DIURETIC EFFECT FROM THE LEAVES OF MAYTENUS MACROCARPA "CHUCHUHUASI" IN ALBINE RATS

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ABSTRACT

Objective: To evaluate the diuretic activity of the aqueous extract obtained from the leaves of Maytenus macrocarpa "Chuchuhuasi" in albino rats.
Material and Methods: 68 male albino rats were used, with average weight of 250 g. The Lipschitz method was used, recording the urine volume per hour and the total volume at the sixth hour. The experimental groups were: control (saline 0.9%), chuchuhuasi 1 (250mg / kg), chuchuhuasi 2 (500 mg / kg), chuchuhuasi 3 (750mg / kg), chuchuhuasi leaves 4 (1000 mg / kg), furosemide 1 (10mg / kg) and furosemide 2 (20mg / kg). For statistical validation Shapiro-Wilk, Turkey and Dunns tests were used.
Results: The largest total volume obtained during the first 24 hours was from the chuchuhuasi 1000 mg/ kg group with 5,17 ml. This was an even greater result than the one obtained with furosemide 20 mg/kg. On the other hand, chuchuhuasi 250 mg/kg group (2.32 ml) achieved less diuretic action than the control group (2,55 ml). Tukey's test indicated no significant difference. Nevertheless, the Xi squared test's results from the biochemical analysis got a p< 0.05 for pH.
Conclusion: A positive diuretic response it's demonstrated in all dose levels tested using ethanolic extract of Maytenus macrocarpa: 250mg/kg, 500mg/kg, 750mg/kg and a greater effect of 1000 mg/kg compared with the control group and furosemide.
Keywords: Diuresis, Furosemide, Traditional Medicine, Ethnopharmacology.

INTRODUCTION

According to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), two thirds of the worldwide population 4 billion people use plants for medical intent (1,2).
Chuchuhuasi, scientifically called *Maytenus macrocarpa* according to Briquet, it is a large tree that grows in Peru, especially in the Amazon jungle, that has particular botanical characteristics such as: whorled roots, entire and coriaceous leaves, oblong seeds with white aril, etc. (3).
Plants related to the *Maytenus* (Celastraceae) genus, are used in popular medicine for their therapeutic properties; some of their effects are well known, but there are many others that remain unknown and may cause adverse reactions, intoxication and even death. Chuchuhuasi use is known for the treatment of a variety of diseases including rheumatism, arthritis, lower back pain, colds, bronchitis, diarrheas, hemorrhoids, infertility and others, being the relieving of gastric disorders its most common use (4,5). Studies were made in order to find the photochemical components of the plants; two eterpenoids, flavonoid glycosides (6), saponins, steroids, phenolic derivatives, vitamins, starch, maytenine, Proanthocyanidin dimers and friedelane triterpene were found (7); in addition, the antioxidant and analgesic activity of this plant was demonstrated (8-10). The previous data confirms the multiple pharmacological properties of the plant.
Even though *Maytenus macrocarpa* is used as a diuretic in popular medicine, no studies have been conducted to demonstrate this ethnopharmacological statement. This is the reason why the objective of this work was to evaluate the diuretic activity of the aqueous extract obtained from the leaves of *Maytenus macrocarpa* "Chuchuhuasi" in albino rats.

MATERIALS AND METHODS:

Study Design: A quasi-experimental preclinical, longitudinal and prospective double-blind study was conducted during the months of March to October 2014.
Samples:

- **Plant Sample:** *Maytenus macrocarpa* leaves collected in Pucallpa (Ucayali, Peru) were used. The taxonomic certification was done under the Cerrate, E.1969 criteria. The ethanolic extract was extracted from the dried and then ground leaves of *Maytenus macrocarpa*. It was macerated in 70% ethanol for a week, then the mixture was filtered and the residue obtained was dried in an oven for 48 hours. The sample obtained was ground in a mortar to a fine powder that was then stored in airtight containers and refrigerated until use.
- **Animal Sample:** 68 male albino rats *Rattus norvegicus*, whose weights ranged between 200 and 300 grams, from the animal facility of the National Institute of Health were used.

