Research Article

Safety Evaluation in Healthy Colombian Volunteers of P2Et Extract Obtained from Caesalpinia spinosa: Design 3+3 Phase I Clinical Trial

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Received 21 June 2021; Revised 21 January 2022; Accepted 25 January 2022; Published 22 February 2022

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1. Introduction

C. spinosa or Dividivi has been traditionally used by Colombian indigenous tribes located in the Caribbean coast. Its traditional medicinal uses include antimicrobial, antioxidant, antitumor, and immunomodulatory properties for different diseases [1–3].

P2Et has shown immunomodulatory and antitumoral properties [3–6]. The immunomodulatory effect of the P2Et seems to be dependent on the basal conditions of host. In healthy C57BL/6 mice treated twice a week for 21 days with the extract, an increase in the number of CD4+ and CD8+ activated T cells, NK cells, regulatory T cells, dendritic cells, and myeloid-derived suppressor cells (MDSC) in lymphoid organs was observed, together with a significant increase in the concentration of serum cytokines such as IL-10, IL-17, IFN-alpha, IL-6, IL-4, and IL-2 [4]. In contrast, when immunocompetent mice orthotopically transplanted with 4T1 tumoral cells (highly metastatic breast cancer cell line) were treated with P2Et, a decrease in primary tumor growth and metastatic foci was observed [4]. In a melanoma murine model, the P2Et induces immunogenic cell death favoring immune system activation, particularly of T cells, allowing generation of specific multifunctional memory T cells, IFN-γ, TNF-α, IL-4, and IL-5 producers which could participate in primary tumor control [7].

To ensure the safety of herbal pharmaceutical preparations, safety and tolerability studies are a fundamental requirement to access the clinical stages efficacy in humans. The P2Et has a potential to be developed as an efficient herbal drug
for the coadjuvant treatment in cancer and diseases with an inflammatory component. Thus, the clinical phase I study was carried out to evaluate the effect of continuous administration of the extract for 28 days in humans.

2. Materials and Methods

2.1. Preclinical Toxicology Test

2.1.1. Animals and Housing Conditions. The animals were housed in specific cages for the species (Rattus norvegicus and Oryctolagus cuniculus) in accordance with current legislation that provides integrity and animal welfare.

The rats (Rattus norvegicus) were kept in a specific room with a temperature between 17.0 and 20.6°C and relative humidity between 54.9 and 70.2%. The rabbits (Oryctolagus cuniculus) were kept in a specific room with a temperature between 17.4 and 20.8°C and relative humidity between 54.9 and 70.2%. Both species were subjected to a photoperiod of 12 hours of light and 12 hours of darkness. The feeding of the animals consisted of conventional food for the species and filtered drinking water. Periodic microbiological analyses were carried out on the water and food, guaranteeing that they were suitable for consumption and did not interfere in the results of the tests.

Animals were maintained in the test facilities according to local and international requirements, based on the Guide for the Care and Use of Laboratory Animals [8]. Animals showing continuing signs of severe distress and/or pain at any stage of the tests were euthanized. Procedures for animal care and criteria for making the decision to euthanize an animal were based on the Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation [9].

The chronic toxicity study was performed in a group of rats and rabbits in a GLP certified center. The study procedures were aligned to ICH M3 (R2) [10]. 12 rabbits of 11 weeks of age were used: 6 experimental animals and 6 control animals with equal number of males and females in each group (Table S1). 20 rats of 9 weeks of age were used for the test: 10 experimental animals and 10 control animals with equal number of males and females in each group (Table S1).

The P2Et was administered at the dose of 1000 mg/kg by oral route once a day, for a period of 28 days, by oral gavage with syringe and gastric tube. Oral administration was chosen due to the intended indicated use of the product.

2.1.2. Dose Calculation. The starting dose of P2Et for the clinical trial in humans was estimated based on the preclinical data and NOAEL of P2Et in the repeated chronic toxicity study according to FDA Guidance for Industry-Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers [11].

2.1.3. Statistical Analysis. Toxicity tests were analyzed using BioEstat 5.3; for parametric data, t-student test was used for comparing between experimental and control groups, and Mann-Whitney test was employed for nonparametric data. The significance level used was $p < 0.05$.

2.2. Clinical Study

2.2.1. Study Volunteers. The clinical study was conducted aligned to Declaration of Helsinki, Good Clinical Practice (GCP), and local regulation [12–14]. The study was reviewed and approved by the independent ethics committee (IEC) of the Hospital Universitario San Ignacio. Trial was registered in clinical trial no. NCT03663881 (https://clinicaltrials.gov/ct2/show/NCT03663881). This is a single-centered clinical trial conducted in the Office of Research of the Hospital Universitario San Ignacio. The subjects were recruited from hospital workers, students, and relatives of patients from different departments of the hospital. All subjects read and signed the informed consent form before performing any study procedure. Subjects were evaluated for eligibility through a medical history, vital signs, physical examination, clinical laboratory tests, and electrocardiogram (ECG).

