Research, Society and Development, v. 11, n. 13, e223111335302, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i13.35302

Assessing the profile and causality of adverse event related to the use of herbal medicines in patients at a public phytotherapy outpatient clinic

Avaliação do perfil e causalidade de eventos adversos relacionados ao uso de fitoterápicos em pacientes de um ambulatório público de fitoterapia

Evaluación del perfil y la causalidad del evento adverso relacionado con el uso de medicamentos a base de hierbas en pacientes de un ambulatorio público de fitoterapia

Received: 09/13/2022 | Revised: 09/26/2022 | Accepted: 09/27/2022 | Published: 10/05/2022

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Abstract
To assess the profile and causality of adverse events related to herbal medicines of widespread use in Brazil, we conducted an open, prospective, before-and-after clinical trial with patients at an outpatient clinic specialized in complementary and alternative medicine. Participants were submitted to laboratory tests prior to and after use of the medicines, as well as following a period of discontinuation in those experiencing adverse events. Occurrence of adverse events and their severity were verified in accordance with the WHO Toxicity Grading Scale for Determining the Severity of Adverse Events, and their causality established via the Naranjo algorithm. Forty-two subjects participated in the trial, of which 25 experienced grade 1 toxicity adverse events, mainly hyperamylasemia, and 14 continued to experience them following discontinuation, mainly hypomagnesemia. Mean/median values of laboratory tests performed in each phase were within specifications. Statistical analysis of these values in pre-treatment and treatment (n=42) and in treatment and post-treatment phases (n=22) showed statistical significance for activated partial thromboplastin time (p=0.020) in the first correlation. The Naranjo algorithm established a possible causal relationship between the use of herbal medicines and the adverse events reported. By signaling a low probability of occurrence, the algorithm indicates the safe use of these medicines under the experimental conditions employed.
Keywords: Causality; Complementary therapies; Drug-related side effects and adverse reactions; Phytotherapy.

Resumo
Para avaliar o perfil e a causalidade dos eventos adversos relacionados aos fitoterápicos de uso generalizado no Brasil, realizamos um ensaio clínico aberto, prospectivo, antes e depois com pacientes de um ambulatório especializado em medicina complementar e alternativa. Os participantes foram submetidos a exames laboratoriais antes e após o uso dos medicamentos, bem como após um período de descontinuação naqueles que apresentaram eventos adversos. A ocorrência de eventos adversos e sua gravidade foram verificadas de acordo com a Escala de Grau de Toxicidade da OMS para Determinação da Gravidade de Eventos Adversos, e sua causalidade estabelecida pelo algoritmo de Naranjo. Quarenta e dois indivíduos participaram do estudo, dos quais 25 experimentaram eventos adversos de toxicidade grau 1, principalmente hiperamilasemia, e 14 continuaram a experimentá-los após a descontinuação,

principalmente hipomagnesemia. Os valores médios/medianos dos testes laboratoriais realizados em cada fase estavam dentro das especificações. A análise estatística desses valores no pré-tratamento e tratamento (n=42) e nas fases de tratamento e pós-tratamento (n=22) mostrou significância estatística para o tempo de tromboplastina parcial ativada (p=0,020) na primeira correlação. O algoritmo de Naranjo estabeleceu uma possível relação causal entre o uso de fitoterápicos e os eventos adversos relatados. Ao sinalizar uma baixa probabilidade de ocorrência, o algoritmo indica o uso seguro desses medicamentos nas condições experimentais empregadas.
Palavras-chave: Causalidade; Terapias complementares; Efeitos colaterais e reações adversas relacionadas a medicamentos; Fitoterapia.

Resumen

Para evaluar el perfil y la causalidad de los eventos adversos relacionados con los medicamentos a base de hierbas de uso generalizado en Brasil, realizamos un ensayo clínico abierto, prospectivo, de antes y después con pacientes en una clínica ambulatoria especializada en medicina complementaria y alternativa. Los participantes fueron sometidos a pruebas de laboratorio antes y después del uso de los medicamentos, así como después de un período de suspensión en aquellos que experimentaron eventos adversos. La ocurrencia de eventos adversos y su severidad se verificaron de acuerdo con la escala de graduación de toxicidad de la OMS para determinar la severidad de los eventos adversos, y su causalidad se estableció a través del algoritmo de Naranjo. Cuarenta y dos sujetos participaron en el ensayo, de los cuales 25 experimentaron eventos adversos de toxicidad de grado 1, principalmente hiperamilasemia, y 14 continuaron experimentándolos después de la interrupción, principalmente hipomagnesemia. Los valores medios/medianos de las pruebas de laboratorio realizadas en cada fase estuvieron dentro de las especificaciones. El análisis estadístico de estos valores en pretratamiento y tratamiento (n=42) y en las fases de tratamiento y postratamiento (n=22) mostró significación estadística para el tiempo de tromboplastina parcial activada (p=0,020) en la primera correlación. El algoritmo de Naranjo estableció una posible relación causal entre el uso de fitoterápicos y los eventos adversos informados. Al señalar una baja probabilidad de ocurrencia, el algoritmo indica el uso seguro de estos medicamentos en las condiciones experimentales empleadas.
Palabras clave: Causalidad; Terapias complementarias; Efectos secundarios y reacciones adversas relacionadas con medicamentos; Fitoterapia.

1. Introduction

Herbs are commonly used to cure, stabilize, prevent diseases and improve general health conditions. They are sometimes preferred over synthetic drugs, mostly for being considered a “natural” and, therefore, safer therapy. However, herbal medicines may cause harm when used irrationally (Aronson & Meyler, 2009). Adverse reactions (AR) related to herbal medicines have been reported in the literature and evidenced by pharmacovigilance systems (Ekor, 2014; Teschke & Eickhoff, 2015). Data from national drug authorities in over 110 countries, gathered by the World Health Organization’s (WHO) global database for adverse drug reactions (ADR) (Vigibase®), has reported hundreds of individual cases of ADR notifications for medicinal plants (World Health Organization, 2019).

Surveillance on ADR to herbal medicines consists mainly of voluntary reports from consumers and health professionals (Jordan et al., 2010). In Brazil, between 2008 and 2012, AE notifications of products based on plant species in the System of Notifications in Health Surveillance (NOTIVISA) was less than 1% of the items notified as medicines. This reflects the fact that users do not consider these products to pose risks, and health professionals do not question their patients about the use of herbal medicines. Both attitudes contribute to reducing AE cause-effect investigations of such products (De Lima et al., 2015).

Another challenge is the large gap between what the population uses and the scientific evidence. Studies point to the need for high quality research on medicinal plants, in search of an "evidence-based phytotherapy", making health care safer (Carmona & Pereira, 2013). Randomized controlled trials are considered the best way to determine the safety and efficacy of a treatment. However, there is a scarcity of this type of study involving medicinal plants and herbal medicines, mainly due to the lack of requirement by regulatory agencies (Tachjian et al., 2010).

