIV/AIDS, we performed the present study.

2 - REVISION OF THE LITERATURE

The immunological research came from the experimental laboratories to find practical clinical application and, today, it is already possible to say that the immunology has been the only clinical specialty these last years, that, instead of going specialized, is being generalized, penetrating the clinical segments, introducing new clinical and diagnostic approaches, as well as new therapies. Especially in the field where the immunology and the infect-logia get mixed, in the combat to the infect-contagious diseases, the immunology has questioned more and more frequently the modern infect-logia: what can be done when the antibiotic or antiviral don't resolve?

It is known that the improvement of the life conditions, provided an increase of the life expectancy, in consequence, an increase of the senior population, which is more susceptible to infections, being in need of antibiotic treatments and with results a lot of times unsatisfactory: the increase of the immunodepressed individuals resulting from immunodepressing therapies by transplants or other clinical situations, the pandemic of AIDS, the resistance to the new anti-microbial and the economical limitations.

Due to those facts, it was concluded that, mainly in relation to HIV, the possibility of restoring the balance agent-host just with anti-retroviral due to the cost, to the effectiveness, to the toxicity, is becoming more and more

necessary the development of viable alternatives. In this point the development of the immunotherapic agents is inserted, whose function in infectology is to help restore the balance agent-host, allowing the cure. However those agents don't act destroying the infectious agent directly, however they give conditions to the own organism of doing it, stimulating the responsible systems for this or correcting certain situation, which is harming their action. It is known that the immunotherapy will hereafter be able to substitute the anti-microbial therapy, however, in the current moment the association of an appropriate anti-microbial therapy, potentiated through the immune-therapy, can obtain positive results. [4]

2.1 Immunomodulators:

Concept:

It is defined as immunomodulator a drug capable to normalize a deficient, inadequate or hyperactive immunological answer, restoring this way a good operation of the defense mechanisms of the host. For instance: the macrophage cannot destroy the mycobacterium in the intracellular medium without the presence of IFNγ, cytokine that is produced by the organism. In its absence, the macrophage will serve as reservoir for the mycobacterium disseminating this way the infection. In this case the presence of IFNγ would act as immunomodulator, correcting the immunological defect.

Another example is the bacterial meningitis, that it is responsible for an intense inflammatory answer of the organism, causing neuronal lesions, sometimes irreversible, that can be moderate in the steroid presence, reducing the intensity of the inflammatory response of the host. We can observe that the

role of the drug was to stimulate a function in the first case and to discourage another one in the second. Both are considered immunomodulators. [4]

2-2 Immunotherapic agents of clinic practice:

2-2.1 Stimulating Immunomodulators:

Some studies are in process to determine the effectiveness of the thymic hormones, with the function of increasing the population of T cells, enlarging the immune answer in AIDS. Representatives of this healthy group are: Thymosin α I, thymopoietin, Thymic Humoral Factor and Cytokines, which already have great clinical application. Among them IL-2, key point of the immunological answer, presenting good results in patients who have AIDS.[5,6]

The IFN α that activates the individual antiviral cellular resistance makes the virus intracellular propagation difficult. It also delays the replication of HIV in up to 90% *in vitro*. Pincus and Wehrly studied the effect of several antiviral agents and immunomodulators recently in culture of infected CD4 cells with HIV, concluding that the most effective combination in the suppression of HIV was the zidovudine (AZT) with IFN α.[7]

IFN γ acts on the Natural Killer (NK) cells, with potent stimulator effect on the cytokines, capacity to activate macrophages and to increase the individual's immunological answer. Its main indication is in the granulomatous diseases as Hansen disease, leishmaniasis. The GM-CSF (Granulocyto-Macrophage Colony-Stimulating Factor) is a cytokine that restores the polymorph-nuclear counting in neutropenia patient, mainly post-chemotherapy, reducing the occurrence of opportunistic infections. Recent studies have demonstrated that GM-CSF has activity in the suppression of the

viral load in individuals infected by HIV, decrease of the viral resistance and increase of the CD4 counts. [8]

2-3 Chemical immunomodulators:

Imidazolone (levemisole) Thiol (N- acetylcysteine), similar of Nucleic Acids.

2-4 Monoclonal Antibodies:

They are antibodies with unique likeness, with the objective of neutralizing the antigen.

2-5 Depressing Immunomodulators:

Corticoids

2-6 Homoeopathic Immunomodulator

2-6.1 Canova

The medicaments of the homoeopathic compound CANOVA® promote a regulating action of the immunological system, through the stimulation of the macrophage. Their immunomodulating activity provokes morphologic alteration in macrophages, which start to present characteristic structure of activated cells, as slack cytoplasm, with more numerous cellular projections, rich nucleus in eucromatin and a substantial increase of the cytoplasmic volume. Besides there is redistribution of some molecules as integrin α and β1, actin filaments and receivers Fc. Physiologic alterations are detected in only 48 hours, when the macrophages showed a reduced production of TNFα. [3] (Piemonte, M. 2000).

