

Therapeutic Use of Guazulma Extract *Ulmifolia lam* of Northern Brazil

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ABSTRACT

*This study aims to analyze the therapeutic use of Guazulma *ulmifolia* Lam extract as an AIDS treatment, describing the management of treatment performed in a clinical report. This work was a literature review. The case reported was a 35-year-old Mozambican, diagnosed with HIV virus in 2008, In March of 2017 she started using Guazulma *ulmifolia lam* extract for 30 days, and has since received successive negative HIV test results. It was concluded that the efficacy of Guazulma has been increasingly proven for the treatment of AIDS, with the advantages being a natural remedy, without any side effects, and there is no ethical-moral impediment to be applied in infected population.*

Keywords

Guazulma *ulmifolia*, HIV, Paciente soropositivo.

Introduction

Acquired immunodeficiency syndrome (AIDS) was identified in 1980 in a high number of patients showing "Kaposi's Sarcoma", Pneumocystis jirovesi pneumonia and immune system impairment. AIDS is caused by the HIV virus to reproduce in the human body in TCD4 + lymphocytes, which makes the body vulnerable to infection by opportunistic diseases [1]. HIV can take years to appear the first symptoms of AIDS, which is defined as asymptomatic phase. The period between HIV infection and the manifestation of the first symptoms depends on the State of health of the person. As in the symptomatic phase, marked the beginning of the treatment with antiretroviral drugs. Front of the exposed, this study aims to analyze the therapeutic use of Guazulma extract *ulmifolia Lam* as AIDS treatment, describing the management of treatment in a clinical report. It's worth mentioning that the study also was based on the published literature on the subject.

Literature Review**Sexually Transmitted Infections (ITS)**

Sexually transmitted infections is so contagious and thus acquired. In your vast majority, the ITS are difficult to detect, since there are few symptoms, and often presents itself so asymptomatic. Among the most common ITSS are chlamydia, gonorrhea, syphilis, genital herpes, human papilloma virus-HPV and AIDS. HPV also known

as genital wart, Crest horse, cockscomb or Condyloma acuminata is set to a universal distribution, disease occurred sexually and infection by contaminated food [2]. It is understood by herpes simplex virus – HSV – Herpesvirus hominis, a virus transmitted by sexual contact, by direct contact with lesions or contaminated objects. After transmitting a variation of four to twelve days for the appearance of the first symptoms [2].

Sexually transmitted infections, AIDS is more relevant because it is a lethal disease, which was not found until the present time cure, but the treatment had a considerable advance [3]. It is worth mentioning that in Brazil the AIDS has evolved from lethal disease in the short term, characterized by physical suffering, verifiable disease treatment. In contemporary times, is already disclosed to the media as a chronic disease that requires regular follow-up treatment, and with that a prolongation of life. In this sense, emerges another profile of people affected by HIV/AIDS, which often remain in activities, maintaining social relationships commonplace, without the striking stigmatisation by society [4]. For Turgeon one can say that AIDS consists of a virus able to damage the human immune system, leaving him susceptible to acquiring diseases, and consider that simple diseases can take the individual HIV carrier to death, since your immune system is compromised.

The Declaration of commitment on HIV/AIDS of the United Nations has created strategies to support both for the individual infected with HIV, and for your family, including the distribution of free

drugs since 1988 [5]. In the second phase, the Federal Government implemented the national program for STD and AIDS control at the national level, involving 22 States from the Federal District, as a response to the growing epidemic. The third stage was a period of grave institutional crisis in the federal Government, which has disrupted the National Program of DTS and AIDS, having consequences would be for the State programs, which fragilizaram on the lack of national perspective. Postures often misguided National Programme team of STD and AIDS control determined disruption of relations with some international bodies [6]. The fourth phase was the restructuring and reorganization of the DTS and AIDS control. Established a new Ministry and so began the process of restructuring and expansion of national responses. Were hired coach and drafted a strategic plan for this new phase, being still being restored relations with States, municipalities and NGOs and resumed international relations [6]. In Brazil, the first cases of AIDS in women took place in 1983 and in 1985 were reported the first cases of perinatal transmission that can occur or postpartum ultraútero by breastfeeding [7].

Epidemiology

The estimate for the cases of STIS per year in Brazil, according to the who, in 2003, was of 937,000 cases of syphilis, gonorrhoea, 1,967,200 1,542,800 for cases of chlamydia, 640,900 cases of genital herpes and HPV cases 685,400 (Gold et al., 2006). The Brazil for being a country with extensive geographical and multicultural area cannot observe homogeneously pipes adopted in the prevention and treatment of AIDS. The pattern of STD/AIDS epidemic also had changes over time, as the reduction of morbidity and mortality on the use of ARVs available free of charge in public health services (Golden, et al., 2006). The United Nations report that most young people don't have the slightest notion of how it's sexually transmitted infections, in which 50% of young people in global terms between 15 and 24 years do not have a correct understanding of how to get the AIDS virus [8]. In 2008, the number of cases in Brazil notified the Ministry of health was 506,499, of these 52,354 occurred between the ages of 13 to 24 years (Dessunti; Kings, 2012). The number of hospitalizations is stable, but the therapeutic adhesion already reaches 75%. For this reason, the Brazilian Government seeks changes in drug legislation in order to reduce costs [9]. In the case of vertical transmission rate, there has been a reduction in the entire country, but with regional variations, because of social, economic, cultural differences and schooling, requiring specific programmes to meet these diversities [9]. Promote the reduction of infection of these diseases is a priority of the National STD and AIDS Programme, which has used as a educational and informative process in order to minimize this problem. Highlights that it is necessary to adopt public policies geared to social factors and vulnerability, enabling prevention strategies and programmatic actions [10].