Most reports on ADR to herbal medicines constitute individual cases, published as single reports or case series, without proper severity and causality assessment (Teschke et al., 2013). However, the use of a standard table for interpreting

and grading abnormal signs, symptoms and laboratory parameters is recommended when investigating adverse events (AEs). The WHO Toxicity Grading Scale for Determining the Severity of Adverse Events (WHO TGS) is used to assess severity in general clinical conditions, making it possible to grade abnormal laboratory parameters as evidence of AEs and quantifying their respective severities (International Centers for Tropical Disease Research Network, 2003a; International Centers for Tropical Disease Research Network, 2003b).

The causality of an AR reflects the degree of probability of a relationship between that reaction and the drug used, and can be assessed by expert analysis, algorithmic and probabilistic methods (Jordan et al., 2010). The Naranjo Adverse Drug Reaction Probability Scale is an algorithmic scale designed for assessing AR in a variety of clinical situations, regardless of the organ affected (García-Cortés et al., 2011). It involves ten ‘‘yes’’, ‘‘no’’ or ‘‘unknown/inapplicable’’ questions, covering factors that should be considered in the assessment of any causality relationship, and the total score obtained provides the following probability categories: definite, probable, possible and doubtful (Edwards & Aronson, 2000; Naranjo et al., 1981).

To investigate the profile of AEs related to herbal medicines traditionally used by complementary and alternative medicine (CAM) in Brazil, we conducted a trial to detect the occurrence of AEs and to assess causality relationships in patients undergoing treatment with herbal medicines.

2. Methodology

We conducted a before/after, uncontrolled, open-label, prospective clinical trial with a single intervention group, composed of adult subjects of both genders (Estrela, 2018).

2.1 Subjects

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committees of the Federal University of Goiás and of the Leide das Neves Ferreira Center for Excellence in Teaching, Research and Projects, both located in Goiás state, Brazil (under protocol nos. 1.705.488 and 1.771.217, respectively). All patients gave their written informed consent and were free to withdraw from the trial at any time.

A total of 100 individuals were recruited at an outpatient clinic specialized in CAM in Goiás state from October/2016 to April/2017. Recruitment was done by a phytotherapist, from his routine of care at the Phytotherapy Outpatient Clinic. These individuals were referred by the collaborating physician for an interview with the investigator. After receiving information about the study, 89 subjects agreed to participate. Inclusion criteria were as follows: male or female, aged between 18 and 60 years, non-use of the herbal medicines under investigation in the previous three months, laboratory tests showing no abnormalities according to WHO TGS and negative results for human immunodeficiency virus (HIV), hepatitis B and C. Patients of childbearing potential were included upon confirmation of a negative serum pregnancy test. Exclusion criteria included hypersensitivity or history of severe AR to prescribed herbal medicines, pregnancy and lactation. Fifty subjects were eligible and 42 completed the study.

Treatment interruption was recorded, and data from those participants were not considered.

2.2 Herbal medicines

This study focused on the clinic’s most commonly prescribed herbal medicines. They were purchased from qualified suppliers in powder form (except for *Cynara scolymus* L., which was supplied shaved and was then powdered in a Willye-type knife mill by Tecnal®, Brazil), in the following batches: *Curcuma longa* L. *Zingiberaceae* – rhizome (Florien, batch 058851),

Cynara scolymus L. *Compositae* – leaves (Florien, batch 058777), *Matricaria chamomilla* L. *Compositae* – flowers (Florien, batch 057523), *Equisetum arvense* L. *Equisetaceae* – aerial parts (Purifarma, batch AUTO0595),

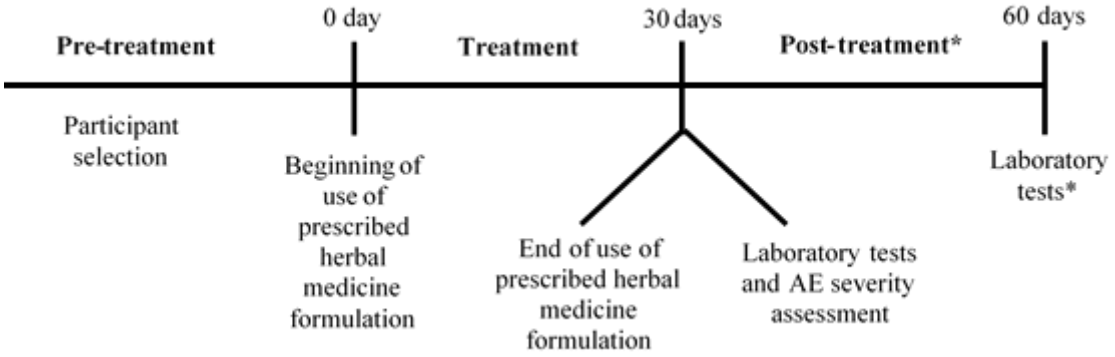
Maytenus ilicifolia Mart. ex Reissek *Celastraceae* – leaves (All Chemistry, batch ALL 061064), *Zingiber officinale* Roscoe *Zingiberaceae* – rhizome (Florien, batch 058733), *Mikania glomerata* Spreng. *Compositae* – leaves (Florien, batch 055650), *Passiflora edulis* Sims *Passifloraceae* – leaves (All Chemistry, batch ALL 063509), *Melissa officinalis* L. *Lamiaceae* – leaves (Florien, batch 059170), *Erythrina mulungu* Benth. *Leguminosae* – barks (Florien, batch 058956), *Bauhinia forficata* Link *Leguminosae* – leaves (All Chemistry, batch ALL 061075) and *Phyllanthus niruri* L. *Phyllanthaceae* – whole plants (All Chemistry, batch ALL 061067). Identity (macroscopic examination, qualitative phytochemical identification of the secondary metabolic used as a marker), physico-chemical (determination of foreign matter, ash, water content, specific gravity) and microbiological quality control tests were performed by suppliers and met specifications.

During regular medical appointments, a phytotherapist prescribed these herbal medicines to patients in different combinations and dosages, according to the clinical indication of each patient. Encapsulation was performed by a pharmacy according to good manipulation practices. Formulations were supplied to participants with enough capsules for 30 days of treatment.

2.3 Study design

A before-and-after clinical trial was conducted in three phases: pre-treatment (PeT), treatment (T) and post-treatment (PoT), with T and PoT lasting 30 days each (Figure 1). In PeT, patients were submitted to laboratory testing with a view to verifying compliance with the inclusion criteria. The researcher registered the drugs being used at that time and over the previous three months, including any self-medication and herbal drugs, as well as ADR history.