CANOVA®, also studied in the Federal University of Pará, proved that this homoeopathic medicament is not mutagenic, doesn't present toxicity, genetoxicity or mutagenicity [9] in human lymphocytes and, as studies *in vitro*

and *in vivo*, is capable to increase the immune answer through functional and structural alterations in macrophages. [10] (data of Master's thesis) Studies at Federal University of Paraná showed that CANOVA® activates human alveolar macrophages in 24 hours, even those of patients who are extremely weakened.[10] In the State University of Rio de Janeiro, activity of the enzyme NADH-oxidize was detected in macrophages treated with CANOVA® - enzyme that characterizes activated macrophages. It was still proven, a significant reduction in the rate of parasite penetration of *Toxoplasma gondii* in macrophages treated with this medicament. (Master's thesis, October, 2001).

3 - METHOD AND CASUISTRY

Between September, 2000 and June, 2001, were selected and accompanied about 46 individuals who have HIV/AIDS, at age between 18 and 55, of both sexes, residents in Curitiba, assisted at the Clinic of Infect-logia of the Clínicas Hospital of the Federal University of Paraná, that presented diagnosis of AIDS according to the criteria of CDC (Center of Disease Control and Prevention) of 1998.

3-1 Criteria of Inclusion:

- Count of T CD4 lymphocytes < 300 cells/mm^3.
- Anti-retroviral use for 6 months.
- The patient's illustrious free consent to be included in that study.

3-2 Criteria of Exclusion:

- Presence of opportunistic infection in activity.
- Presence of Neoplasia.

- Previous use of immunomodulators or MC (CANOVA®).

3-3 Model of the Study

Controlled placebo randomized study, with the objective of studying the effectiveness of CANOVA® in patients who have AIDS, for 6 months, on anti-retroviral use with two or three drugs and prophylaxis for pneumonia P.Carinii in the cases that presented CD4 < 200 cells/mm^3. The presentations of CANOVA® were: sub-lingual drops (10 drops, 4 times a day) plus the inhalant way (4 ml, 3 times a day, for 4 minutes), using ultrasonic inhaler. The control group or placebo had the same procedure, but the flasks (with the same aspect) contained distilled water plus 0,01% of cereal alcohol. The patients were oriented to agitate the flasks of the medicaments before using them, according to the principle of Homeopathy. Neither the researcher nor the participants of the study knew the content of the flask. The anti-retroviral treatment was considered as failure or therapeutic success according to the norms of "recommendations for anti-retroviral therapy in adults and adolescents infected by HIV produced by Ministry of Health, year 2000".[11] It must be considered as therapeutic success a great reduction in their values, >= log or 90% of the viral load in the first 4 to 6 weeks, thus remaining. And the therapeutic failure is defined as the occurrence of clinical deterioration and/or worsening of the laboratorial parameters or increase of the viral load.

The clinical and laboratorial valuations were accomplished in the moment of the admission and, weekly, until completing the first 4 weeks and to proceed, monthly, until completing 6 months. Parameters of the renal function, liverwort, hematological, viral load, count of T CD4 and CD8 lymphocytes were used. Identification records were filled out where relative data to the risk

factor were collected, beginning of the disease, previous opportunistic infections and beginning of the use of anti-retroviral drugs.

3-4 Medicament in Study

Immunomodulator CANOVA ®

CANOVA® consists of a homoeopathic medicament, therefore highly diluted and dynamized, that doesn't present toxicity, suitable in the diseases where the immunological system is depressed. CANOVA® is a medicament that results from the combination of the active principles of Aconitum Napellus (Ranunculaceae) 20dH + associations.

This homoeopathic formulation is diluted in distilled water, containing in the final dilution 0,01% of cereal alcohol. The sequence of the combination of the components is essential to the action process of this medicament. The medicament is manipulated for the treatment of patients who have cancer and other immune depressing illnesses as AIDS and bottled with rigid quality control, innocuity, toxicity and pyrogen.

3-5 Laboratorial evaluation

The T CD4 and CD8 lymphocytes were appraised in the moment of the admission, 4 weeks after and in the end of the sixth month. The quantitative determination of the sub-population of the T lymphocytes was accomplished by the technique of flow cytometry by direct immunofluorescence, equipment FACSVantage® (Becton Dickinson), using antibodies CD8 FITC/CD4 RD1 / CD3 PECY5 (CYTO-STAT triCHROME® (COULTER), CD45 FITC (Becton Dickinson), in the Laboratory of Immune-phenotype of the Clinical Analyses Service of Clínicas Hospital of the Federal University of Paraná.