Aids in Brazil

Paker (1999) reports that the Acquired Immunodeficiency Syndrome (AIDS), was discovered in 1981 in the United States, when the first cases were reported in male homosexuals. In 1983, was identified the causative agent, the virus called Human

Imundeficiência virus (HIV). After HIV infection, gradually decrease the number and activity of lymphocytes (CD4), mainly of compromised cellular immunity, and AIDS a late manifestation and advanced this process, that is, there is a difference between be a carrier of the HIV virus and being sick of AIDS. Tchaicka (2013) reports that since the appearance of the first cases and the identification of the HIV virus, AIDS is configured with one of the major public health problems in the world, transmission: occurs through sexual intercourse, shared use of contaminated syringes, transfusion of infected blood and HIV positive mother to fetus (vertical transmission) at time of delivery or breastfeeding the baby. Second Parker [6] in addition to the actions in the field of health, the HIV/AIDS pandemic is now a central issue in the fight for human rights, making it much more than the spread of an infectious disease, high lethality and high social cost. In the complex process of spread of HIV, proliferates a 'epidemic' meanings, which leads to constant combating stigma and discrimination that are obstacles to the commitment to prevention and care. The AIDS pandemic has your highest concentration in developing countries, causing a great socio-economic impact, especially in countries of Sub-Saharan Africa, Eastern Europe, Central America and Asia, where, especially the productive age population and reproductive health, is strongly affected. As projection of the World Health Organization (who), 70 million of lives will be affected for the next 20 years if it is not implemented an effective action at the global level to halt the spread of HIV/AIDS. In 1986, with the creation of the special program of AIDS of who started the official responses to the fight against AIDS. These past two decades, Governments and organized civil society have committed technical and social mobilization efforts to build responses for the control of AIDS. Toledo (2012) declares that for the Brazilians, the news of the spread of the AIDS virus, arrived labeled with punitive and stigmatizing, terrorizing the population, once, sexuality and sex are treated by a large number of the population, as taboo or sin. The media made public a fact that in a short time becomes one of the major problems of challenge to public health. To spread the disease, the press also released and gave emphasis to reactions of prejudice, fear and moralism. In early 1980 the media announced the celebrities infected by HIV, as Cazusa and Renato Russo, but to transmit this information, contributed in the construction of prejudice around AIDS, when pointing the same as homosexuals, promiscuous and rebels, with the exception of the sociologist Betinho seen as victim of the disease by being a hemophilic, became a martyr of the epidemic.

Para Galvão [11]

The media, in the initial years of the epidemic, was instrumental in presenting to the public that new disease. And in the case of Brazil, importing the American model of explanation of sexual transmission and HIV infection, being victims, children, those infected by blood transfusion and women (victims of their partners) and the culprits, those who infected sexually or by injection drug use. There is a subtle violence, generated by the prejudices, that promises a homosexual is being punished for a fault that loads. It's not a sick; It is a relapse. To fight AIDS were established guidelines to be followed, which are laid out in articles 1 to 6, of the Ordinance

No. 236, among which are to adopt the guidelines for the control of AIDS and that with this setting, as well as coordinate, at the national level, actions for the epidemiological monitoring of AIDS or AIDS.

Policy to the ITS /AIDS

As conquest can be stressed that with the creation of the specific policy of IST/AIDS the success in the fight against the AIDS epidemic in Brazil, was the reduction in mortality and morbidity from 1996 [11]. With the creation of law n° 9,313/96 (BRAZIL, 1996) "establishing the gratuitousness of antiretroviral drugs under the SUS and also to the expansion of free access to diagnostic HIV and AIDS treatment" [13]. AIDS is a chronic disease that causes viral replication and consequently the fall of the immune system, but can be treated with the introduction of antiretroviral therapy; although your use bring benefits there are some adverse effects to the use of antiretroviral drugs [13]. It should be noted that the side effects in the use of anti-retroviral therapy consist of:

Physical deterioration: dyslipidemias (which consists in the increase of cholesterol and triglycerides), lipodystrophy (changes in the distribution of body fat), peripheral resistance to insulin (resulting in increased blood sugar) and acidosis metabolic (for mitochondrial dysfunction) [12].

Before the foregoing we perceive the adverse effects caused by the use of "cocktail" (a term popularly used for the use of two or three antiretroviral drugs associated with, which is characterised by the taking of a large number of capsules or tablets per day). In General, the combination is made with two reverse transcriptase inhibitors and protease inhibitor. The indication of the number and your dosage medications should always be determined by the physician according to the condition, viral load and CD4 cell number of the patient for the treatment of HIV/AIDS, which requires more frequent visits of patients to services specialized care.

To the person who receives the diagnosis of HIV positive, happens to be traumatic, Yes, even know that the disease is associated with prejudices and discrimination on the part of the alien society scientific knowledge about the disease, it causes the individual to know the diagnosis has some resistance in initiating the treatment, because it is a chronic disease and will have to live with it. At the beginning of treatment it was noted that there was a significant decline in the number of deaths among AIDS patients, which underscores the importance of adherence and treatment continuity as well as the strengthening of public health policies, in particular the IST/AIDS-specific.

Use of Medicinal Plants

As Felipe et al. [14], research on the use of medicinal plants have been significant in recent times, being intensified your use in the treatment and cure of diseases. During millennia the humanity uses medicinal plants for the treatment, cure and prevention of disease, being this knowledge passed from generation to generation, to spread with the birth of modern civilizations. Glehn and Rao [15] referred to in your job for the existence of treaties are evidence of the use of medicinal plants in human health with 1700 BC dates People Romans, Greeks, Egyptians and Chinese, have some

of those treaties that include descriptions of the plants and your use. According to Raj (2004), the use of medicinal plants has been widely used in the treatment and prevention of disease for the population in recent years. During millennia the indigenous peoples from all over the world have been using plants for medicinal purposes and these skills have been passed down for generations to diffuse with the emergence of modern civilizations. In search of new ways to treat diseases, studies on the therapeutic properties of medicinal plants have been developed by the scientific community. These studies are conducted in many countries; and in Brazil, by possessing a rich and varied flora, beyond the culture of use of these plants as antifungal, anti-inflammatory, antibacterial, among others, has much to contribute in this direction [16]. Silva [8] says that the knowledge passed on from generation to generation in the traditional communities, therapeutic resources of plants found in your natural environment can be an important instrument, for example, for the pharmaceutical industry in development of new drugs. For medical and commercial vested interest, the global scientific community has been working to establish the popular use of medicinal plants, thus contributing to the safe use and the discovery of new drugs. According to Ganta (2016) information about plants, people and culture, associated with an experimental record of use, with proven biological effect are analyzed and studied by disciplines such as ethnobotany and Ethnopharmacology.