Figure 1. Experimental study design. *Post-treatment (PoT) performed only with participants showing adverse events (AEs) in accordance with WHO Toxicity Grading Scale for Determining the Severity of Adverse Events (WHO TGS) at the end of treatment (T).



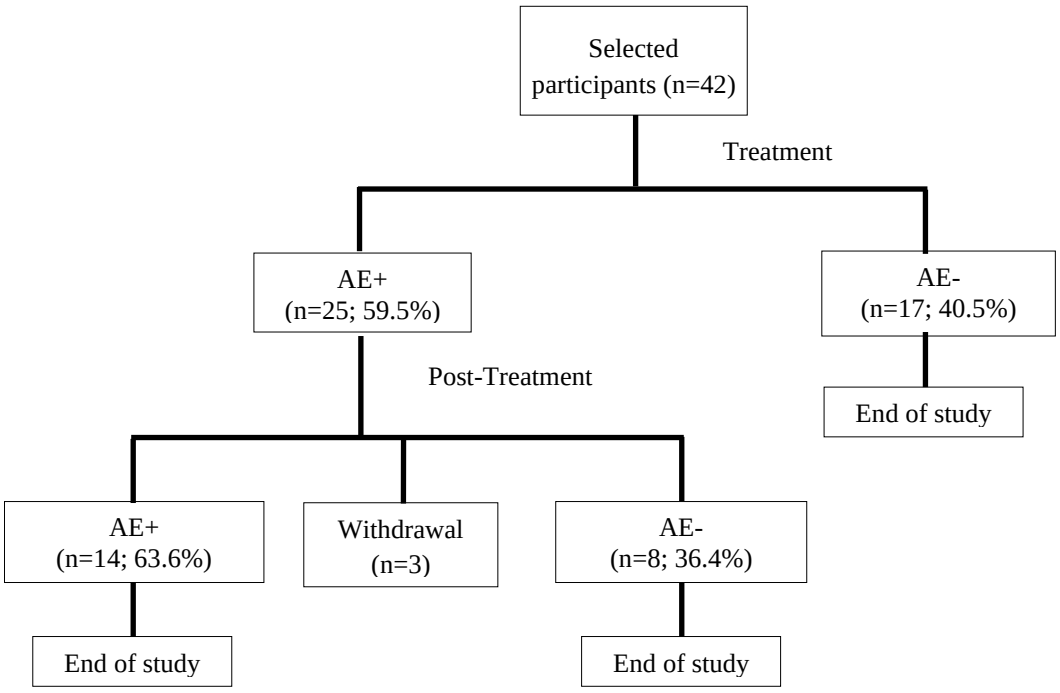
Source: The authors.

Figure 1 provides a representative scheme of the study's experimental design, describing the phases of the study, the time frames of these phases, and the respective actions performed in each time period.

During T, participants (n=42) made use of the herbal medicine formulations prescribed by the clinic's phytotherapist and underwent further laboratory tests afterwards. Results were assessed according to WHO TGS, and patients experiencing no AEs (AE-; n=17) had their participation terminated. Subjects experiencing AEs (AE+; n=25) had their prescriptions suspended

and continued during PoT (except for three withdrawals). By the end of PoT (n=22), laboratory exams were performed and the study was concluded (Figure 2).

Figure 2. Flowchart of allocation of participants in Treatment and Post-Treatment stages, according to the results of laboratory tests and WHO TGS. AE+ = Subjects experiencing AEs; AE- = subjects experiencing no AEs.



Source: The authors.

Figure 2 demonstrates the allocation of subjects into the groups AE+ and AE-, respectively characterized by the occurrence or non-occurrence of AE according to WHO TGS, and the path taken by these subjects throughout the phases of the study.

Participants were instructed to report, during T and PoT, the use of any concomitant medication, whether prescribed or over-the-counter, including all kinds of natural products, food or dietary supplements.

2.4 Laboratory testing

Laboratory tests were performed in PeT (baseline), T and PoT phases and included: hemoglobin; platelet count; neutrophil count; prothrombin time; activated partial thromboplastin time; serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase, amylase, fasting glucose, electrolytes (potassium, sodium, calcium, magnesium, phosphate), albumin, bilirubin (total, direct and indirect), creatinine, blood urea nitrogen, cholesterol (total, HDL, LDL, VLDL) and triglycerides; urinalysis (proteinuria and hematuria). Serological tests for HIV, hepatitis B and C viruses were performed only in PeT, as well as those for human chorionic gonadotropin (hCG) β-subunit in female patients of childbearing potential.

2.5 AE severity assessment

Laboratory tests during T were analyzed according to WHO TGS. Results that conformed to this scale were considered AEs and were classified in four grades of toxicity, from grade 1, the mildest, to grade 4, the most severe, as shown in Table S1 (International Centers for Tropical Disease Research Network, 2003a). For parameters expressed as times the Upper Limit of Normal (x ULN), we considered reference values reported by the laboratory in accordance with the technique employed (Table 1).

Table 1. Laboratory parameters, toxicity severity values in accordance with the WHO Toxicity Grading Scale for Determining the Severity of Adverse Events (WHO TGS), laboratory methodologies employed and respective reference values.

Laboratory test	Grade 1 toxicity	Grade 2 toxicity	Grade 3 toxicity	Grade 4 toxicity	Laboratory methodologies	Laboratory reference values
Hemoglobin	9.5-10.5 g/dl	8.0-9.4 g/dl	6.5-7.9 g/dl	< 6.5 g/dl	Photometry and microscopy	11.5 – 15.5 g/dl (female); 13.5 – 17.5 g/dl (male)
Neutrophil count	1000-1500/mm^3 (µl)	750-999/mm^3 (µl)	500-749/mm^3 (µl)	< 500/mm^3 (µl)	Fluorescence flow cytometry and microscopy	1500 – 7500/µl
Platelet count	75000-99000/mm^3 (µl)	50000-74999/mm^3 (µl)	20000-49000/mm^3 (µl)	< 20000/mm^3 (µl)	Electrical impedance and microscopy	150000 – 500000/µl
Prothrombin time	1.01-1.25 x ULN	1.26-1.5 x ULN	1.51-3.0 x ULN	> 3.0 x ULN	Clot formation, laser reading	11.00 – 15.00 s
Activated partial thromboplastin time	1.01-1.66 x ULN	1.67-2.33 x ULN	2.34-3.0 x ULN	> 3.0 x ULN	Clot formation, laser reading	25 – 45 s
AST	1.25-2.50 x ULN	2.60-5.00 x ULN	5.10-10.00 x ULN	> 10.00 x ULN	Kinetics (UV)	10 – 37 U/L (female); 11 - 39 U/L (male) or 0 – 40 IU/L (both)
ALT	1.25-2.50 x ULN	2.60-5.00 x ULN	5.10-10.00 x ULN	> 10.00 x ULN	Kinetics (UV)	5 – 38 U/L or 0 – 41 U/L
GGT	1.25-2.50 x ULN	1.60-5.00 x ULN	5.10-10.00 x ULN	> 10.00 x ULN	Modified Szasz	≤ 38 IU/L (female); ≤ 55 IU/L (male) or ≤ 40 IU/L (both)
Alkaline phosphatase	1.25-2.50 x ULN	1.60-5.00 x ULN	5.10-10.00 x ULN	> 10.00 x ULN	Kinetic IFCC method	27 – 100 U/L or ≤ 115 U/L
Amylase	1.1-1.5 x ULN	1.6-2.0 x ULN	2.1-5.0 x ULN	> 5.1 x ULN	Kinetics (UV)	≤ 90 U/L
Hyponatremia	130-135 mmol/L	123-129 mmol/L	116-122 mmol/L	<116 mmol/L	Ion-selective electrode	135 – 145 mmol/L