The determination of the viral load of each patient was accomplished in the moment of the admission, 4 weeks after and in the end of the sixth month. NASBA, Organon-Teknika® was used HIV.RNA Q (a test of amplification of the nucleic acid for quantitative determination of RNA of HIV-1 in plasma and human serum) whose detection limit is of 80 copies/ml. These exams were accomplished in the Molecular Biology Sector of the General Laboratory of Paraná State (LACEN).

3-6 Statistical analysis

The collected data in the groups that used PLACEBO plus ANTI-RETORVIRAL (P+ARV) and CANOVA plus ANTI-RETROVIRAL (MC+ARV) were analyzed by the test χ^2 followed by the test Z for standardized residues and the nonparametric test of Mann-Whitney for comparisons of the averages. The differences were considered statistically significant when $p < 0,05$.

4 - RESULTS

During the period of the study, the sample was reduced from 46 to 40 patients for the following reasons: 1) 4 patients changed the residence for another city; 2) the wife and the daughter of one of the patients died, having this patient entered in deep depression, coming to die soon afterwards; 3) one of the patients grew worse. It was necessary to have him hospitalized in the ITU, for presenting septicemia. It was chosen then to open the protocol, having been identified as belonging to the group placebo+ARV. It was added CANOVA® then in intravenous presentations, orally and nebulization and the patient developed with improvement of the septic condition. He left the ITU and persists in ambulatory attendance. After the opening of the protocol, he was removed from the study group. The 40 individuals were composed of 21 men and 19 women with average age at 37,28, who were randomized to receive (20 individuals) Canova - 10 sub-lingual drops 4 times a day and nebulization with 4 ml of Canova for 4 minutes, three times a day, or placebo (20 individuals) plus the anti-retroviral. 5 (25%) patients of the control group and 10 (50%) of the group MC presented reduction of the viral load in the first month of the treatment >90%, thus remaining. 3 (15%) patients in the control group and 6 (30%) in the group MC showed reduction of the viral load < 90%, and, 6 of those of the group MC, 50% suffered a reduction of the viral load >90% in the 6th month. The therapeutic failure happened in 9 (45%) of the patients of the control group (they increased the viral load) and 4 (20%) of the group MC.

In the end of the 6th month of treatment the therapeutic effectiveness with reduction of the viral load >90% was statistically significant with p < 0,05, presenting 13 (65%) in the group MC+ARV against 5 (25%) of the placebo + ARV (χ^2=8,46;gl=2;P=0,014). The therapeutic failure characterized

by the increase of the viral load, at superior or the same levels to the initial ones was of 45% in the control group and 20% of the group MC (tab. 2).

The statistical analyses done with the data obtained on the T CD4 and T CD8 cells didn't result in significant differences. In spite of the low rates of these cells in the two groups, just a patient (5%) of the sample treated with CANOVA® plus anti-retroviral showed a opportunistic disease (peritoneal tuberculosis). He was hospitalized, remaining part of the study, while 7 patients (35%) of the control group showed opportunistic infections, considering that, when compared to the group that took MC plus anti-retroviral, it showed statistically significant difference ($p < 0,05$). The diseases detected in the sample placebo + ARV during the period were: lung tuberculosis, seborrheic dermatitis, infections of superior aerial canals, pneumonia, intestinal infections (diarrheas), *Herpes zoster* and piodermites. (Tab. 5 and 6).

The tests that evaluated VHS (blood-sedimentation speed), TGP (transaminase glutamil-piruvic), LEU (leukocytes), HB (hemoglobin), EOS (eosinophils), TRIG (triglycerides) and HDL (lipoproteins of high density) showed significant difference among the samples that used Placebo plus anti-retroviral and Canova® plus anti-retroviral, as evidenced in the Table 4. The results in the blood count as the anemia absence, neutropenia were better in the group that used anti-retroviral plus Canova® than in the control one.

RESULTS

TABLE 1 - Characteristics of the Studied Sample

Characteristics	P+ARV n=20	MC+ARV n=20	P
Average of age	39,38 (+\-8,47)	35,8 (+\-8.47)	NS
Men	13 (65%)	8 (40%)	NS
Women	7 (35%)	12 (60%)	NS
Average CD4(/ul)	164,25 (17-419)	147,27 (21-611)	NS
Average CD8(/ul)	829,23	712,66	NS
Average RNA viral(Cop/ml)	38.408(80-800.000)	50.166(80-1.100.000)	NS
% CD4 50-100	5/20 (25%)	4/20 (20%)	NS
I.Os(Previous) %	9 (45%)	14 (70%)	S
IOs(During) %	7 (35%)	1 (5%)	S
Anti-retroviral therapy			NS
2N+1NN	4 (20%)	8 (40%)	
2N+1IP	14 (70%)	10 (50%)	
2N	2 (10%)	2 (10%)	