Following were inclusion criteria: participants should be healthy, between 18 to 65 years old, without significant comorbidities, and with adequate hepatic, renal, and hematological function. Additionally, eligible female subjects are not pregnant or lactating and if they were of childbearing age, they must be using at least one effective contraceptive method. The exclusion criteria were the following: (1) active disease or infection such as HIV positive or history of severe hepatic, cardiac, pulmonary, endocrine, or psychiatric morbidity; (2) history of allergic reactions to polyphenol-type compounds; (3) use of dietary supplements or phyto-medicines <15 days prior to entry and during the study or strong or moderate inducers and inhibitors of CYP3A7 drugs prior to the start of study treatment; (4) history of drug or alcohol abuse or use of an investigational product and/or participation in another clinical study.

2.2.2. Study Design. This study was open label, single arm, representing a phase 1 study for the safety evaluation of the P2Et in healthy subjects, with dose escalation to establish the maximum tolerated dose (MDT) and toxicity at dose limits (TDL). A classic 3 + 3 design was used for dose escalation (Figure 1).

Dosage escalation of P2Et was planned with 4 dosage levels: 600 mg, 1200 mg, 2400 mg, and 4800 mg per day. At least 3 different subjects would have to be enrolled at each dose level. If TDL was not observed in the first 3 subjects treated at 28 days, they would proceed to escalation to the next dose level. If one or more TDL occurred at any dose level in a subject, 3 subjects would be added to that cohort. Any dose level at which 2 out of 6 subjects experienced a TDL was considered intolerable, and MDT would be considered if escalation was discontinued. In the case that the MDT was observed in the lowest dose level, and according to the clinical toxicity events, this could be set as the MDT.

2.2.3. Study Medication/Herbal Extract. Pods of C. spinosa (Feuillé ex Molina) Kuntze (Divi-divi or tara) were collected in Villa de Leyva, Boyacá, Colombia, and identified by Carlos Alberto Parra of the National Herbarium of Colombia (copy number of voucher COL 588448). The P2Et
is produced as described from the pods of *C. spinosa* [1, 16]. Briefly, fresh pods from *C. spinosa* were dried under airflow in a solar oven at 35°C and ground down to obtain plant material dried. This plant material was certified according to FDA Guidelines for Herbal Medicinal Products. Subsequently, the plant material was extracted with ethanol (96%) in a recirculating percolator. The ethanol crude extract was concentrated under vacuum and trapped on silica gel, and excess humidity was removed at 25°C. Afterward, the ethanol extract was fractionated with ethyl acetate. This herbal drug is manufactured in a validated production process under GMP conditions in LABFARVE laboratories. The standardization of the extract was carried out according to the FDA Guidance for Herbal Drugs [17]. For the standardization, multiple compounds derived from gallic acid were identified such as ethyl gallate, methyl gallate, ethyl 4,5-digaloyl quinate, methyl 4,5-digaloyl quinate, ethyl 3,5-digaloyl quinate, 4,5-digaloylquinic, ethyl 3,4,5-trigaloyl quinate, ethyl 5-galloyl quinate, among others [5] (Figure 2). Three main compounds were used for the normalization of P2Et: Ethyl gallate, methyl gallate, and gallic acid. Ethyl gallate is between 71.45 and 153.49 µg/mL, methyl gallate is between 7.89 and 27.30 µg/mL, and gallic acid is between 11.34 and 98.54 µg/mL. Also, the cytotoxic activity is measured in 4T1 and MCF-7 cell lines, where the CI50 could not be higher than 50.81 µg/mL and 132.75 µg/mL for each cell line, respectively.

With these specifications and the standardized production, qualitative and quantitative identification of these molecules is carried out in each batch produced, guaranteeing high batch-to-batch consistency for P2Et. The studies of natural and accelerated stability of P2Et were carried out according to the ICH and WHO regulations for zone IVB [10], where the P2Et is stable for at least 12 months, maintaining chemical and therapeutic integrity. To start producing a batch, plant material and P2Et must meet quality controls established by regulatory guide 2266 of 2004 of herbal drugs in Colombia, article 561 from USP 34 NF 29, and WHO guideline "Quality Control Methods for Herbal Materials" and FDA guideline "Botanical Drug Development Guidance for Industry."

In this trial, the P2Et was used in a dosage capsule of 600 mg of active ingredient with starch as excipient to be taken daily and manufactured in LABFARVE laboratories under GMP conditions.

The presentation of extract was a bottle with 28 capsules, with a label according to requirements of the local regulation for clinical trials. The investigational product was stored in the research center from Hospital Universitario San Ignacio at a temperature below 30°C and humidity below 70.0 guaranteeing the stability.