Laboratory test	Grade 1 toxicity	Grade 2 toxicity	Grade 3 toxicity	Grade 4 toxicity	Laboratory methodologies	Laboratory reference values
	(mEq/L)	(mEq/L)	(mEq/L)	(mEq/L), or mental status changes, or seizures	Enzymatic	136 – 146 mmol/L
					Potentiometry	136 – 145 mmol/L
Hypernatremia	146-150 mmol/L (mEq/L)	151-157 mmol/L (mEq/L)	158-165 mmol/L (mEq/L)	>165 mmol/L (mEq/L), or mental status changes, or seizures	Ion-selective electrode	135 – 145 mmol/L
					Enzymatic	136 – 146 mmol/L
					Potentiometry	136 – 145 mmol/L
Hypokalemia	3.0-3.4 mmol/L (mEq/L)	2.5-2.9 mmol/L (mEq/L)	2.0-2.4 mmol/L (mEq/L), or intensive replace-ment therapy required or hospital-zation required.	<2.0 mmol/L (mEq/L), or paresis or ileus or life-threate-ning arrhyth-mia	Ion-selective electrode	3.5 – 5.5 mmol/L
					Potentiometry	3.5 – 5.1 mmol/L
					Enzymatic	3.5 – 5.1 mmol/L
Hyperkalemia	5.6-6.0 mmol/L (mEq/L)	6.1-6.5 mmol/L (mEq/L)	6.6-7.0 mmol/L (mEq/L)	>7.0 mmol/L (mEq/L), or life-threatening arrhythmia	Ion-selective electrode	3.5 – 5.5 mmol/L
					Potentiometry	3.5 – 5.5 mmol/L
					Enzymatic	3.5 – 5.5 mmol/L
Hypocalcemia (corrected for albumin)	8.4-7.8 mg/dl	7.7-7.0 mg/dl	6.9-6.1 mg/dl	<6.1 mg/dl, or life-threatening arrhythmia or tetany	Endpoint colorimetric method	8.8 – 11.0 mg/dl
					Enzymatic colorimetric	8.8 – 10.5 mg/dl
Hypercalcemia (correct for albumin)	10.6-11.5 mg/dl	11.6-12.5 mg/dl	12.6-13.5 mg/dl	>13.5 mg/dl, or life-threatening arrhythmia	Endpoint colorimetric method	8.8 – 11.0 mg/dl
					Enzymatic colorimetric	8.8 – 10.5 mg/dl
Hypomagnese-mia	1.4-1.2 mEq/L	1.1-0.9 mEq/L	0.8-0.6 mEq/L	<0.6 mEq/L or life-threatening arrhythmia	Mann and Yoe method	1.60 – 2.40 mg/dl
Hypophospha-temia	2.0-2.4 mg/dl	1.5-1.9 mg/dl or replace-ment therapy required	1.0-1.4 mg/dl or intensive therapy or hospitaliza-tion required	<1.0 mg/dl or life-threatening arrhythmia	Endpoint UV	2.5 – 4.8 mg/dl
					Kinetics (UV)	2.5 – 4.5 mg/dl

Laboratory test	Grade 1 toxicity	Grade 2 toxicity	Grade 3 toxicity	Grade 4 toxicity	Laboratory methodologies	Laboratory reference values
Hypoglycemia (fasting)	55-64 mg/dl	40-54 mg/dl	30-39 mg/dl	<30 mg/dl or mental status changes or coma	Enzymatic colorimetric	65 - 99 mg/dl
Hyperglycemia (fasting)	116-160 mg/dl	161-250 mg/dl	251-500 mg/dl	>500 mg/dl or ketoacido-sis or seizures	Enzymatic colorimetric	65 - 99 mg/dl
Hyperbilirubi-nemia	1.1-1.5 x ULN	1.6-2.5 x ULN	2.6-5.0 x ULN	> 5.1 x ULN	Colorimetric	0.10 – 1.20 mg/dl
Creatinine	1.1-1.5 x ULN	1.6-3.0 x ULN	3.1-6.0 x ULN	> 6.0 x ULN or required dialysis	Kinetic colorimetric Kinetic	0.40 – 1.40 mg/dl 0.60 –1.20 mg/dl
Blood urea nitrogen	1.25-2.50 x ULN	2.60-5.00 x ULN	5.10-10.00 x ULN	> 10.00 x ULN	Fixed-time kinetics	15 - 40 mg/dl
Proteinuria	1+ or <0.3% or <3 g/L or 200 mg^{-1} g loss/day	2-3+ or 0.3%-1,0% or 3-10 g/L or 1-2 g loss/day	4+ or >1.0% or >10 g/L or 2-3.5 g loss/day	Nephrotic syndrome or >3.5 g loss/day	Combur10-test® strip with automatic reading	≤ 30.0 mg/dl
Hematuria	Microscopic only	Gross, no clots	Gross + clots	Obstructive or required transfusion	Combur10-test® strip with automatic reading and microscopy with Neubauer chamber	≤ 10000/ml

Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; x ULN, times the Upper Limit of Normal (informed by the laboratory according to the technique used). Source: International Centers for Tropical Disease Research Network (ICTDR), 2003a

Table 1 presents the WHO TGS, a scale for grading the severity of AEs, and the criteria defined for classifying these AEs into 4 degrees of toxicity, with grade 1 being the least severe and grade 4 the most severe. The methodologies and reference values adopted by the laboratory in performing laboratory tests to investigate the occurrence of these AEs are also described.