Guazulma Ulmifolia

Lopes et al. [17] report that the determination of the stability of pharmaceutical products is based on concern for public health. Measure the chemical stability of extracts is considered a challenge by virtue of your complexity, to include hundreds of compounds. A *Guazulma ulmifolia* Lam (Sterculiaceae), known as Mutamba is a tropical plant used in Gen. report for treatment of Burns, inflammations and alopecia. However, no studies on the stability of the constituents of the dried extracts of *g. ulmifolia*. The importance on the stability of the components in the extract of *g. ulmifolia* is important, since the pharmacological properties depend on the chemical feasibility of extract [17]. Gouveia [18] in your article aimed to identify the effectiveness of the tannin of *Guazulma ulmifolia*, viral replication inhibitor in evidentiary examinations tests for HIV. Performed in vitro tests and retests in vitro for HIV, with inhibition of HIV 100% in bovine lymphocytes cells without cytotoxicity, in which Elisa tests were carried out for HIV. *Guazulma extract ulmifolia* was used for 30 consecutive days. The results showed that the patient was diagnosed with HIV L.T. on day 14 October 2014, with Elisa test. The load test held on 10 November 2014 resulted 254,924 copies with cd4 598 [18]. Viral load test on 29 January 2015 with 370 612 copies with cd4, viral load test 09/04/2015 with 42 copies, 861 cd4, viral load test 30/07/2015 with 40 copies, with cd4 < 766, viral load 12/11/2015 not detected, with 798 cd4, viral load test 08/03/2016 with viral load, cd4, 822 40 < conducted test on 15/09/2016 day number 18312, Elisa enzyme immunoassay method (immnocomb-bispot) with two indeterminate samples and the third sample being released as inconclusive for HIV. With this, the author concluded that the tannin extracted from *Guazulma ulmifolia* was effective in inhibiting HIV in 100%, in retest in vitro and in patient L.T [18].

Berenquer et al. [19] explain that a *Guazuma ulmifolia* Lam. is a member of the malvaceae (or Sterculiaceae) family with antioxidant, antimicrobial and antihypertensive drugs company. Most research focuses on the bark of this plant by the high concentration of Proanthocyanidins antioxidant, however its flowers and leaves can be used as a remedy for renal, gastrointestinal diseases, fever and diabetes. Magos et al. [20] investigated the cardiovascular activity in vivo and in vitro of a split family procianidina from the bark of the *Guazulma ulmifolia*, used as antihypertensive agent. The authors concluded that a shell of *Guazulma* has reacionadas to antihypertensive drugs company *ulmifolia* endothelium, where the TSA is involved nitric.

Materials and Methodology

This work was a literature review, which according to Gil, is developed from material already prepared, consisting mainly of books and scientific articles. According to Markoni and Lakatos [17], the bibliographical research consists of eight phases, which:

- a) determination of objectives;
- b) work plan;
- c) identification of the sources;
- d) location of the sources and obtaining the material;
- e) reading the material;
- f) taking notes;
- g) production records.
- h) writing the work.

The same way it preceded this research. The bibliographic survey occurred in secondary sources in the virtual health library (VHL) and included the articles indexed in the library of articles SciELO (Scientific Electronic Library Online). The criteria for inclusion of articles defined were: be full research article, be available electronically, be published in Portuguese, English language publication date between 2007 and 2017, i.e. published in the last 10 years and as exclusion criteria: repeated articles in the database mentioned above. In the survey conducted with the keywords "HIV" and "Guazulma" in the criteria of *Ulmifolia* research cited. This happened in December 2017. After the selection of the works that followed the criteria of inclusion, the selected material was analyzed and the results were discussed for the better understanding of the reader.

Ethical aspects

The confidentiality of participants will be kept as recommendations of Leopardi [22], which States that ethical legal procedures include the ethical care necessary to preserve secrecy about data sources and on the approval of the process where will carry out the research. It will therefore be guaranteed for all participants the right to confidentiality of information obtained (anonymity) and fairness (principle of Justice) so that any risks or benefits are shared equally, without any embarrassment or biased treatment of people who give up to participate in the survey at any time of your implementation, as well as ensuring access of participants to the researcher for clarification of any doubts and/or complications. With regard to the principle of autonomy, will be clarified that the participation of the subjects is voluntary, with no damage to your

person or injurious treatment if you do not accept provide some information, or even want to stop your participation in research. Ethical guidelines also will appear in an informed consent (TFCC), which the participants will sign as a way of agreeing with the study.

- This study, although it is not a laboratory experiment, involves human beings, and so require special attention with regard to ethical issues, in accordance with the provisions of resolution 196/96 to research with human beings. In this sense, it is of fundamental importance to make it clear in this project:
- The participants will only be considered to be searched when you sign the consent form that accept to participate in the survey, without any act of coercion for this;
- The consent form aims to enlighten the participants about the scientific and social relevance of research; What are the goals, what your duration; What are the responsibilities of the researchers; What are the possible risks and; What are the expected social benefits in order to obtain your free consent to participate as researched;

The subjects of the research are not identified by official names, as well as your information will be kept in one piece, whereas their testimony only makes sense as part of the global set of information; The subject directly and/or indirectly involved in the research process and other people interested in this study, may have access to your conclusive documents, which should be forwarded to the coordination of educational institution. From this, the project proposal will then be submitted to the Committee of ethics in research (CONEP) in compliance with the guidelines established by resolution 466/12 of the National Council of health-CNS that addresses the research involving human beings, characterized as a search that individually or collectively, involve, directly or indirectly, in your entirety or from them, including the management of information or materials.