2.2.4. Tolerability and Safety Assessment. Adverse events, tolerability, and safety were evaluated based on the National Institute of Health Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [18] at the visit day 1 or baseline, day 8, day 15, and day 28, or final visit, which included clinical follow-up, measuring vital signs (pulse, blood pressure), and physical examination (including weight and height). The clinical laboratory tests were performed at baseline and final visit in the laboratory of the Hospital Universitario San Ignacio. The safety assessments included kidney, hepatic, electrolyte, metabolic, and hematological parameters (Table 1).

All adverse events that occurred during the trial were recorded along with the duration, severity, and relationship to the P2Et. TDL was considered as any grade 3 or greater hematological and nonhematological toxicity, observed during the 28 days of treatment according to CTCAE version 4.0. The tolerability and safety profile were assessed by the clinical team; they classified the laboratories out of range and if they had clinical and safety implications on the volunteers.

The evaluation of immunological parameters was included given background of the P2Et, which was assessed immunophenotype in peripheral blood, protein...
2.2.5. Statistical Analysis. An intention-to-treat analysis was performed using SPSS, version 23. Descriptive analysis methods were used for demographic data and vital signs. Frequencies and percentages for the analysis of adverse events were recorded. Data from the safety and immunological laboratories were analyzed with mean, standard deviation, in some cases median with ranges, and the percentage of change from the baseline visit to completion. \( p < 0.05 \) is considered significant.

3. Results

3.1. Preclinical Toxicity Results. There was no statistical difference between initial and final weights and food intake in rats and rabbits between the groups (Table S1). Also, there was no difference in the absolute and relative weights of liver, spleen, and kidneys in both groups. The analysis of the

| Table 1: Safety assessments: kidney, hepatic, electrolyte, metabolic, and hematological parameters. |
|-------------------------------------------------|-------------------------------------------------|
| Baseline visit \((n = 7)\) | Extract P2Et 600 mg/d \((n = 7)\) | \% Change |
|----------------------------------|----------------------------------|----------|
| Urea nitrogen blood (mg/dL)     | 13.7 [11.1, 20.4] | 13.0 [9.10, 23.7] | 2.36 [−20.2, 73.0] |
| Serum creatinine (mg/dL)        | 0.870 [0.630, 1.09] | 0.860 [0.650, 1.14] | −1.15 [−9.09, 10.7] |
| AST (U/L)                       | 20.0 [12.0, 22.0] | 22.0 [13.0, 50.0] | 8.33 [−11.1, 127] |
| ALT (U/L)                       | 19.0 [14.0, 23.0] | 21.0 [14.0, 44.0] | 13.3 [−21.1, 214] |
| Total bilirubin (mg/dL)         | 0.690 [0.470, 1.08] | 0.660 [0.420, 0.920] | −8.33 [−28.6, 58.6] |
| Sodium (mmol/L)                 | 139 [136, 141] | 138 [136, 141] | 0.00 [−2.16, 0.735] |
| Potassium (mmol/L)              | 4.40 [4.00, 4.80] | 4.50 [4.00, 5.00] | 0.00 [−6.98, 12.5] |
| Calcium (mg/dL)                 | 9.50 [9.10, 9.70] | 9.50 [9.00, 9.70] | 0.00 [−5.26, 4.40] |
| Magnesium (mg/dL)               | 2.00 [1.80, 2.10] | 2.00 [1.80, 2.10] | 0.00 [−5.26, 11.1] |
| Plasma glucose (mg/dL)          | 84.0 [78.0, 99.0] | 88.0 [78.0, 96.0] | 1.18 [−3.03, 4.76] |
| Total cholesterol (mg/dL)       | 170 [122, 250] | 178 [98.7, 226] | −3.47 [−19.4, 4.66] |
| Triglycerides (mg/dl)           | 69.7 [47.7, 147] | 70.2 [40.4, 161] | −6.33 [−48.7, 84.9] |
| Sht (Uu/mL)                     | 1.24 [0.690, 3.42] | 1.44 [0.630, 2.09] | −8.70 [−38.9, 55.6] |
| C-reactive protein (mg/dL)      | 0.100 [0.03, 0.750] | 0.180 [0.05, 2.17] | 133 [−64.0, 570] |
| Lactate dehydrogenase (U/L)     | 159 [130, 174] | 161 [128, 210] | 3.12 [−4.47, 31.8] |
| Hemoglobin (g/dl)               | 14.7 [13.6, 17.9] | 14.5 [14.1, 16.7] | 0.621 [−6.70, 6.62] |
| Hematocrit (%)                  | 43.0 [40.4, 51.0] | 43.1 [41.0, 47.9] | 1.05 [−7.06, 5.12] |
| Medium corpuscular volume (fL)  | 89.0 [87.1, 97.2] | 88.3 [87.4, 98.2] | 0.344 [−2.97, 1.03] |
| Mean corpuscular hemoglobin (pg) | 30.5 [29.6, 33.3] | 30.6 [29.7, 33.8] | 0.338 [−1.98, 1.50] |
| Mean corpuscular hemoglobin concentration (g/dl) | 34.0 [33.5, 35.1] | 34.1 [33.1, 35.2] | 0.285 [−2.93, 2.67] |
| MPV (fL)                        | 7.10 [6.90, 9.20] | 7.50 [6.70, 9.00] | 1.35 [−3.80, 7.04] |
| Erythrocyte sedimentation rate (mm/h) | 2.00 [2.00, 10.0] | 5.00 [2.00, 16.0] | 0.00 [−50.0, 700] |