2.6 Causality assessment

AEs reported during T in patients who completed all stages of the study (concluded PoT) had their causality assessed by a team of pharmacists, employing the Naranjo Adverse Drug Reaction Probability Scale. To identify previous conclusive reports on the events evaluated (question 1), we performed a comprehensive search on MEDLINE (via PubMed), Science Direct and Virtual Health Library from October to December/2017, using several combinations of descriptors to cover all herbs investigated and the AEs in question. Full-text publications available through the Journal Portal of Brazil's Coordination for the Improvement of Higher Education Personnel (CAPES) were selected.

As regards temporality of occurrence, question 2 was answered affirmatively for all cases. To answer question 3, the AE's continuity of occurrence after discontinuation (PoT) was considered. Question 4 was marked "unknown/inapplicable",

since there was no subsequent administration of the herbal medicines related to AE in the study design. Occurrence of the same AE after previous use of the herbal medicines was assessed according to past ADR history reported by the participants during PeT (question 9).

Alternative causes to the AE (question 5) were evaluated by investigating each drug, medicinal plant, herbal medicine, food or dietary supplement concomitantly used during T, as informed by the participants. For that purpose, we searched for evidence relating these substances and the AEs in UpToDate®. For medicinal plants and herbal medicines, not commonly found in that database, searches were conducted on MEDLINE/Pubmed and linked the name of the plant species (scientific and common) and the AE. For AEs related to liver damage, the LiverTox® website was also consulted. Medical history and alcohol consumption reported by participants in PeT were also considered.

As regards objective confirmation of AEs (question 10), laboratory findings indicating toxicity (in line with WHO TGS) were viewed as an objective piece of evidence of AE occurrence.

Placebo administration, increased or decreased doses of the herbal medicines under investigation and analysis of their plasma concentration were not evaluated in this study. Questions 6, 7 and 8 were marked "unknown/inapplicable".

2.7 Statistical analysis

Frequency was established for patients' characteristics (gender and health complaints). Age was expressed as mean and standard deviation (SD).

Laboratory test results were expressed as mean and SD for parametric data or median and interquartile range for nonparametric data, considering all the individuals enrolled in each study phase. Two-tailed *t*-test (parametric data) and the Kolmogorov-Smirnov test (nonparametric data) were used to correlate the means/medians of these results between PeT and T and later between T and PoT. For laboratory test results expressed qualitatively (proteinuria and hematuria), McNemar paired-samples test was used.

A p-value < 0.05 was considered to indicate statistical significance. Statistical analyses were performed via Système Portable d'Analyse – SPAD (version 7.4) and free software R (version 3.6.2 for Windows).

3. Results and Discussion

3.1 Subjects' characteristics

The socio-demographic characteristics of the 42 selected participants are presented in Table 2. The median age of the 42 participants was 46.1 years (SD $\pm$ 10.2), and 92.9% were female subjects.

Table 2. Socio-demographic characteristics of the 42 selected participants.

Variables	Absolute Frequency	Relative Frequency (%)
GENDER		
Female	39	92.9
Male	3	7.1
AGE (years)		
25-29	4	9.5
30-39	6	14.3
40-49	15	35.7
50-59	16	38.1
60	1	2.4
LEVEL OF EDUCATION		
Complete college degree	16	38.1
Complete high school	13	30.9
Incomplete high school; complete elementary school	4	9.5
Incomplete elementary school	3	7.1
Incomplete college degree	2	4.8
SELF-DECLARATION OF RACE		
Multiracial	17	40.5
White	14	33.3
Black	7	16.7
Asian	2	4.8
Indigenous	2	4.8

Source: Authors.

Table 2 describes the socio-demographic profile of the study participants regarding gender, age, level of education and self-declaration of race, showing a greater predominance of multiracial women, aged between 50-59 years, with higher education.

3.2 Safety evaluation

Mean/median values of each laboratory test performed during PeT and T, considering all participants selected (n=42), are shown in Table 3. Statistical significance was established for activated partial thromboplastin time (p=0.020). Nevertheless, the totality of results is within the range considered favorable by WHO TGS (Table 1).

Mean/median values of each laboratory parameter evaluated in T and PoT for the 22 patients that completed PoT are shown in Table 3. No unfavorable values (in accordance with WHO TGS - Table 1) were observed, and there was no statistically significant difference when comparing these values in both phases.

There was no statistical significance between PeT and T for proteinuria and hematuria (p=1 and p=0.07364, respectively). The same was reported when assessing these parameters during T and PoT (p=1 for both tests).

Table 3. Laboratory parameters expressed as mean ± standard deviation or median and interquartile range for each phase of the study, using the WHO Toxicity Grading Scale for Determining the Severity of Adverse Events (WHO TGS) as reference.

Laboratory test	Pre-treatment (n=42)	Treatment (n=42)	*p*-value	Treatment (n=22)	Post- treatment (n=22)	*p*-value	Unfavorable values
Hemoglobin	13.4 ± 1.2	13.4 ± 1.0	0.853	13.0 ± 1.0	13.2 ± 0.9	0.432	≤ 10.5 g/dl
Neutrophil count	3394 ± 1169	3519 ± 1416	0.661	4012 ± 1623	3941 ± 1345	0.875	≤ 1500/mm^3 (µl)
Platelet count	254000 (215000 - 328000)	267881 ± 74845	0.965	284500 ± 71864	290409 ± 70750	0.785	≤ 99000/mm^3 (µl)
Prothrombin time	0.80 ± 0.07	0.83 ± 0.09	0.052	0.83 ± 0.11	0.85 ± 0.06	0.416	≥ 1.01 x ULN
Activated partial thromboplastin time	0.72 ± 0.11	0.64 (0.58 – 0.75)	0.020*	0.67 ± 0.11	0.66 ± 0.08	0.751	≥ 1.01 x ULN
AST	0.76 ± 0.15	0.83 ± 0.27	0.161	0.94 ± 0.31	0.83 ± 0.23	0.224	≥ 1.25 x ULN
ALT	0.46 (0.39 – 0.60)	0.54 (0.37 – 0.71)	0.436	0.74 ± 0.42	0.62 ± 0.33	0.323	≥ 1.25 x ULN
GGT	0.46 (0.34 – 0.63)	0.53 ± 0.27	0.426	0.53 (0.34 - 0.79)	0.53 (0.34 – 0.90)	0.632	≥ 1.25 x ULN
Alkaline phosphatase	0.56 ± 0.19	0.60 ± 0.20	0.480	0.63 ± 0.18	0.62 ± 0.17	0.880	≥ 1.25 x ULN
Amylase	0.8 ± 0.2	0.8 ± 0.3	0.878	0.9 ± 0.3	0.8 ± 0.3	0.973	≥ 1.1 x ULN
Sodium	140 ± 3	140 ± 3	0.442	140 ± 3	141 ± 2	0.234	≤ 135 or ≥ 146 mmol/L (mEq/L)
Potassium	4.4 ± 0.5	4.5 ± 0.4	0.306	4.4 ± 0.4	4.6 ± 0.5	0.117	≤ 3.4 or ≥ 5.6 mmol/L (mEq/L)
Calcium (corrected for albumin)	9.2 (9.0 – 9.6)	9.3 ± 0.5	0.468	9.3 ± 0.6	9.3 ± 0.5	0.823	≤ 8.4 or ≥10.6 mg/dl
Magnesium	1.5 ± 0.2	1.6 (1.4 – 1.7)	0.355	1.5 ± 0.2	1.6 ± 0.2	0.400	≤ 1.4 mEq/L
Phosphate	3.4 ± 0.5	3.4 ± 0.4	0.839	3.5 ± 0.4	3.5 ± 0.4	0.908	≤ 2.4 mg/dl
Fasting glucose	88 (82 – 91)	87 (82 - 93)	0.791	90 ± 14	87 ± 12	0.407	≤ 64 or ≥116 mg/dl
Bilirubin total	0.5 ± 0.2	0.5 ± 0.2	0.632	0.5 ± 0.2	0.4 (0.3 – 0.7)	0.821	≥ 1.1 x ULN
Creatinine	0.6 (0.5 – 0.6)	0.6 (0.5 – 0.7)	0.436	0.6 ± 0.1	0.6 (0.6 – 0.7)	0.223	≥ 1.1 x ULN
Blood urea nitrogen	0.65 ± 0.16	0.71 ± 0.21	0.133	0.73 ± 0.18	0.74 ± 0.30	0.887	≥ 1.25 x ULN

Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; x ULN, times the Upper Limit of Normal (informed by the laboratory according to the technique used).
Correlation analysis between Pre-treatment vs. Treatment phases and Treatment vs. Post-treatment phases using two-tailed *t*-test (parametric data) and the Kolmogorov-Smirnov test (nonparametric data). *statistical significance (p<0.05).Source: The authors

Table 3 presents the mean/median values of each laboratory parameter evaluated in PeT, T and PoT phases, and according to the values defined by WHO TGS, none of them was considered unfavorable. It also brings results of the statistical correlation of the mean/median values of laboratory parameter between PeT and T, and later between T and PoT, demonstrating a statistically significant difference for activated partial thromboplastin time (p=0.020) between PeT and T.

3.3 AE incidence and severity assessment

The occurrence of AE was evaluated by comparing the laboratory test results with the values considered AE by WHO TGS (Table 1). At the end of T, 25 of the 42 participants (59.5%) had laboratory test results indicating 38 different AEs (in line with WHO TGS), all of them categorized with grade 1 toxicity severity (Table 1). AEs reported are hyperamylasemia (n=9; 23.7%); hypomagnesemia (n=8; 21.1%); hematuria (n=5; 13.2%); AST and ALT increase (n=3; 7.9%, each); hyperglycemia and hypocalcemia (corrected for albumin) (n=2; 5.3%, each); hypercalcemia (corrected for albumin); GGT increase; thrombocytopenia; prothrombin time elevation; blood urea increase and proteinuria (n=1; 2.6%, each). The majority of participants experienced one AE (n=17), while some experienced two (n=4), three (n=3) and four (n=1) AEs.

3.4 AE causality assessment

Causality assessment was conducted in each of the 35 AEs observed during T in the patients who completed all phases of the study (n=22). From the 25 subjects who experienced AE during T, 22 repeated the laboratory tests and concluded PoT. The other 3 individuals dropped out of the study at this stage, alleging unavailability to come to the laboratory for the tests. The loss of these 3 patients did not impact the study, since the causality assessment was performed from the individual's perspective, considering the study design (before/after).

The Naranjo algorithm was selected to assess causality because it is a simple and widely applicable method that has been used to evaluate AE causality in several clinical situations, including the use of medicinal plants (Bilgi et al., 2010; Necyk et al., 2014). In addition, the application of the Naranjo scale reinforced the individual approach adopted in the study's experimental design, since each AE associated with each participant was investigated individually.

Table 4 lists participants' characteristics; herbal medicines prescribed and their respective dosages and combinations; drugs, natural products, food or dietary supplements concomitantly used during T; AEs identified and AE causality in accordance with the Naranjo scale.

Table 4. Participants' characteristics; health complaints; herbal medicines prescribed and their respective dosages and combinations; drugs, natural products, food or dietary supplements concomitantly used during treatment (T); adverse event observed; adverse event causality according to the Naranjo algorithm.

Participant	Health complaints	Herbal medicines prescribed	Drugs, food or dietary supplements, medicinal plants and/or herbal medicines used during T	Adverse event	Causality
1B.1	Recurrent urinary tract infection	*C. longa* 250 mg + *E. arvense* 250 mg - 1 capsule 2 times/d	Loratadine (2-d use); dexchlorpheniramine (2-d use)	Hypocalcemia (corrected for albumin)	Possible
1B.2	Anxiety, hypertension	*E. mulungu* 250 mg + *M. officinalis* 200 mg - 1 capsule 2 times/d	Oral rehydration formulation (15-d use); cefadroxil 1g/d (8-d use); nimesulide 4 times/d (8-d use); bromazepam 3mg/d, continuous use (5 y); atenolol 50mg/d, continuous use (8 m); amlodipine + losartan 5+100mg/d, continuous use (8 m); estradiol + norethisterone 1 time/d, continuous use (13 m)	Hematuria	Possible
1B.3	Generalized body pain	*E. mulungu* 300 mg + *M. officinalis* 200 mg - 1 capsule 2 times/d	Estradiol valerate 1mg/d, continuous use (11 y); calcium + vitamin D 1 time/d, continuous use (2 y); bupropion 1 time/d, continuous use (5 m); dimeticone 35 drops/d (5-d use); dipyrone (metamizole) + orphenadrine + caffeine (11-d use); *Ageratum conyzoides* L. and *Myristica fragrans* Houtt. tea 2 times/d (5-d use)	Hyperamylase-mia	Possible
1B.4	Headache	*Z. officinale* 300 mg + *C. longa* 500 mg - 1 capsule 2 times/d	Medroxyprogesterone 10mg/d (10 d-use); *Foeniculum vulgare* Mill. tea and castor oil 1 time/d daily	Hyperamylase-mia	Possible
1B.5	Anxiety	*P. edulis* 300 mg - 1 capsule 2 times/d	Atenolol 25mg/d continuous use (5 y); venlafaxine 225mg/d continuous use (2 y); alprazolam 0.5mg/d continuous use (2 y); *Rosmarinus officinalis* L. tea 1 time/d 3 times/week, continuous use (5 m)	Hyperamylase-mia and AST increase	Possible (both AE)