The animals were used by the ethical codes of International Guiding Principles for Biomedical Research Involving Animal (1985)

- **Chemical Sample:** Furosemide in ampoules, batch 071011, sanitary registration (RS) GBE 10008-03-07 (expiration date 08/14).

Diuresis measurement: The animals were kept for 3 days in adaptation to the experimental conditions with a temperature of 27 ± 1 ° C, relative humidity of 60 ± 5% and 12-hour light/dark cycles.

They were housed in aluminum cages with metal grid at 3 animals per cage. They were deprived of food 24 hours before starting the experiment and drinking water an hour before. The rats were placed in metabolic cages and it was proceeded to record the volume of urine excreted at 1, 2, 3, 4, 5, 6 and 24 hours post administration using 2 micropipettes of 5 and 10 ml, depending on the volume of urine to be measured.

Biochemical determinations: Biochemical analysis was performed after a greater volume than 0.5 ml (minimum volume required for the biochemical analysis) was obtained from each rat. Urine reagent test strips were used for this purpose, testing for the presence and levels of bilirubin, urobilinogen, ketones, glucose, proteins, hemoglobin, nitrites, pH and leucocytes in the urine samples.

Groups:

Group 1: Control
Group 2: Furosemide 10 mg/kg (11)
Group 3: Furosemide 20mg/kg (12)
Group 4: Chuchuhuasi (*Maytenus macrocarpa*) 250 mg/kg
Group 5: Chuchuhuasi (*Maytenus macrocarpa*) 500 mg/kg
Group 6: Chuchuhuasi (*Maytenus macrocarpa*) 750 mg/kg
Group 7: Chuchuhuasi (*Maytenus macrocarpa*) 1000 mg/kg

Statistical analysis:

A database in Microsoft Excel was created, including the urine volumes measured and the results of the biochemical analysis. Statistical analysis was performed using the Statistical Packages GraphPrism 5.0 and the Statistical Packages for the Social Sciences (SPSS) for Windows (version 12.0)

Quantitative variables were expressed as mean and standard deviation, in order to observe the volume variation according to the hour the samples were obtained. Additionally, only urine volumes obtained 24 hours after administration were taken in account for the analysis of the variance and correlation, using Shapiro-Wilk's test (to know if the results were in Gaussian distribution) to compare the means using the appropriate test later.

For the results of the biochemical determinations a qualitative analysis via contingency tables was performed. Chi square test was completed for each possible paired combination.

Ethical aspects: This study was approved by the Ethics Committee of the *Universidad de San Martin de Porres* after being evaluated by the *Centro de Investigación de Medicina Tradicional y Farmacológica de la Universidad de San Martin de Porres,* whose members constantly monitored the research process.

RESULTS

Quantitative analysis

The largest total volume obtained during the first 24 hours was 5,17ml for Chuchuhuasi of 1000 mg / kg, getting to have even greater value than furosemide 20 mg / kg. Furthermore, Chuchuhuasi of 250 mg / kg (2.32) group resulted obtaining lower diuretic action than the control (2.55 ml) group, as shown in Table No. 1

The Shapiro-Wilk test showed a non-Gaussian distribution, where quantitative analysis did not show statistical differences by Dunns test, This result was verified by performing the Tuckey test assuming a Gaussian distribution. However,the graph No. 1 shows some differences between the progress of urine volume in 24 hours.