Clinical Case Report

N.j. B, 35-year-old Mozambique born in Quelimane, Mozambique and resident in Stuttgart, Germany. The patient was diagnosed HIV virus positive in 2008, however, began treatment with retrovirales (art) only in 2014. She started taking *Guazulma* *Iam ulmifolia* extract in March of 2017. On April 10 of 2017, the patient N.J.B performed HIV test pcr quantitative, obtaining as a result 70 copies per ml and positive for HIV 1. In may of 2017, the patient performed the same test again, featuring 29 copies per ml, being positive for HIV 1. In September of 2017, HIV1-quantitative pcr, being negative for HIV 1 and quantitative PCR negative. The patient held quick test for HIV, giving negative. In October of 2017, the patient performed new HIV test 1-quantitative PCR, resulting in negative for HIV 1 and quantitative PCR negative.

Discussion

The case considered in this study is a disease characterized by severe dysfunction of the immune system infected with the human immunodeficiency virus-HIV. In the case described were considered the background of the results of the patient, which according to Gouveia [18] for the diagnosis of HIV-the techniques are used to quantify antibodies, genetic material and direct virus isolation. Detect-if antibodies by serology in average period of 6 to

12 weeks after initial infection. GSM monitoring with the patient was similar to those cited by Gouveia [18], that during eight months of treatment, performed specific tests of PCR, as blood tests that detect viral load.

How to test for diagnosis of HIV, Machado e Costa (1999, p. 139) describe:

The most direct laboratory method to detect HIV-1 in patients, is the culture of the virus through the blood mononuclear cells, plasma or ganglion cells, with stimulation of Peripheral Blood Mononuclear Cells-PBMCs non-infected donors. The culture is read as positive by the formation of (i.e., giant cells, resulting from a merger cell-cell), detection of reverse transcriptase activity or presence of p24 antigen in the supernatant of culture, these events that translate viral replication. This method has a high cost, require a team of trained technicians, isolation and safety laboratory conditions (confinement PII or PIII). This technique presents reproducibility defects linked to the variability of the mononuclear cells from donors. Many of these techniques are based on the detection of antibody response against the HIV, more than in the detection of the virus itself.

In this case, the patient was guided by her doctor in Germany to suspend the anti-retroviral therapy-A, and repeating every three months the Elisa test for HIV, antiretroviral being described by Cosi et al. [23] as virus reverse transcriptase inhibitors to prevent and alter the genetic code in DNA-RNA to make use of Guazulma, which in your time *ulmifolia* acts as George (2014), on reverse transcriptase inhibition, preventing the reapplication viral. After using *Guazulma ulmifolia*, the patient presented successive negative HIV test results, meaning that the virus stopped the multiplication in the patient's body.

Conclusion

The case reported in this study and found to support this research demonstrated the effectiveness of *Guazulma* has been increasingly proven to AIDS treatment, with the benefits being a natural remedy, without side effects, there is no ethical-moral impediment to be applied in the infected population.

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TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O (A) Sr (a). Natalia Joaquim Belua.....está sendo consultado (a) no sentido de autorizar a utilização de dados clínicos, laboratoriais e lâminas histológicas de seu caso clínico/cirúrgico e documentação radiológica que se encontram em sua ficha de prontuário médico, para apresentação do mesmo em encontro médico científico e publicação do caso em revista científica como "Relato de caso". Nosso objetivo é discutir as características de sua patologia em meio científico, em função das particularidades de apresentação de sua doença e metodologia de diagnóstico. A sua autorização é voluntária e a recusa em autorizar não acarretará qualquer penalidade ou modificação na forma em que é atendido (a) pelos médicos assistentes e pesquisadores. Os pesquisadores irão tratar a sua identidade com padrões profissionais de sigilo. O relato do caso estará à sua disposição quando finalizado. Seu nome ou o material que indique sua participação não será liberado sem a sua permissão. O (A) Sr (a). não será identificado (a) em nenhuma publicação. Este termo de consentimento encontra-se impresso em duas vias, sendo que uma cópia será arquivada pelo pesquisador responsável, e a outra será fornecida ao (a) Sr (a).

Eu, Natália Joaquim Belua _____, portador (a) do documento de Identidade 13AFCG322 fui informado (a) a respeito do objetivo deste estudo, de maneira clara e detalhada e esclareci minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações. Declaro que autorizo a utilização de dados clínico-laboratoriais de meu caso. Recebi uma cópia deste termo de consentimento livre e esclarecido e me foi dada a oportunidade de ler e esclarecer as minhas dúvidas.

stuttgart 17 de Dezembro de 2017

NomeAssinatura participanteData
Natalia Belua

NomeAssinatura pesquisador Data
Paulo Antônio R. Eyzenski

NomeAssinatura testemunhaData
Jo. C. das Santos

Labcode: 0611164769 Ext-ID: Auftr.Nr: FA 71934550 Seite: 1/2

Patient: BELUA, NATALIA JOAQUIM Entnahme: 10.05.2017 Kasse: AOK Syntab MZ
Geb.: 24.12.1982 Eingang: 10.05.2017 13:50 Abrechnung: KV Max-Lang-Str. 58
Geschlecht: W Ausgang: 12.05.2017 18:20 Kennz: 32021 70771 Leinfelden
Klinische Angaben: Asymptomatische HIV-Infektion (Humane Immundefizienz-
Malaria: 1x EDTA-Blut, 1x Serum/Vollblut Tel. 0711-9033-0