Participant	Health complaints	Herbal medicines prescribed	Drugs, food or dietary supplements, medicinal plants and/or herbal medicines used during T	Adverse event	Causality
1B.6	Back pain, insomnia, depression, menopause	*M. officinalis* 300 mg + *E. mulungu* 200 mg - 1 capsule 2 times/d	Hydrochlorothiazide 25mg /d continuous use (15 y); enalapril 40mg/d continuous use (15 y); calcium 600mg/d continuous use (6 m); omega 3, 6, 9 fatty acids 1 time/d continuous use (6 m)	Hematuria	Possible
1B.7	Low immunity, cramps, recurrent urinary tract infection	*E. arvense* 300 mg + *P. niruri* 200 mg - 1 capsule 2 times/d	Famciclovir 250mg/d (5-d use)	Hypomagnese-mia	Possible
1B.8	Swelling in inferior members and legs	*E. arvense* 300 mg + *B. forficata* 300 mg - 1 capsule 2 times/d	-	Hypomagnese-mia	Possible
1A.1	Depression	*C. longa* 250 mg + *Z. officinale* 250 mg - 1 capsule 2 times/d	Enalapril 10mg/d continuous use (5 y); dipyrone (metamizole) + orphenadrine + caffeine 1 tablet (single use)	Hyperamylase-mia	Possible
1A.2	Muscle pain, fibromyalgia, spine curvature disorders	*B. forficata* 500 mg - 1 capsule 2 times/d	-	Hypomagnese-mia and ALT increase	Possible (both AE)
1A.3	Anxiety, irritability, stress	*E. mulungu* 250 mg + *M. officinalis* 200 mg - 1 capsule 2 times/d	Dipyrone (metamizole) + orphenadrine + caffeine (2-d use); carisoprodol + sodium diclofenac + paracetamol + caffeine, (2-d use); dipyrone (metamizole) + isometheptene + caffeine (2-d use)	Hyperamylase-mia, hypocalcemia (corrected for albumin) and thrombocytope-nia	Possible (the three AE)
1A.4	Allergy, rhinitis	*M. chamomilla* 250 mg + *M. officinalis* 250 mg - 1 capsule 2 times/d	Papaverine + dipyrone (metamizole) + *Atropa belladona* L. fluid extract 1 tablet (single use); dipyrone (metamizole) 1 tablet (single use); ethinylestradiol + cyproterone 1 time/d continuous use (1 year)	Hyperamylase-mia	Possible

Participant	Health complaints	Herbal medicines prescribed	Drugs, food or dietary supplements, medicinal plants and/or herbal medicines used during T	Adverse event	Causality
1A.5	None (follow-up consultation)	*M. chamomilla* 250 mg + *M. officinalis* 250 mg - 1 capsule 2 times/d	Flaxseed oil 1400mg/d continuous use (2 m); multivitamin/ multimineral supplement 1 time/d continuous use (2 m); vitamin D3 2000 IU/d continuous use (2 m); simvastatin 20 mg/d continuous use (2 m); levothyroxine 50 mcg/d continuous use (5 y); isoflavone 150mg /d continuous use (10 y); *Cimicifuga racemosa* L. 40mg/d continuous use (10 y); *Morus nigra* L. 2 times/d continuous use (6 m)	Hyperamylase-mia and hypomagnese-mia	Possible (both AE)
1A.6	Weight loss difficulty	*B. forficata* 200 mg + *E. arvense* 200 mg + *E. mulungu* 200 mg - 1 capsule 3 times/d	Caffeine + gingerol + catechins + synephrine 2 tablets/d (46-d use)	Hypomagnese-mia and hypercalcemia (correct for albumin)	Possible (both AE)
1A.7	Essential tremor, body pain, sinusitis, rhinitis, depression	*M. officinalis* 250 mg + *E. mulungu* 250 mg - 1 capsule 2 times/d	Propranolol 80mg /d continuous use (many years); vitamin B-complex 1 time/d continuous use (3 m); cyclobenzaprine 1 time/d if pain, occasional use (2 m); chlorpheniramine + ascorbic acid + dipyrone (metamizole) 4 times/d (7- d use); *Origanum vulgare* L. and *Sysygium aromaticum* (L.) Merr. & L.M.Perry tea (7-d use)	Hematuria	Possible
1A.8	Insomnia, back pain	*E. mulungu* 300 mg + *M. officinalis* 200 mg - 1 capsule 2 times/d	*Cinnamomum zeylanicum* bark tea 1 time/d frequent use (not daily); *Cymbopogon citratus* DC. (Stapf.) tea 1 time/d frequent use (not daily); *Cinnamomum zeylanicum* and *Z. officinale* Roscoe tea 1 time/d frequent use (not daily)	Hypomagnese-mia; AST, ALT and GGT increase	Possible (the four AE)
1A.9	Headache, allergic rhinitis	*Z. officinale* 200 mg + *C. longa* 300 mg - 1 capsule 2 times/d	Venlafaxine 37.5mg/d (24-d use); cyclobenzaprine 10mg/d (10-d use); ketoprofen 200mg/d (10-d use);	ALT increase	Possible

Participant	Health complaints	Herbal medicines prescribed	Drugs, food or dietary supplements, medicinal plants and/or herbal medicines used during T	Adverse event	Causality
			medroxyprogesterone intramuscular every 3 m, for 7 m; *Hibiscus rosa-sinensis* L. tea 500 mL/d (15-d use)		
1A.10	Allergy, hypothyroidism	*B. forficata* 250 mg + *C. scolymus* 250 mg - 1 capsule 2 times/d	Levothyroxine 150mcg/d continuous use (8 y); magnesium chloride 2 times/d (15-d use)	Hyperglycemia	Possible
1A.11	Migraine	*C. scolymus* 250 mg + *E. mulungu* 250 mg - 1 capsule 2 times/d	Ascorbic acid (vitamin C) 1g/d (30-d use); zinc + magnesium + vitamin B6 120g/d (30-d use); leucine + isoleucine + valine 20mg/d (30-d use); glutamine 10mg/d (30-d use); *Tribulus terrestris* L. 1g/d (30-d use); desogestrel + ethinylestradiol 1 time/d continuous use (14 y)	Prothrombin time elevation	Possible
1A.12	Follow-up consultation	*B. forficata* 250 mg + *C. scolymus* 250 mg - 1 capsule 2 times/d	-	AST increase	Possible
1A.13	Memory loss	*P. edulis* 250 mg + *E. mulungu* 250 mg - 1 capsule 2 times/d	-	Hematuria, hyperglycemia and proteinuria	Possible (all three AE)
1A.14	High uric acid level	*C. longa* 500 mg + *Z. officinale* 500 mg - 1 capsule 2 times/d; *P. niruri* 500 mg - 1 capsule 2 times/d	Atenolol + chlortalidone 50 mg /d continuous use (15 y); colchicine 0.5mg/d continuous use (10 y); multivitamin/ multimineral supplement 1 time/d continuous use (2 y); omega 3 fatty acid 2 capsules/d continuous use (2 y); vitamin E 1 time/d continuous use (2 y); Iron glycinate 1 time/d continuous use (2 y); glucosamine sulfate 1500mg/d continuous use (10 y), with interruptions every 6 m	Hyperamylase-mia, hypomagnese-mia and hematuria	Possible (all three AE)

Abbreviations: AE: adverse event; d: day; F: female; M: male; m: month; y: year. Source: Authors.