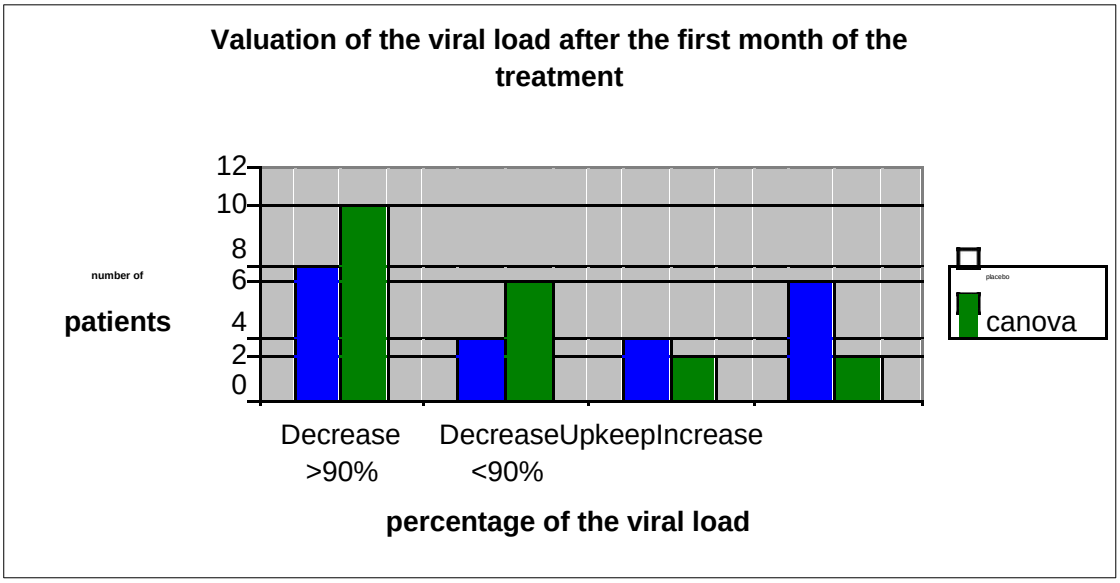
Note: NS (non-significant) S (significant) IOs (opportunistic infections) 2N+1NN (2 similar nucleosides + 1 non nucleosides) 2N+1IP (2 similar nucleosides + 1 protease inhibitor).

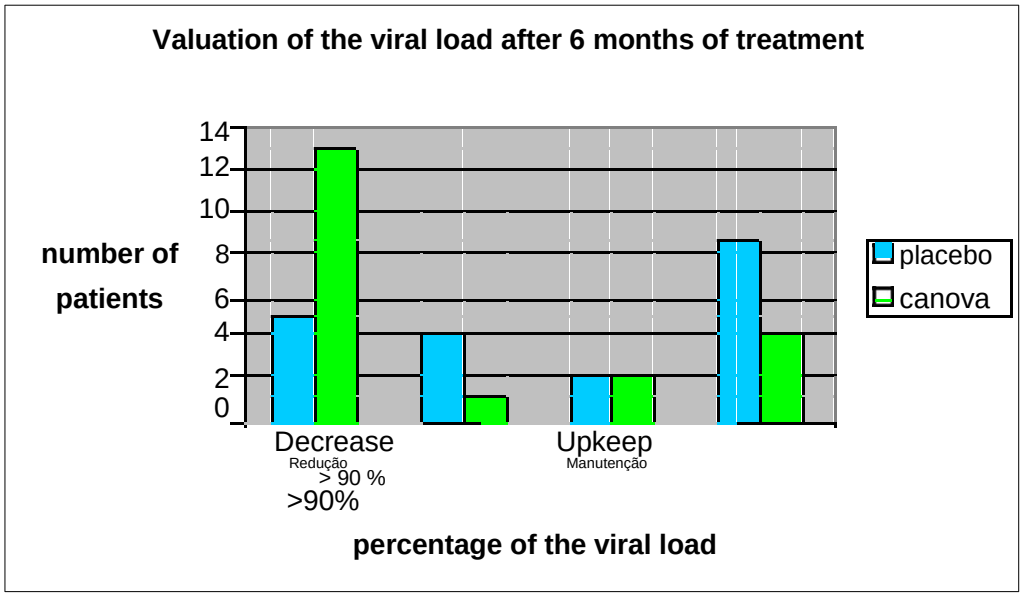
TABLE 2 - VALUATION OF THE ANSWER TO THE TREATMENT AS FOR THE REDUCTION IN PERCENTAGE OF THE VIRAL LOAD