Untersuchung	Ergebnis / Einheit	Vorwerte	Referenzbereich	Endbefund	Methode
Grosses Blutbild:					
Leukozyten	6,6 x10 ⁹ /µl	3,3 (16.12.2016)	3,5 - 9,8		FCM
Erythrozyten	4,4 x10 ¹² /µl	4,5 (16.12.2016)	4,1 - 5,1		FCM
Hämoglobin	12,2 g/dl	11,3 (16.12.2016)	12,0 - 18,0		PHO
Hämatokrit	39,8 %	36,3 (16.12.2016)	36,0 - 48,0		RECH
MCV	90,2 fl	81,1 (16.12.2016)	80,0 - 96,0		RECH
MCH	27,7 pg	25,3 (16.12.2016)	28,0 - 33,0		RECH
MCHC	30,7 g/dl	31,2 (16.12.2016)	32,0 - 36,0		RECH
Thrombozyten	222 x10 ⁹ /µl	192 (16.12.2016)	140 - 440		FCM
Neutrophilie	48,5 %	40,1 (07.05.2016)	43 - 75		FCM
Lymphozyten	38,0 %	26,6 (07.05.2016)	16 - 45		FCM
Monozyten	3,8 %	9,6 (07.05.2016)	2,6 - 8,2		FCM
Eosinophilie	0,4 %	2,9 (07.05.2016)	0,4 - 6,6		FCM
Basophilie	0,4 %	0,6 (07.05.2016)	0,3 - 1,3		FCM
Sonstige (LUC)	2,0 %	1,4 (07.05.2016)	< 4,5		FCM
Neutrophilie abs.	3069 /µl	1700 - 6900			RECH
Lymphozyten abs.	2508 /µl	900 - 3400			RECH
Monozyten abs.	222 /µl	200 - 650			RECH
Eosinophilie abs.	251 /µl	30 - 380			RECH
Basophilie abs.	26 /µl	20 - 80			RECH
T-Lymphozyten CD3	▼ 69,0 %	40,0 (07.05.2016)	80,0 - 85,0		RECH
T-Lymph. CD3+ abs.	1480 /µl	404 (07.05.2016)	700 - 2200		FCM
T-Helfer-Ly. CD4	▼ 12,8 %	5,0 (07.05.2016)	29,0 - 58,0		RECH
T-Helfer-Ly. CD4 abs.	▼ 321 /µl	81 (07.05.2016)	450 - 1400		RECH
T-Suppressor-Ly. CD8	▲ 41,1 %	34,0 (07.05.2016)	16 - 40		RECH
T-Suppr.-Ly. CD8 abs.	▲ 1031 /µl	344 (07.05.2016)	250 - 850		RECH
T-Helf./Suppr.-Index	▼ 0,31	0,15 (07.05.2016)	1,1 - 2,3		RECH

Aufgrund der Verteilung der Lymphozyten-Subpopulationen ergeben sich deutliche Anzeichen für eine HIV-Infektion.
Die Befunde passen zur Kategorie A2-C2 (revid. CDC-Klassifikation ab 1.1.93) bzw. zum Stadium II (Lymph-Adenopathie-Syndrom bzw. AIDS related Complex, ARC): Helferzellen >200 und <500/µl; Helfer/Suppressor-Quotient <1; Suppressorzellen erhöht; Leukozyten >4000/µl.

ASAT(GOT)	20 U/l	10 - 35		PHO
ALAT(GPT)	10 U/l	10 - 35		PHO
Kreatinin (Jaffe, JMS)	13 µmol/l	< 40,0		PHO
eGFR(MDRD-Kurz)	0,69 ml/min/1,73m ²	0,49 - 0,96		PHO
Geschätzte GFR n. MDRD-Formel (Kurzform)	104 ml/min/1,73m ²			RECH

Falls GFR > 60 ml/min: Im Bereich >60 ml/min ergibt diese Abschätzung ungenau und i.d.R. zu niedrige Werte. In diesem Fall sollten bevorzugt andere Verfahren wie CystatinC-GFR oder die MDRD-GFR-Formel in der Langform (Ermittlung aus Serum-Kreatinin, -Albumin, -Hämstoff) angefordert werden (insbesondere wenn zur Dosisanpassung von Medikamenten verwendet).

Erseiner: Herm Dr. med. Werner Simon Facharzt für Allgemeinmedizin, Ötztal Str. 32, 70327 Stuttgart

13.04.2017 18:05

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Zentralinstitut für Klinische Chemie und Laboratoriumsmedizin
mit Laborpraxis am Katharinenhospital
Kriegsbergstraße 62, 70174 Stuttgart
Tel: 0711/278-34801 Fax: 0711/278-34806

Name: Belua, Natalia Joaquim
Geb.-Datum: 24.12.1982
Pat.Nummer: PAIKEY-7396265
Fallnummer: 0090106512
Erseiner: Praxis Stuttgart Dr. Zsolnai

Auftragsnummer: 60343431
Abnahme: 17.01.17 13:39

Klinische Chemie		Ergebnis	Referenzbereich
Kreatinin	0,5 - 0,9 mg/dl	0,8	
KreaCl(MDRD)	> 60 ml/min	82	
Bilirubin (ges.)	< 1,1 mg/dl	0,3	
Cholesterin	120 - 200 mg/dl	152	
Triglyceride	< 200 mg/dl	91	
AST (GOT)	< 31 U/l	17	
ALT (GPT)	< 34 U/l	17	
G-GT	< 38 U/l	18	
AP	< 130 U/l	62	
CRP	< 0,5 mg/dl		

Blutbild		Ergebnis	Referenzbereich
Hämoglobin	12 - 18 g/dl	13,2	
Hämatokrit	35 - 45 Vol%	41,6	
Erythrozyten	4,10 - 5,10 Mio/µl	4,93	
MCV	80 - 95 fl	86,1	
MCH	27 - 34 pg	27,2	
MCHC	32 - 36 g/dl	31,6	
Thrombozyten	150 - 350 Tsd/µl	218	
Leukozyten	4,00 - 10, Tsd/µl	6,28	
Mikrozytose	neg		
Hypochromasie	neg		

FACS		Ergebnis	Referenzbereich
Lymph. Typ. CD4/8	s. Bef.	8 u.	
T-Zellen (CD3)	60 - 85 %	58,4	
T-Zellen abs. (CD3)	850 - 3000/µl	1518	
Helferzellen (CD4)	35 - 65 %	12,4	
Helferzellen abs. (CD4)	450 - 1600/µl	322	
Zyto/Suppr.zellen (CD8)	17 - 35 %	42,9	
Zyto/Suppr.zellen abs. (CD8)	250 - 1000/µl	1115	
CD4/CD8 Ratio	0,8 - 2,8	0,29	