Table 4 presents the causality assessment of the evidenced AEs, as well as the aspects considered in this assessment according to the Naranjo algorithm. The laboratory alterations indicative of toxicity were all characterized as possible AEs to

the use of the investigated herbal medicines.

When assessing causality, it is imperative to gauge whether an AE fits the pharmacological pattern of the substances under investigation (Edwards & Aronson, 2000). Although we have conducted an extensive and systematic search of the literature, no evidence was found relating the herbal medicines used by participants and the AEs experienced by them. In fact, some studies described opposite effects to what we observed. Participants 1B.4 and 1A.14, who respectively experienced hyperamylasemia and hematuria, used *C. longa*, which has been reported as effective in reversing serum amylase elevation in mice with acute pancreatitis (Yu et al., 2011) and in significantly reducing hematuria in a clinical trial with lupus nephritis patients (Khajehdehi et al., 2012).

Another aspect of major importance involves considering the temporal relationship between exposure to a substance and the emergence of an AE, as well as behavior following withdrawal, and analyzing objective parameters of organ functions both before (baseline) and after substance use (Edwards & Aronson, 2000). This study was designed as a before-and-after clinical trial, followed by a withdrawal phase, allowing the establishment of baseline laboratory parameters and the evaluation of subsequent alterations related to the use of herbal medicines for 30 days of treatment, and the same suspension interval. The decision to set the 30-day period for T was based on an observational study that investigated liver injury among users of herbal medicines, and clinical safety studies of phytotherapeutic medicines (Jeong et al., 2012; Nascimento et al., 2009; Tavares et al., 2006). All AEs submitted to the Naranjo algorithm were objectively confirmed through laboratory test alterations, which yielded a temporal relationship with the use of the herbal medicines prescribed.

Diagnosing AEs must also take into account any other drugs or natural products consumed by subjects as possible causal agents. For that, we investigated the role of each product consumed by participants during T, which are described in Table 4, as an alternative cause to the AE in question. As regards hyperamylasemia, participant 1B.3 made use of estradiol, calcium and bupropion, described as capable of causing elevation in amylase seric concentration and pancreatitis (Bupropion, 2018; Vege, 2018). Pancreatitis is also an ADR to venlafaxine, enalapril, sodium diclofenac and cyproterone, used respectively by subjects 1B.5, 1A.1, 1A.3 and 1A.4 (Cyproterone, 2018; Diclofenac (systemic), 2018; Enalapril, 2018; Venlafaxine, 2018). Acute drug-induced pancreatitis is caused by simvastatin and chlortalidone, used by 1A.5 and 1A.14, respectively (García Gavilán et al., 2017; Vege, 2018).

AST and ALT increase are described in the literature as being related to drugs such as atenolol and alprazolam used by participant 1B.5, ketoprofen and medroxyprogesterone used by 1A.9, and venlafaxine used by both (Alprazolam, 2017; Alprazolam, 2019; Atenolol, 2017; Atenolol, 2019; Ketoprofen, 2018; Ketoprofen, 2019; Progestins, 2020; Venlafaxine, Desvenlafaxine, 2020).

In addition to these drugs, patient 1A.9 reported the use of hibiscus tea. Fakeye et al. (2009) have shown significant ALT elevation in rats that received an aqueous extract of *Hibiscus sabdariffa* Linn.

Chlortalidone, used by participant 1A.14, is a thiazide diuretic that inhibits magnesium reabsorption and can hence be related to the hypomagnesemia observed in that patient (Chlorthalidone, 2019; Yu, 2018). Thrombocytopenia is a known ADR to paracetamol and diclofenac, both used by participant 1A.3 (Arnold & Cuker, 2019; Diclofenac (systemic), 2018).

Besides gathering information and analyzing each product consumed as a potential alternative cause to AEs, it is important to determine how long these substances were being used. The majority are medications used continuously for more than six months prior to the study, which corroborates the causality attributed to the herbal medicines, given that they primarily represent the only difference between PeT and T.

In addition to the products consumed by participants during T, other factors may yield alternative causes to AEs. Participant 1A.6 experienced hypercalcemia during T, with a calcium concentration (corrected for albumin) equal to 10.8

mg/dL. Calcium and magnesium compete with each other for carriers in the Henle loop, and hypercalcemia may lead to a reduction in magnesium concentration (Yu, 2018); hence, it may cause the hypomagnesemia detected in that patient. Female subject 1A.13 reported being in the end of the menstrual period when T-phase laboratory tests were performed, which corresponds to an alternative cause to the transient hematuria registered. Alcohol consumption, in turn, was not considered an alternative cause since participants reported the use of acceptable quantities, in line with Friedman (2019); these are lower than 210 g and 140 g of alcohol for men and women per week, respectively, for a minimum of two years.

4. Conclusion

Total scores yielded by the Naranjo scale show that all AEs registered are possible ADRs caused by the investigated herbal medicines at dosages and combinations listed. A "possible" relationship corresponds to a low probability of occurrence of an AE by a product under investigation, under the present experimental conditions.

Results obtained with the individual approach proposed by the Naranjo algorithm were corroborated by the global analysis of participants. Comparisons of laboratory parameters evaluated prior to, during use, and after discontinuation of the consumption of herbal medicines failed to show statistical significance. In fact, this monitoring of toxicity and AEs profile through laboratory testing is one of the strengths of this work.

Moreover, the results showed that using WHO TGS in the detection of AEs and the Naranjo algorithm in causality assessment is a useful and effective strategy to optimize the benefits and minimize the risks of this therapy in clinical practice, thus contributing to the rational use of herbal medicines.

As perspectives for future research, it is recommended to conduct controlled, double-blind, randomized clinical trials, using herbal medicines detected as "possible" causes of AE, in order to deepen the investigation of the data obtained.

Acknowledgments

This research was supported by Fundação de Apoio à Pesquisa do Estado de Goiás (FAPEG) via the program "Programa de Pesquisa para o SUS" (PPSUS 2013) and the provision of a scholarship [grant no. 201410267000317 and 201510267001250, respectively]. The authors are grateful to Professor Claudio Rodrigues Leles, PhD, and pharmacist Flávia Neri Meira, PhD, for their assistance with the study design.

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