Data are in median [minimum, maximum] range; \((n = 7)\). AST, aspartate aminotransferase; ALT, alanine aminotransferase; SHT, stimulating hormone thyroid; MPV, medium platelet volume.
results of hemogram (erythrogram and leucogram), coagu- 
gulogram, and kidney function indicate the absence of re- 
levant alterations in rats and rabbits (Tables 2–3). There 
were no systemic clinical signs of toxicity during 28 days 
of observation, and macroscopic findings in the necropsy 
were not observed between groups. These results showed that no 
observed adverse effect’s level (NOAEL) for P2Et is 1000 mg/ 
kg for rabbits and rats. 
The rabbit’s weight was used for the calculation of the 
starting dose in the clinical trial as we did not find differences 
between rabbits and rats; we selected rabbits as superior 
specie. Once a starting dose level has been determined, other 
levels of doses were duplicates. The dose calculated was 
600 mg/d for 28 days in the clinical trial. 
The P2Et was previously assessed for genotoxicity and 
mutagenicity activity in a previous study. It did not show 
genotoxicity and mutagenicity activity [16].

3.2. Clinical Results

3.2.1. Subject Characteristics. Eleven subjects were screened, 
of which 7 were enrolled in the study at the first dosage level. 
3 subjects completed 600 mg/d level and the remainder were 
discontinued from the study due to adverse events with a 
reasonable possibility of relationship to the investigational 
product.

The maximum sample size planned was between 12 and 
24 if we could scale the doses level, but TDL was observed at 
the first dose level; 4 volunteers showed adverse events and 
discontinued the intervention; considering that at any dose 
level at which at least 2 subjects experienced TDL was 
considered intolerable, we set the MDT at the first dose level 
(600 mg/d). Table 4 summarizes the main baseline charac- 
teristics of the subjects. The range of exposure to the in- 
vestigational product was between 2 and 28 days. The 
adherence during the exposure period was greater than 80%.

3.2.2. Safety. The MTD of the P2Et was 600 mg/d. In the first 
cohort of 3 subjects, 1 presented TDL requiring the inclusion 
of 3 additional subjects, of which 2 subjects presented TDL. 
The TDL reported in these 3 subjects was defined by the 
investigator since the adverse events were clinically signif- 
icant and led to the discontinuation of these study subjects.

A total of 42 nonserious adverse events (AEs) were 
reported; the majority of AEs were grade 1 in severity (95%) 
according to CTCAE and a reasonable possibility of a re- 
lationship with the P2Et. There were no serious adverse 
events (SAEs) in the subjects participating in the clinical 
study. The most frequent AEs were gastrointestinal events 
such as epigastralgia and nausea in 23.8%; only one subject 
had a slight increase in AST who recovered without sequelae. 
The detail of the incidence of reported adverse events is 
described in Table 5.

No changes outside the normal range were observed 
from baseline to final visit in vital signs and physical ex- 
amination in all subjects. Cardiovascular function did not 
present any alteration in most of the subjects, except for one 
subject who presented an abnormality in the 
electrocardiogram with a marked sinus bradycardia, in- 
complete right bundle branch block, and left fascicular block 
since the baseline visit, being classified as clinically not 
significant and considered as a medical history.

The hematological parameters evaluated were within 
normal ranges at the initial and final visit, except for LDH, 
which was found with a median below the lower normal 
limit, as well as the mean platelet volume (MPV). In the 
evaluation of renal function, no changes outside the normal 
range were observed, only a slight increase in the upper limit 
of the range of the median blood urea nitrogen at the initial 
and final visit. Likewise, hepatic, electrolyte, and metabolic 
function remained within normal ranges; only the AST level 
at the completion visit showed a slight increase in the upper 
limit of the median range. Table 1 shows the changes in 
blood chemistry markers evaluated at the initial and final 
visit.

3.2.3. Immunological Parameters. As seen in Table 6, the 
results of the peripheral blood immunophenotype for en- 
rolled subjects since baselines to end visit showed a median 
frequency of LT CD3+/CD5+/CD45+, CD3+/CD4+, and 
CD3+/CD8+ below the normal lower limit at both times. The 
above was not observed in the absolute count of these cell 
populations.