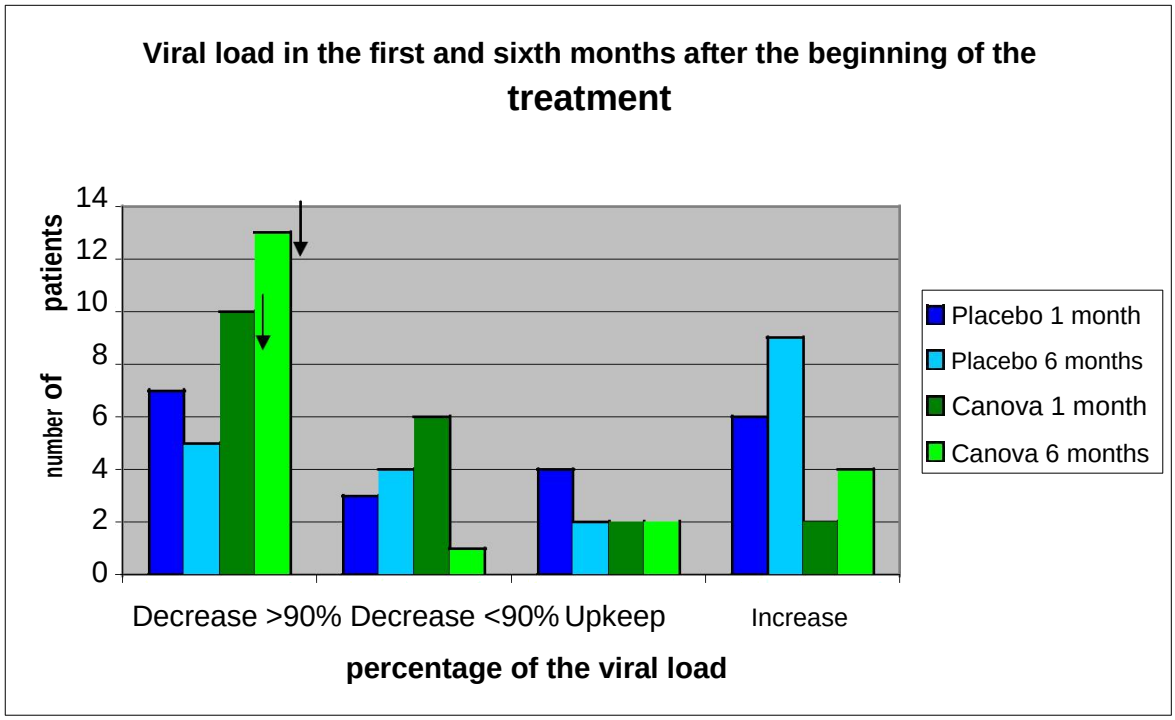
	Placebo + antiretroviral			MC + antiretroviral	
	1 month	6 months		1 month	6 months
Pct 1	94,66	94,66	Pct 1	-22,33	-18,36
Pct 2	90,71	57,14	Pct 2	68,90	90,26
Pct 3	-36,36	-19,09	Pct 3	68,27	-27,31
Pct 4	-38,46	-49,77	Pct 4	0	0
Pct 5	-16,5	-36,88	Pct 5	-15,86	61,29
Pct 6	78,9	78,9	Pct 6	0	0
Pct 7	99,84	99,84	Pct 7	96,8	96,8
Pct 8	-19,00	-26,4	Pct 8	54,1	-34,41
Pct 9	98,04	-14,66	Pct 9	99,46	99,46
Pct 10	-82,9	-17,3	Pct 10	99,3	99,88
Pct 11	0	-88,75	Pct 11	97,13	98,86
Pct 12	99,99	99,99	Pct 12	83,75	99,99
Pct 13	15,78	15,78	Pct 13	94,32	99,78
Pct 14	99	96,8	Pct 14	99,63	99,63
Pct 15	19,4	89,61	Pct 15	87,55	92,22
Pct 16	0	-20	Pct 16	49,49	-8,48
Pct 17	0	0	Pct 17	98,9	90,26
Pct 18	99,3	99,41	Pct 18	99,3	99,88
Pct 19	0	0	Pct 19	98,8	99,22
Pct 20	-84,8	-18,3	Pct 20	94,3	99,35

Blue = reduction of the viral load >90% (THERAPEUTIC SUCCESS), Green = reduction < 90%, black = maintenance and red = increase of the viral load. (it suggests therapeutic failure)

Significant difference of the reduction of the viral load exists between the beginning and the end of the treatment, among the groups of patients that used placebo + anti-retroviral and patients treated with Canova + anti-retroviral.
(test χ^2, $p < 0,05$ and test Z for standardized residues).