Endokrinologie/Si-offwechsel		Ergebnis	Referenzbereich
LH	0,5 - 80 U/l	14,76	
FSH	1,5 - 30 U/l	6,3	
Prolaktin	3 - 25 µg/l	1,3	
Freies T3	2,18 - 3,98 ng/l	2,19	
Freies T4	0,76 - 1,46 ng/dl	0,87	
TSH basal	0,30 - 3,50 mIU/l	1,09	

Molekularbiologie/PCR		Ergebnis	Referenzbereich
HV PCR (quant.)	neg.	Kop/ml	70

1) Ausgehend vom Plasmakreatinin nach MDRD errechnete GFR. (Deutsches Ärztebl., Jg.106, Heft 51/52, S.849ff, 21.12.03). Die Berechnung wurde nur für Pat. zw. 18 u. 70 J. validiert.

Labcode: 0611164769 Ext-ID: Auftr.Nr: FA 71934550 Seite: 1/2

Patient: BELUA, NATALIA JOAQUIM Entnahme: 28.08.2017 Kasse: AOK Syntab MZ
Geb.: 24.12.1982 Eingang: 28.08.2017 14:16 Abrechnung: KV Max-Lang-Str. 58
Geschlecht: W Ausgang: 28.08.2017 18:09 Kennz: 32021 70771 Leinfelden
Klinische Angaben: Asymptomatische HIV-Infektion (Humane Immundefizienz- unklare Dermatitis
Malaria: 1x EDTA-Blut, 1x Serum/Vollblut Tel. 0711-9033-0

Untersuchung	Ergebnis / Einheit	Vorwerte	Referenzbereich	Endbefund	Methode
Grosses Blutbild:					
Leukozyten	7,6 x10 ⁹ /µl	6,6 (10.05.2017)	3,5 - 9,8		FCM
Erythrozyten	4,7 x10 ¹² /µl	4,4 (10.05.2017)	4,1 - 5,1		FCM
Hämoglobin	12,9 g/dl	12,2 (10.05.2017)	12,0 - 18,0		PHO
Hämatokrit	41,2 %	36,3 (10.05.2017)	36,0 - 48,0		RECH
MCV	88,3 fl	90,2 (10.05.2017)	80,0 - 96,0		RECH
MCH	27,0 pg	27,7 (10.05.2017)	28,0 - 33,0		RECH
MCHC	30,6 g/dl	30,7 (10.05.2017)	32,0 - 36,0		RECH
Thrombozyten	214 x10 ⁹ /µl	222 (10.05.2017)	140 - 440		FCM
Neutrophilie	54,5 %	46,5 (10.05.2017)	43 - 75		FCM
Lymphozyten	29,2 %	38,0 (10.05.2017)	16 - 45		FCM
Monozyten	4,5 %	3,8 (10.05.2017)	2,6 - 8,2		FCM
Eosinophilie	▲ 8,7 %	9,4 (10.05.2017)	0,4 - 6,6		FCM
Basophilie	0,6 %	0,4 (10.05.2017)	0,3 - 1,3		FCM
Sonstige (LUC)	2,5 %	2,0 (10.05.2017)	< 4,5		FCM
Neutrophilie abs.	4142 /µl	3069 (10.05.2017)	1700 - 6900		RECH
Lymphozyten abs.	2219 /µl	2508 (10.05.2017)	900 - 3400		RECH
Monozyten abs.	342 /µl	251 (10.05.2017)	200 - 650		RECH
Eosinophilie abs.	▲ 861 /µl	620 (10.05.2017)	30 - 380		RECH
Basophilie abs.	46 /µl	26 (10.05.2017)	20 - 80		RECH
T-Lymphozyten CD3	68,8 %	69,0 (10.05.2017)	80,0 - 85,0		FCM
T-Lymph. CD3+ abs.	1482 /µl	1480 (10.05.2017)	700 - 2200		RECH
T-Helfer-Ly. CD4	▼ 17,4 %	12,8 (10.05.2017)	29,0 - 58,0		FCM
T-Helfer-Ly. CD4 abs.	▼ 386 /µl	321 (10.05.2017)	450 - 1400		RECH
T-Suppressor-Ly. CD8	▲ 46,0 %	41,1 (10.05.2017)	16 - 40		FCM
T-Suppr.-Ly. CD8 abs.	▲ 988 /µl	1051 (10.05.2017)	250 - 850		RECH
T-Helf./Suppr.-Index	▼ 0,39	0,31 (10.05.2017)	1,1 - 2,3		RECH

Aufgrund der Verteilung der Lymphozyten-Subpopulationen ergeben sich Anzeichen für eine HIV-Infektion. Die Befunde passen zur Kategorie A2-C2 (revid. CDC-Klassifikation ab 1.1.93) bzw. zum Stadium II (Lymph-Adenopathie-Syndrom): Helferzellen >400 und <500/µl; Helfer/Suppressor-Quotient <1; Leukozyten >4000/µl; Suppressorzellen erhöht.
Seit dem Vorbesuch stieg die Helferzellzahl an. Das Helfer/Suppressorverhältnis erhöhte sich.