The count of LT CD4+/CD8+ presented a 40% of 
median of percentage of change between baseline and 
final visit; relation CD4/CD8 was preserved. Population 
of B lymphocytes (LB) CD19+/CD20+/CD38+/CD45+ had 
a median below the normal lower limit in basal and final 
visit, with a percentage of change lower than 20%. Ad- 
ditionally, a percentage of positive change from the initial 
to final visit of 66% was observed in the median per- 
centage of monocyte-derived dendritic cells CD45 ++/CD38 + /CD4 –. Other cell populations assessed were 
within the normal range.

In protein electrophoresis, the mean of the alpha 1 
fraction remained outside the normal range (1% to 2.8%) 
between baseline (3.49%) and final visit (3.84%), with a 
percentage change of 10.6%. The levels of the different 
immunoglobulins evaluated and the level of complement 
were in normal limits in both visits.

4. Discussion

Polyphenols have been used in healthy humans for the 
prevention of diseases such as cancer, cardiovascular dis- 
eease, diabetes, neurological disorders, infections, among 
others. The above, given its antioxidant, anti-inflammatory, 
antiallergy, and even immunomodulatory effects [19–26], is 
also useful for the treatment of some respiratory diseases 
[27].

Although its potential for use is extensive, the side effects 
generated by the consumption of natural products prepared 
in the form of standardized extracts have not been sys- 
tematically evaluated. The development of formal clinical 
studies makes it possible to strengthen knowledge on dosage, 
population characteristics, and possible adverse events and
Table 2: Effects of P2Et extract on biochemical parameters and hematological parameters in rats following 28 days of administration.

| Parameters (reference values) | Test (N = 10) | Control (N = 10) | p value |
|-------------------------------|--------------|----------------|--------|
| **Kidney function**           |              |                |        |
| Urea (18.0 to 45.0 mg/dL)     | 46.42 ± 7.04 | 49.15 ± 4.64   | 0.53   |
| Creatinine (0.05 to 0.65 mg/dL)| 0.28 ± 0.03  | 0.29 ± 0.04    | 0.54   |
| Sodium (135.0 to 146.0 mmol/L)| 144.47 ± 1.74| 144.27 ± 4.09  | 0.52   |
| Potassium (4.0 to 5.9 mmol/L) | 6.487 ± 0.55 | 7.87 ± 0.98    | 0.48   |
| Chloride (96.0 to 107.0 mmol/L)| 103.63 ± 1.62| 99.66 ± 3.12   | 0.60   |
| Calcium (5.3 to 11.6 mg/dL)   | 10.70 ± 0.63 | 10.99 ± 0.75   | 0.73   |
| **Liver function**            |              |                |        |
| ALT (20 to 61 U/L)            | 59.30 ± 10.89| 78.2 ± 12.29   | 0.12   |
| Phosphatase (16 to 302 U/L)   | 214.90 ± 54.03| 300.30 ± 91.81| 0.08   |
| AST (39.0 to 111.0 U/L)       | 123.30 ± 14.79| 209.00 ± 71.43| 0.16   |
| GGT (0 to 6 U/L)              | 4.20 ± 0.63  | 4.40 ± 0.97    | 0.70   |
| Bilirubin (0.1 to 0.7 mg/dL)  | 0.34 ± 0.14  | 0.54 ± 0.10    | 0.61   |
| Cholesterol (20 to 92 mg/dL)  | 5.92 ± 0.43  | 6.28 ± 0.42    | 0.70   |
| Triglycerides (27 to 108 mg/dL)| 111.28 ± 46.38| 116.65 ± 25.88| 0.24   |
| **Erythrogram**               |              |                |        |
| Erythrocytes (5.1 to 7.9 millions/mm³)| 6.06 ± 0.63| 6.06 ± 0.75 | 0.76   |
| Hemoglobin (10.0 to 17.4 g/dL) | 13.36 ± 1.05 | 12.98 ± 1.13  | 0.45   |
| Hematocrit (33.0 to 50.0%)    | 42.16 ± 2.74 | 40.80 ± 3.05  | 0.88   |
| MVC (57.8 to 66.5 uI)         | 64.94 ± 2.40 | 57.83 ± 5.95  | 0.87   |
| MHC (17.1 to 23.5 pg)         | 20.54 ± 0.67 | 21.55 ± 1.45  | 0.49   |
| MCHC (9.0 to 37.0 g/dL)       | 31.68 ± 1.04 | 31.85 ± 2.07  | 0.33   |
| Total protein (5.4 to 8.5 g/dL)| 5.92 ± 0.33 | 6.10 ± 0.45   | 0.38   |
| **Leukogram**                 |              |                |        |
| Leukocytes (5.2 to 12.5 thousands/mm³)| 4.48 ± 1.15| 5.95 ± 2.01 | 0.33   |
| Neutrophil (20 to 75%)        | 5.56 ± 1.06  | 63.67 ± 7.89  | 0.46   |
| Eosinophil (1 to 4%)          | 0.00 ± 0.00  | 0.17 ± 0.41   | 0.19   |
| Lymphocytes (30 to 83%)       | 41.80 ± 18.05| 33.00 ± 8.15  | 0.30   |
| Monocytes (1 to 4%)           | 2.60 ± 1.14  | 3.17 ± 1.47   | 0.11   |
| Platelets (250 to 650 thousands/mm³)| 418.60 ± 102.50| 473.67 ± 76.62| 0.52   |
| **Coagulogram**               |              |                |        |
| PT (7.2 to 7.8 seconds)       | 7.44 ± 0.32  | 7.35 ± 0.26   | 0.44   |
| PTT (35 seconds)              | 21.38 ± 1.09 | 22.73 ± 2.23  | 0.38   |