Valuation of the viral load after the first month of the treatment
number of
patients
12
10
8
6
4
2
0
placebo
canova
Decrease >90%
DecreaseUpkeepIncrease <90%
percentage of the viral load


Valuation of the viral load after 6 months of treatment
number of
patients
14
12
10
8
6
4
2
0
placebo
canova
Decrease
Redução
> 90 %
>90%
Upkeep
Manutenção
percentage of the viral load



Representative graphs of the distribution of the percentages of viral load, in order to have a better visualization of the differences in the results obtained in the two samples of patients (test χ^2 and test Z for standardized residues, p < 0,0 5).

TABLE 4 - Comparative analysis among the results of laboratorial valuations of the group Placebo + anti-retroviral and Canova + anti-retroviral

	P+ARV				MC+ARV					
			Limit of Trust				Limit of Trust			
	N	Average	-95%	95%	N	Average	-95%	95%	p	
VHS	126	43,51	34,57	52,45	124	30,20	26,36	34,04	0,008	S
CREA	127	0,72	0,70	0,74	126	0,73	0,70	0,75	0,866	NS
TGP	126	19,53	16,43	22,62	126	24,89	21,81	27,97	0,003	S
TGO	127	21,94	20,76	23,13	126	25,65	23,45	27,85	0,198	NS
LEU	168	5723,62	5447,49	5999,74	161	5409,32	5075,43	5743,20	0,010	S
HEM	169	3,80	3,70	3,91	161	3,84	3,70	3,99	0,986	NS
HT	167	39,46	38,58	40,34	161	39,33	38,77	39,88	0,068	NS
HB	169	13,66	13,17	14,15	161	13,24	13,04	13,43	0,039	S
BT	160	5,29	4,75	5,83	161	5,98	5,39	6,56	0,057	NS
EOS	160	6,04	5,27	6,80	161	4,89	3,98	5,79	0,001	S
SEG	162	50,07	47,96	52,17	161	50,11	48,15	52,07	0,874	NS
MON	153	7,38	6,97	7,79	151	7,68	7,18	8,17	0,537	NS
NEU	81	55,36	52,25	58,46	89	57,37	54,94	59,81	0,407	NS
LIN	161	30,40	28,45	32,34	161	29,42	27,91	30,92	0,764	NS
COLES	69	194,45	184,69	204,20	66	203,36	187,86	218,87	0,281	NS
TRIG	69	201,65	166,73	236,58	66	134,45	118,17	150,74	0,001	S
LDL	26	156,34	129,76	182,92	15	124,93	87,52	162,34	0,074	NS
HDL	67	42,30	31,36	53,24	66	47,64	39,16	56,12	0,000	S
GLI	73	99,40	92,08	106,71	66	93,83	90,76	96,91	0,152	NS

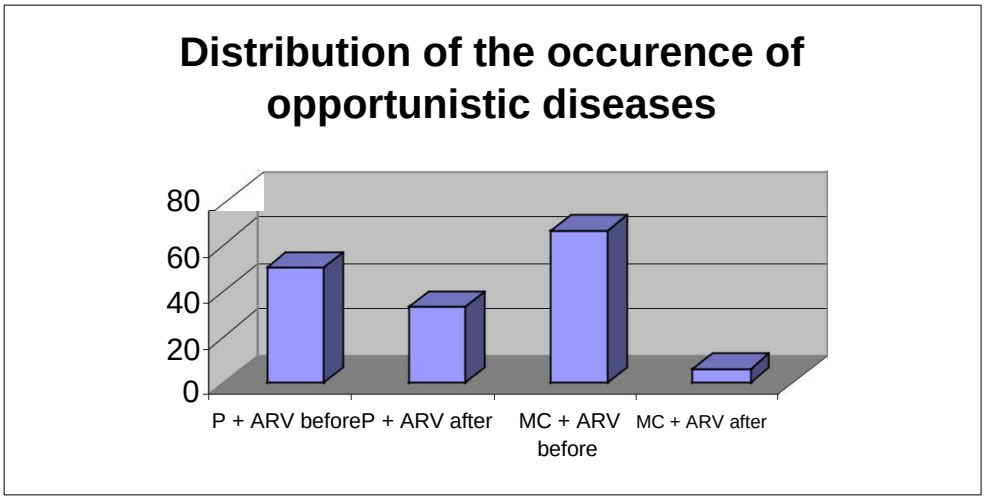
Non-parametric test for comparisons of Mann-Whitney averages.

TABLE 5 - Analysis of the present opportunistic diseases in the patients of the samples, before and after they enter the protocol

	With disease	Without disease
P+ARV before	9	11
P+ARV after	7	13
MC+ARV before	14	6
MC+ARV after	1	19

In percentage

	With disease
P+ARV before	45%
P+ARV after	35%
MC+ARV before	70%
MC+ARV after	5%



Note: P+ARV = placebo plus anti-retroviral, MC+ARV = Canova plus anti-retroviral, before and after they enter in protocol.