ALAT(GPT)	10 U/l	10 (10.05.2017)	10 - 35	PHO
Gesamt-Eiweiß	8,20 g/dl	8,1 (10.05.2017)	6,0 - 8,2	PHO
Elw.-Elektrophorese				
Albumin	▼ 51 %		55,8 - 66,1	KEL
α1-Globulin	4,7 %		2,9 - 4,9	
α2-Globulin	9,6 %		7,1 - 11,8	
β1-Globulin	6,2 %		4,7 - 7,2	
β2-Globulin	5,4 %		3,2 - 6,5	
γ-Globulin	▲ 23,1 %		11,1 - 18,8	

Immunglobulin E ▲ 1623 kU/l < 120

HIV-1-RNA quant. negatv 2,9 (10.05.2017) < 120

HIV-1-RNA n o t detected.
The sensitivity of the assay is approx. 25 Copies/ml.
(Little Sample volume. The LOD had to be changed.)

geprüft durch: Chrysioua Seliou Erseiner: Herm Dr. med. Werner Simon Facharzt für Allgemeinmedizin, Ötztal Str. 32, 70327 Stuttgart

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Name: Belua, Natalia Joaquim
 Geb.-Datum: 24.12.1982
 Pat.Nummer: PAIKEY-73396265
 Fallnummer: 0090107757
 Einsender: Praxis 2 Stuttgart Dr. Zsolnai

Auftragsnummer: 59003293
 Abnahme: 10.10.17 13:43

Klinische Chemie		
Kreatinin	0.5 - 0.9 mg/dl	0.7
eGFR CKD-EPI	60 - 180 ml/min	113
Bilirubin (ges.)	< 1.1 mg/dl	0.2
Cholesterin	120 - 190 mg/dl	143
Triglyceride	< 200 mg/dl	101
AST (GOT)	< 31 U/l	23
ALT (GPT)	< 34 U/l	15
G-GT	< 38 U/l	15
AP	< 130 U/l	74

Blutbild		
Hämoglobin	12 - 16 g/dl	11.5
Hämatokrit	35 - 45 Vol%	35.8
Erythrozyten	4.10 - 5.10 Mio/ μ l	4.14
MCV	80 - 95 fl	86.4
MCH	27 - 34 pg	27.7
MCHC	32 - 36 g/dl	32.1
Thrombozyten	150 - 350 Tsd/ μ l	235
Leukozyten	4.00 - 10. Tsd/ μ l	6.27
Hypochromasie	neg	+

FACS		
Lymph. Typ. CD4/8	s. Bef.	su
T-Zellen (CD3)	60 - 85 %	61.4
T-Zellen abs. (CD3)	850 - 3000/ μ l	1205
Helperzellen (CD4)	35 - 65 %	17.4
Helperzellen abs. (CD4)	450 - 1600/ μ l	342
Zyto/Suppr.zellen (CD8)	17 - 35 %	40.7+
Zyto/Suppr.zellen abs. (CD8)	250 - 1000/ μ l	799
CD4/CD8 Ratio	0.8 - 2.8	0.43

Endokrinologie/Stoffwechsel		
TSH basal	0.30 - 3.50 mIU/l	1.99

Molekularbiologie/PCR		
HIV PCR (quant.)	neg. Kop/ml	su

1) Die eGFR nach der CKD-EPI-Formel ist bei Personen über 70 Jahren nur eingeschränkt validiert, aber auch in dieser Altersgruppe der MDRD-Formel überlegen (Kilbride et al. Am J Kidney Dis. 2013;61(1):57-66)
 2) hämolytisch
 3) HIV-PCR quantitativ:
 < 40 HIV-1-RNA-Kopien/ml Plasma.
 In der PCR zeigte sich ein schwaches Signal.
 Errechneter Wert: nicht quantifizierbar.
 Engmaschige Verlaufskontrolle empfohlen.

Seite 1 von 1



Flowchart 1: Liquid-liquid partition of aqueous extract of *Guazuma ulmifolia*.

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Figure 1: Aqueous extract of *Guazuma ulmifolia* lam.



Thin layer chromatography:



Fractions:

- 1) Aqueous extract after filtering;
- 2) Aqueous Fraction;
- 3) Butanol Fraction;

Eluent:

Ethyl acetate: Formic Acid: Water (90:5:5)

Disclosing solution:

Anisaldehyde solution.

3.2 Antiviral action test of the fractions (BUI and AQJ) and extract (TI) of *Guazuma ulmifolia* using MT4 cells.

3.2.1 **Standard virus:** NL4-3 isolate (subtype B, standard) purified by passage in MT-4 cell culture. Title: 103 TCID50 / ml.

3.2.2 **Cell line used:** MT-4, lymphocyte cell line established in culture, CD4+, expressing co-receptors CCR5 and CXCR4 of HIV-1. Syncytium-inducing (SI) cells and very sensitive to infection by HIV-1.

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Figure 2: Thin layer chromatography.

4. Results

The table below shows the raw data for the assay with the samples of *Guazuma ulmifolia*.

Experiment 1:

ug/ml	1	2	3	4	5	6	7	8	9	10
3218.536	3787.642	3054.273	3029.623	3050.815	2942.556	2838.061	3266.952	3856.086	21463.08	
3742.886	3349.446	2993.495	2921.36	3504.554	3313.594	3368.63	3508.849	3811.135	14058.51	
3791.326	3862.401	3165.824	3175.966	3471.753	3284.441	3324.773	3071.067	4265.1	20294.67	
3111.216	19477.83	4366.21	2674.602	3761.903	3076.742	6052.958	3048.72	7240.043	7477.002	
3035.973	11976.43	8528.929	5251.845	4034.446	6046.562	9489.978	7767.46	10664.81	12772.81	
3018.325	10846.91	24493.36	10675.08	10511.61	11035.92	13717.92	7460.173	11631.7	14904.05	
4678.774	4786.826	3640.615	3197.269	2969.581	3167.663	3161.772	2945.539	2893.992	15247.06	
3348.367	4698.749	3396.308	3033.221	2937.966	3177.718	3185.137	2912.807	3171.715	16203.14	
4505.607	4855.646	3050.558	3060.215	2851.461	3222.067	3087.069	3152.385	3232.763	19429.31	
3478.071	11082.36	20798.67	3092.523	6175.344	9931.44	6314.826	6791.282	7868.27	11966.43	
3464.056	18769.24	23575.37	6290.354	11040.51	12574.45	6861.942	10059.75	20465.64	22468.87	
3466.57	30047.38	42701.53	20304.38	21610.14	33459.76	23847.6	20340.6	24218.94	29270.19	
3780.665	6687.205	4030.617	3735.877	3343.416	3349.616	3335.424	3387.817	3535.693	21099.38	
3725.434	9439.628	3754.924	3216.574	3391.877	3571.244	3594.279	3599.979	3483.987	20940.39	
3699.26	6130.598	3697.187	3453.84	3268.927	3461.059	3536.97	3485.578	3595.887	28091.88	
3706.6	9131.073	9456.156	4017.367	6634.354	4801.022	3718.691	4119.047	19062.17	10836.41	
3751.745	13926.65	13667.83	5648.854	28670.71	11885.63	6658.553	3977.721	6637.935	13064.59	
3786.029	21043.8	35402.97	5648.854	40455.04	36367.43	14835.80	9279.815	14016.52	23878.32	

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Administrative department: Av. José da Rocha Bonfim, nº 214 - Edifício Londres - Cond. Praça Capital

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Figure 3: Results.