N: number of animals in the group; data expressed as mean ± standard deviation. *Statistically significant difference p < 0.05.

As a result of the preclinical toxicity study, 600 mg/d was defined as the MDT of the P2Et in healthy subjects. It was evidenced that gastrointestinal AEs were the main AEs associated with the consumption of P2Et with an incidence of 52% (n = 22/42); the most frequent event was epigastric pain, nausea, constipation, reflux, abdominal distention, and flatulence, with nonserious characteristics, severity grade 1, and 85% with reasonable possibility related to the product. In the same way, other phase 1 clinical trials [29–33] have reported this type of adverse events such as nausea, diarrhea, and mild abdominal pain, with a frequency of 16% to 91% with the use of other polyphenols (turmeric, epigallocatechin gallate, resveratrol, and sahastara) in healthy humans; the above could have an impact on adherence and quality of life of people due to adverse event severity.

In addition to the above, other types of adverse events were reported in various functional systems in 48% (n = 20/42) such as myalgia, asthenia, adynamia, and respiratory symptoms (odynophagia, rhinopharyngitis, and cough). Likewise, dermatological and central nervous system adverse events such as pruritus, dermatitis, dizziness, headache, and drowsiness were reported in other clinical studies of polyphenols in healthy subjects with a frequency greater than 30%, which seem to be signs of toxicity that they are not normally associated with this type of natural product [29, 32, 34–36]. Thus, there is great diversity in the adverse events that polyphenols can cause in healthy humans, which may be associated with their route of administration, duration of consumption, and the metabolism of each of the polyphenols.

The analysis of hepatic function parameters did not show significant alterations, only one outside the normal range at the limit of the maximum range of the median in the AST level, taking into account that only one subject had an AST increase during the study that was resolved without complication and sequel.

Alterations in hepatic function are the most severely monitored toxic effects that can occur in healthy people who
of resveratrol and most of them associated with the use of high doses in fact, these hepatic abnormalities are reflected in increased drug-induced liver injury in the United States. In the phenomenon of herbal supplements has been reported, significant increase in cases of hepatic damage attributed to consumption of polyphenols or phytomedicines, even without the most frequent alteration, given the severity of its appearance, it must always be monitored and taken into account for the safety evaluation after the consumption of polyphenols in healthy humans.

In the same way, kidney function can be affected by the consumption of polyphenols since during the metabolism, some of its products can generate kidney damage; however, in this study only levels of urea nitrogen were evidenced outside the normal range in the limit of the maximum range of the median, which was not considered significant. There are some intrinsic factors that can increase the risk of kidney injury, such as the quality of polyphenol manufacture, overdose, interactions with other medications, and pathological conditions of the individual, including gender and age. For example, increased levels of urea nitrogen and creatinine have been linked to the use of high doses of polyphenols such as sahastara, Crocus sativus, and Bacopa monnieri. Other polyphenols have not shown alteration in kidney function or in some studies it is un-known because it was not evaluated.

The mean of the metabolic parameters evaluated in this study was within the normal range, with the exception of