Decrease of occurrence of opportunistic diseases among the patients of the group that was treated with Canova plus anti-retroviral compared with the one that used Placebo plus anti-retroviral is significant (test χ^2, p 0,0 5).

TABLE 6 - Frequency of opportunistic infections (IOs) type and hospitalizations

Clinical event	Placebo+AR	MC+AR	P
IOs during study	7 (20) 35%	1 (20) 5%	0,05
History of ´previous IOs	9 (20) 45%	14 (20) 70%	0,05
CD4 50-100 cells	5 (20) 25%	4 (20) 25%	NS
Category of IOs			0,05
Ganglionic tuberculosis	2	1*	
Pneumonia P.carinii	1		
Herpes zoster	2		
Infectious diarrhea	1		
No infection during the study	13(65%)	19(95%)	0,05

*** Hospitalized patient**

5 - DISCUSSION

This is the first clinical study of a homoeopathic medicament accomplished in AIDS that demonstrated therapeutic effectiveness when reducing the plasmatic levels of viral RNA in patients with AIDS on anti-retroviral use. The viral load decreased < 80 copies in 65% of the patients treated with Canova plus anti-retroviral when compared with the control group that presented 25% of the reduction of the viremia with the use of the anti-retroviral. It is known that these agents produce a fast decrease of the viral load during the first 4 weeks up to 10 weeks of treatment, following by an increase of plasmatic viral RNA, due to the development of resistant phenotype or the lack of adherence to treatment.[12] In contrast, CANOVA® produced a decrease of the viral load and maintained the answer sustained during its use. Studies *in vitro* have demonstrated that CANOVA® can influence in the decrease of the production of Tumor Necrosis Factor α by thermacrophages.[3] It is known that this cytokine plays an important role in the viral replication and that high levels of TNFα are associated to the progress of the disease.[13,14] On the other hand the patients who have AIDS have several infections with multiple agents, not just with the virus HIV, what elevates the levels of TNFα . Some authors consider the dosage of the levels of TNFα as a marker of prognostic of the disease. [15] The statistical analyses done with the data obtained on the TCD4 and TCD8 cells didn't result in significant differences. In spite of the low rates of these cells in the two groups, only 1 patient (5%) of the sample treated with the CANOVA® plus anti-retroviral presented opportunistic disease (peritoneal tuberculosis) and was hospitalized, remaining in the study. While 7 patients (35%) of the control group presented opportunistic infections, considering that, when compared to the study group, it showed statistically significant

difference. The opportunistic diseases that were detected in the sample placebo plus anti-retroviral during the period were: lung tuberculosis, pneumonia, intestinal infections, candidiasis, Herpes zoster. CANOVA® seems to offer a better protection in the prevention of the opportunistic infections, still considering the number of previous opportunistic infections that was of 70% in the group CANOVA® against 45% of the group placebo. Seventy percent of the patients of the control group used therapeutic outline with three drugs (2N+1IP) while 50% of the patients of CANOVA's group used the same outline (2N+1IP) considered as associations of better results in terms of therapeutic effectiveness when compared with other associations.[16] The therapeutic failure characterized by the increase of the viral load at superior or the same levels to the initial ones was larger in the placebo group with 45% while in the MC group it was 20%. The alterations of the transaminases, cholesterol, triglycerides, HDL and anemia, were more evident in the control group when compared with the group that used CANOVA® with statistically significant results, showing a smaller toxicity of the anti-retroviral. This study demonstrated that the homoeopathic medicament Canova doesn't have side effects, or toxicity, that possesses immunomodulator action and could be a new natural biological agent for the treatment of HIV. However studies will be necessary to appraise and understand its action mechanism better.

6 - CONCLUSIONS

1. **THE REDUCTION OF THE VIRAL LOAD** among the patients of the group that used immunomodulator CANOVA® along with the anti-retroviral, within the parameters that are considered **THERAPEUTIC SUCCESS** by Ministry of Health was significant.

2. **THE DECREASE OF THE OCCURRENCE OF OPPORTUNISTIC DISEASES** among the patients of the group that used immunomodulator CANOVA® plus the conventional medication was significant.

3. **THE DECREASE OF THE ANTI-RETROVIRAL TOXICITY** was shown significant through the decrease of **TGP, Triglycerides, HDL and hemoglobin** rates**,** when used with immunomodulator CANOVA®.

4. Due to the decrease of the viral load, opportunistic diseases, toxicity effects and general organic improvement, there was an **IMPROVEMENT IN THE LIFE QUALITY**.

GENERAL CONCLUSION

The present placebo-controlled randomized clinical study demonstrated the effectiveness of the medicament called immunomodulator CANOVA® in the therapeutics of patients who have AIDS, on anti-retroviral use. However more studies should be performed for a better knowledge of the medicament.