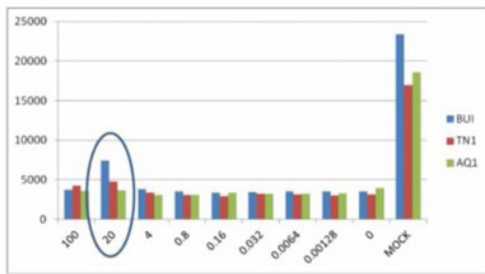


Figure 1: Survival of the MT4 cell with different concentrations of samples BU1, TN1, and AQ1. Partial protection of the cells can be seen with the BU1 sample (30%) at the concentration of 20ug/ml.

Experiment 2:

The table below shows the data for repetition of the experiment with the BU1 sample.

ug/ml	120	24	4.8	0.96	0.192	0.0384	0.00768	0.001536	0	MOCK
1823.828	7267.684	3611.812	3179.984	3606.229	3365.61	3273.242	4442.716	4141.916	33535.09	
1754.799	7431.17	3269.485	3695.089	3082.308	4431.423	3857.433	3150.869	3247.724	33003.2	
1744.599	6982.898	4333.729	4048.463	3943.282	4972.181	3180.402	3760.187	4009.271	31025.81	
1723.314	7768.199	3425.291	4360.88	3168.434	3841.711	3847.31	3339.885	3148.082	35635.67	
1745.994	26911.6	31401.27	34002.62	32780.6	31191.5	33799.98	34284.94	3049.602	35846.08	
1811.232	27341.41	35005.58	33018.74	33096.76	34080.29	34062.86	35448.51	3335.248	33422.74	

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Figure 4: Experiment 2.

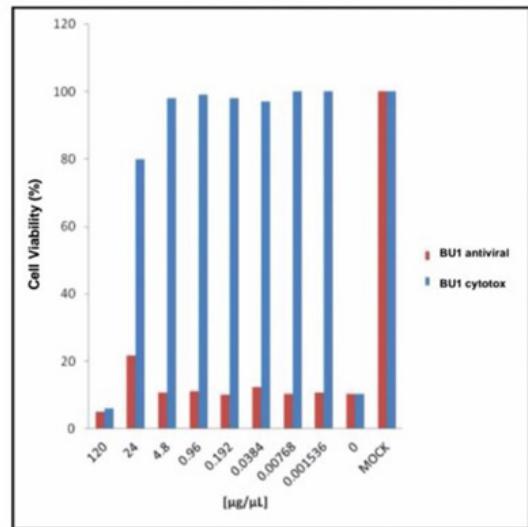


Figure 2: Repetition of the previous experiment with the extract BU1. The histogram shows survival of the MT4 cell infected by the HIV-1 pNL43 virus with a MOI of 0.01 and simultaneously, the cytotoxicity with different concentrations of the BU1 sample. A partial protection of the cells can be seen with the BU1 sample (30%) at the concentration of 24ug/ml. Note that the cytotoxicity of BU1 is still very high and masks the antiviral effect.

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Laboratory: Rua Lauro Vannucci, nº 1020 - Jd. Sta. Cândida

Campinas - SP CEP: 13087-548 Fone: 55 (19) 4062-8090 / (11) 4063-8090 Ramal: 1100

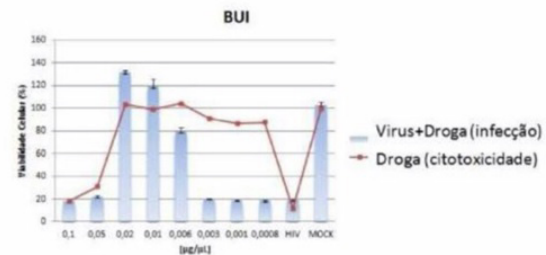
Administrative department: Av. José da Rocha Bonfim, nº 214 - Edifício Londres - Cond. Praça Capital

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Figure 5: Repetition of the previous experiment with the extract BU1.

Graphical RETEST



1	2	3	4	5	6	7	8	HIV	MOCK
9338.524	11880.56	13560.28	11891.04	2264.312	2108.455	1959.657	1948.475	1830.21	10660.57
1849.289	2326.237	13908.07	12645.02	7847.857	2110.309	1048.768	1922.236	1935.398	9648.168
1909.445	2177.255	4002.308	3012.97	8360.524	3199.707	6822.804	1911.822	1990.976	10282.93
1918.236	5165.838	11592.02	12440.57	12962.84	8503.254	8099.72	8089.708	1278.772	9899.466
1889.712	3030.055	13189.9	8678.102	9141.528	8827.542	8571.334	8574.43	1316.385	10234.12
1904.304	3580.364	7986.334	10219.24	10768.34	11379.97	10779.35	11050.6	1167.071	11464.49

More data about Mutamba extract (BU1) which had given activity. Now we had a narrower variation of concentration of extract (2 X fold). We note that a good activity on 20ug/ml range protecting 100% of the cells that were dead by default of NL43 virus no cytotoxic effect (red curve superimposed). Retest with 100% inhibition of HIV NL 43 cells vitro lymphocytes in bovine without cytotoxic.

Figure 6: Graphical retest.