| Parameters and reference values | Test (N = 5) | Control (N = 6) | p value |
|---------------------------------|-------------|----------------|---------|
| **Kidney function**             |             |                |         |
| Urea (13.0 to 52.0 mg/dL)        | 42.32 ± 11.9 | 43.68 ± 6.98   | 0.66    |
| Creatinine (0.80 to 1.80 mg/dL) | 1.34 ± 0.46  | 1.19 ± 0.33    | 0.91    |
| Sodium (138.0 to 148.0 mmol/L)  | 142.48 ± 1.18| 144.35 ± 3.76  | 0.93    |
| Potassium (3.3 to 6.9 mmol/L)   | 4.81 ± 0.46  | 4.78 ± 0.47    | 0.55    |
| Chloride (92.0 to 112.0 mmol/L) | 99.64 ± 2.55 | 98.38 ± 4.56   | 0.58    |
| Calcium (5.6 to 12.0 mg/dL)     | 12.86 ± 0.32 | 12.88 ± 0.41   | 0.93    |
| ALT (31 to 60 U/L)              | 162.00 ± 111.60 | 108.17 ± 21.21 | 0.62    |
| Phosphatase (90 to 145 U/L)     | 145.00 ± 32.52 | 137.33 ± 44.53 | 0.50    |
| AST (42.0 to 98.0 U/L)          | 103.80 ± 95.05 | 95.50 ± 53.78  | 0.65    |
| GGT (4 to 12 U/L)               | 6.20 ± 2.49  | 6.00 ± 1.67    | 0.81    |
| Bilirubin (0.30 to 0.80 mg/dL)  | 0.44 ± 0.07  | 0.53 ± 0.10    | 0.23    |
| Cholesterol (35 to 60 mg/dL)    | 44.00 ± 0.00 | 43.67 ± 0.52   | 0.26    |
| Triglycerides (124 to 156 mg/dL)| 108.34 ± 75.81 | 76.17 ± 37.28  | 0.81    |
| **Erythrocytes (5.1 to 7.9 millions/mm³)*** | 6.06 ± 0.63  | 6.06 ± 0.75    | 0.76    |
| **Hemoglobin (10.0 to 17.4 g/dL)** | 13.36 ± 1.05 | 12.98 ± 1.13   | 0.45    |
| **Hematocrit (33.0 to 50.0%)**  | 42.16 ± 2.74 | 40.80 ± 3.05   | 0.88    |
| **MVC (57.8 to 64.5 µ/μ)**      | 64.94 ± 2.40 | 67.83 ± 5.95   | 0.87    |
| **MHC (17.1 to 23.5 pg)**       | 20.54 ± 0.67 | 21.55 ± 1.45   | 0.49    |
| **MCHC (29.0 to 37.0 g/dL)**    | 31.68 ± 1.04 | 31.85 ± 2.07   | 0.33    |
| **Total proteins (5.4 to 8.5 g/dL)** | 5.92 ± 0.33 | 6.10 ± 0.45   | 0.38    |
| **Leukocytes (5.2 to 12.5 thousands/mm³)** | 4.48 ± 1.15 | 5.95 ± 2.01   | 0.33    |
| **Neutrophil (20 to 75%)**      | 55.60 ± 17.10 | 63.67 ± 7.89 | 0.46    |
| **Eosinophil (1 to 4%)**        | 0.00 ± 0.00  | 0.17 ± 0.41    | 0.19    |
| **Lymphocytes (30 to 85%)**     | 41.80 ± 18.05 | 33.00 ± 8.15  | 0.30    |
| **Monocytes (1 to 4%)**         | 2.60 ± 1.14  | 3.17 ± 1.47    | 0.11    |
| **Platelets (250 to 650 thousands/mm³)** | 418.60 ± 102.50 | 473.67 ± 76.62 | 0.52    |

N: number of animals in the group, data expressed as mean ± standard deviation. *Statistically significant difference p < 0.05.

Table 4: Baseline characteristics in healthy volunteers.

| Level 1 600 mg/d, n = 7 |
|-------------------------|
| N (%)                   |
| Male                    | 7 (100)       |
| Female                  | 4 (57.1)      |
| Age (years, mean ± SD)  | 33.3 (7.76)   |
| Weight (kg, mean ± SD)  | 62.6 (13.0)   |
| Height (mt, mean ± SD)  | 1.68 (0.05)   |
| Systolic blood pressure (Hg/mm, mean ± SD) | 107 (8.41) |
| Diastolic blood pressure (Hg/mm, mean ± SD) | 74.3 (7.87) |
| Heart rate (beats per minute, mean ± SD) | 75.7 (13.5) |

consume polyphenols or phytomedicines, even without having risk factors for hepatic disease. In recent years, a significant increase in cases of hepatic damage attributed to the phenomenon of herbal supplements has been reported, being the second most frequent (16.53%) after antibiotics to cause drug-induced liver injury in the United States. In fact, these hepatic abnormalities are reflected in increased levels of total bilirubin, AST, and alkaline phosphatase [29, 33], most of them associated with the use of high doses of resveratrol and Bacopa monnieri and self-limited with the discontinuation of their consumption. Therefore, hepatic toxicity seems to be dose dependent and although it is not very frequent and depend on other factors, should be closely monitored when using this kind of products.

The mean of the metabolic parameters evaluated in this study was within the normal range, with the exception of...
total cholesterol, which was reported slightly outside the maximum range of the median from the initial visit persisting until the end visit, without a significant change percentage. Similarly, there was a positive percentage change of 18% in median HDL from baseline to final visit. Given the effects on the prevention of cardiovascular disease of some polyphenols, there are studies that report a benefit such as a decrease in total cholesterol and triglycerides and an increase in HDL [25, 32]. In contrast, some clinical studies have not reported changes in lipid profile [39, 42–44] and even negative effects such as that high doses of carvacrol induced a decrease in HDL and an increase in triglycerides [34].