"The History will judge us harshly if we fail to of the alone now, and right now."

Nelson Mandela

BIBLIOGRAPHICAL REFERENCES

1. Finzi D, Hermankova M, Pierson T, et al**. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science,** 1997. 278: 1295-1300**.**

.

2. Cohen. O; Weissman D.; Fauci, A; **Immunopathogenesis of HIV Infection. Fundamental Immunology**.. Raven. Lippincott, 1999.

3. Piemonte MR & Buchi DF. – **Analyses of Il-2, IFN** g **and TNF** a **production,** a**5,** b**1 integrins and actin filament distribution in peritoneal mouse macrophages treated with na homeopathic medicament,** J Submicro Cytrolpathol, in press, 2001.

4. Finger E.; Schinberg M [a], **Agentes Immunoterápicos, Tratado de Infectologia ,** S. Paulo, Atheneu, 1996

5. Katlama C, Duvivier C, Chouquet C, et all. ILSTIM (ANRS 082) **– A randomized comparative open-label study of Interleikin w (IL-2) in patients with CD4 > 200/mm^3 despite effective HAART. Program and abstracts of the 7th Conference on Retroviruses and Opportunistic Infections.** January 30- February 2, 2000; San Francisco, Calif. Abstract 543.

6. Kovacs JA, Vogel S, Albert JM. **Controlled trial of interlerikin-2 infusion in patients infected with the human immunodeficiency virus. N Engl J Med** 1996, 335: 1350-6.

7. Pincus S H & Wehrly K. **AZT demonstrates Anti-HIV-1 activity in persistently infected cell lines: Implicatins for combination Chemotherapy and Immunotherapy. J Infect. Dis.** 162: 1233, 1990**.**

8. Brite C, Badaro R, Sasaki, MGM, et al. **A Randomized, Placebo-Controlled Trial of Granulcyte-Macrophage Colony-Stimulating Factor and Nucheoside Analogue Therapy in AIDS. J. Infec. Dis,** 2000;182:1531-5.

9. Seligmann, I.C., Lima, P.D.L., Bahia, M. O., Khayat, A . S., Bushi, D. F. & Burbano, R. R. **– Departamento de Biologia do CCB- Universidade Federal do Pará. Departammento de Patologia do CCB – UFPa. Departamento de Biologia Celular do SCB-UFPr. Ausência de efeitos genotóxicos do composto medicamentoso Método Canova em linfócitos humanos estimulados pela fitohemaglutinina. 46º Congresso Nacional de Genética, setembro de 2000, Águas de Lindóia/SP. Genetics and Molecular Biology,** vol. 23- 3 – supplement

10. Azambuja A P; Telles G. ; Buchi, DF.; **Efeito Método Canova em Macrófagos Alveolares Humanos; Departamento de Biologia Celular SCB-UFPR;** 2000.

11. Recomendações para Terapia Anti-retroviral em Adultos e Adolescentes infectados pelo HIV-2000 MS.

12. UIP**,** DE**. Perspectivas no Tratamento da AIDS. JBM**, 2000. 1: 27-30**.**

13. Juahani Lähdevirta, M.D., C.P.J. Maury, M.D., Anna-Maija Teppo, M.S., Heikki Repo, M.D., Helsinki Finland**. Elevated Levels of Circulating Cachectin / Tumor Necrosis Factor in Patients with Acquired Immunodeficiency Syndrome. The American Journal of Medicine, vol**. 85, 289-291, setembro 1998.

14. Bruce Beautler, MD, Dallas, Texas. **The Presence of Cachection/Tumor Necrosis Factor in Human Disease States. The American Journal of Medicine,** september 1988, vol. 85 – 287:288.

15. Pal Aukrust, Nina-Beate Liabakk, Fredrik Müller**, Egil Lien, Terje Espevik, and Stig S. Froland. Serum Levels of Tumor Necrosis Factor-α (TNFα) and TNF Receptors in Human Immunodeficiency Virus Type 1 Infection – Correlations to Clinical, Immunologic, and Virologic Parameters. The Journal of Infections Diseases,** 1994; 169:420-4. University of Chicago**.**

16. Bhiva Writing Commitee on Behalf of the Bhiva Executive Committee Bristlsh HIV Association (BHIVA). **Guidelines for the treatment of HIV. Infected Adults with Anti-retroviral therapy**. January 28, 2000.

17. Azevedo, N.L.; Borges, S.G.; Stumbo, C.A.; Maia, S.F.; **Estudo da interação entre macrófagos tratados pelo Método Canova e *Toxoplasma gondii.*** Dept° de Histologia e Embriologia da Universidade Estadual do Rio de Janeiro. Tese de Mestrado, outubro/2001.

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