Thus, the data associated with the possible effect on lipid metabolism of polyphenols in healthy subjects are not conclusive and are divergent, suggesting that the differences, not only in these parameters, but in those previously mentioned, may be due to molecular diversity of polyphenols, as well as the variations inherent in the populations studied.

Now, part of the secondary endpoints of the study was the evaluation of immunological parameters, taking into account the preclinical history of immunomodulation of the P2Et. The quantification of the lymphoid and myeloid population remained within normal ranges, as were the levels of IgM, IgA, IgG, and complement C3 protein, with the exception of the percentage of LT CD3+/CD5+/CD45+; CD3+; CD5+; CD4+; CD3+; CD8+; and LB CD19+; CD20/CD38; CD45+ that were reported below the lower normal limit from the baseline to final visit, with percentages of positive change less than 30%. It is possible that the normal values of our population are different from those currently used by the clinical reference laboratory.

Additionally, there was a 66% positive change percentage from the initial to final visit in the median percentage of monocyte-derived dendritic cells CD45++/CD38+/CD4+. In the same way, the absolute value of these cells also showed an increase from the baseline to the end visit with a change percentage of 34%. The aforementioned findings were not clinically significant, but they show a considerable increase in the dendritic cell population when the P2Et is consumed. These findings could suggest an increase in the production of their precursors in the bone marrow, in the generation of DCs derived from monocytes, or in their maturation; however we do not know the real origin of this increase and its impact on the functionality of these cells after the use of P2Et. However, this immunostimulatory effect of increasing antigen-presenting cells in healthy humans could be associated with a state of readiness for a more effective immune response in case of exposure to antigens. In the case of P2Et, congruence between preclinical data in animals and clinical data in healthy subjects is evidenced, confirming its immunomodulatory effect. In fact the P2Et has shown anti-tumor and immunomodulatory activity in some types of cancer such as breast cancer, leukemia, and melanoma [1 3–7, 45] and its administration also modulates the immune response of healthy mice [4]. In humans, it is still controversial. It is possible that the effects of certain polyphenols on the immune system may depend on the type of polyphenol,
its dosage, and exposure time. There are other polyphenols for which their immunomodulatory activity has been studied in healthy people, finding a significant decrease in total LT, LT CD8+, and NK [46]; or on the contrary, others present an immunostimulatory effect with an increase in cytotoxic LT, NK, and LB [47–49].

5. Conclusions

The P2Et consumed was safe for use in healthy humans with a maximum tolerated dose of 600 mg/d. There was no severe toxicity, only mild adverse events for the most part, with no significant out-of-range changes in the safety parameters evaluated. Changes in immunological parameters were observed that could suggest the immunomodulatory activity of P2Et also observed in animal models. Levels higher than 600 mg/d were not reached because of the gastrointestinal events showed for the volunteers at the first dose level. Despite the number of recruited volunteers, the MDT was set at 600 mg/d according to the 3+3 design for clinical trials. The foregoing will allow continuing with the clinical development of the extract given its preclinical history; it could be in a population with cancer, diseases with an immunological component, and even infectious diseases.

Data Availability

The data used to support the findings of this study are available from the corresponding author on reasonable request.

Disclosure

An earlier version of the manuscript was presented as a poster in "Integrative Medicine for Cancer Prevention and Cancer Patient Management October 27–30 2020."

Conflicts of Interest

SF and CU are inventors of a granted patent related to P2Et. SF, CU, and RB are partners of the DreemBio Company that...
has a licensee of related patents. The rest of the authors declare no conflicts of interest.

Acknowledgments

The authors want to express their gratitude to the volunteers who participated in this clinical trial. This project has the authorization of the Ministry of the Environment, for the use of genetic resources through the Contract of Access to Genetic Resources No. 220 of 2018 for Colombian plant material. The research received financial support from Fondo de Ciencia y Tecnología del Sistema General de Regalías of the Republic of Colombia, under Grant 2013000100196. RB was funded in the last months for the writing of the article by the Ministerio de Ciencia, Tecnología e Innovación (Colciencias), Ministerio de Educación Nacional, Ministerio de Industria, Comercio y Turismo e ICETEX (792–2017 2° Convocatoria Ecosistema Científico–Colombia Científica para la Financiación de Proyectos de I + D + I), and Vicerrectoría de Investigaciones, Pontificia Universidad Javeriana, Bogotá, Colombia (contract no. FP44842-221-2018).

Supplementary Materials

Table S1. Mean intake of food/water as well as initial and final body weight in rats and rabbits following 28 days of administration. (Supplementary Materials)

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