Table 1. Comparison of average volumes of urine at 24 hours

Groups	Solution	Min	Max	Mean	SD
G1	Control	0.5	5.8	2.5	2.0
G2	Furosemida 10 mg/kg	1.1	5.7	2.8	1.5
G3	Furosemida 20 mg/kg	1.3	10	4.2	3.2
G4	Chuchuhuasi 250 mg/kg	0.2	8	2.3	2.8
G5	Chuchuhuasi 500 mg/kg	0.5	9.4	3.7	3.4
G6	Chuchuhuasi 750 mg/kg	1.1	5.6	2.9	1.8
G7	Chuchuhuasi 1000 mg/kg	2	7	5.1	2.0

* The Shapiro Wilk test showed no Gaussian distribution

** The Tukey test showed no statistical difference in any relation.

Qualitative analysis

The chi-square test using table in biochemical results gave us a $p < 0.05$ value for the pH between the control group and

the Chuchuhuasi group of 750 mg / kg (p = 0.036), and the group of furosemide 20 mg / kg and Chuchuhuasi 750 mg / kg (p = 0.027). Also obtained by the same test, p <0.05 value for leukocytes from the groups: furosemide 10 mg / kg and Chuchuhuasi 250 mg / kg (p = 0.036), furosemide 10 mg / kg and Chuchuhuasi 500 mg / kg (p = 0.030), furosemide 10 mg / kg and Chuchuhuasi 1000 mg / kg (p = 0.009).

DISCUSSION

In the present study a dose-response diuretic effect of Furosemide between 10 and 20mg/kg was evidenced, as was a superior diuretic action compared to the one obtained in the control group, which only received saline solution (ClNa0.9%). These results confer validity to the methodology used as well as our results, because it shows normal physiological conditions in the animals studied.

Among the results of Maytenus macrocarpa, the 1000 mg/kg dose resulted in greater diuresis at 24 hours than the one obtained with the 250mg/kg dose, 500mg/kg dose and even with the 750mg/kg dose, which gives an outline of a possible range of peak response. We must emphasize that the effect of this dose also exceeded the effect given by Furosemide 10 mg/kg and Furosemide 20 mg/kg in 24 hours.

Furosemide has a half-life of 30 minutes, an effect duration of 8 hours depending on the dose and a 50% bioavailability (5). A similar effect had Xanthiumstru marium L. at 400mg/kg dose in relation to Furosemide at 5mg/kg dose, which reached a superior diuretic effect of the drug since the 1st hour of application (13). Unlike C. alata and P.americana, which managed to equal the Furosemide's diuretic power at 5 hours of being applied (14). Another study concludes that Maytenus krukovii at a 1000 mg/kg dose also has a hypotensive activity, giving evidence of a possible diuretic effect of this plant, which belongs to the same genre of the one studied in this work. (15)

We can notice that the 1000mg/kg dose of Maytenus macrocarpa has a long term diuretic activity, similar to Furosemide which could indicate that the mechanism of action also has a similarity.

The Furosemide's main mechanism of action is blocking the Na+/K+/2Cl- symporter pump in the thick ascending limb of the loop of Henle, which alters the flow of ions and water across the cell membranes, this process increases the sodium, potassium and chloride excretion and concomitantly generates water excretion, which explains the diuretic effect.

Additionally, there is a change in the electrical potential of the cell which disturbs the dynamics of the Ca 2+ and Mg 2+ transportation (8), it is also thought that it produces the Na+/K+/2Cl- symporter inhibition or the carbonic anhydrase inhibition in the epithelial cells, inflammatory cells or cholinergic neural pathways (9). Furosemide is subtype specific noncompetitive blocker for the GABA-A receptor that gets through a process of glucuronization and is a reversible antagonist of the evoked streams of GABA- a62. (16)

It had been recognized that the main active metabolites present in medicinal plants that may have diuretic effect are: essential oil, saponoside, potassium salts, and flavonoids, the latters were found in Maytenus macrocarpa in previous studies. Their main site of action is the glomerulus, rather than the tubuli. This causes an increase of the renal circulation and an increased glomerular filtration rate and urine primary formation. It is also considered that cardiotonic heterosides could be involved in the diuretic activity. (1-9)

About the biochemical analysis, the significant difference in pH indicates an acidification effect on urine, this is explained by the Furosemide inhibitory effect on the Na-K-2Cl symporter in the thick ascending limb of the loop of Henle, and it would also improve the H+ and K+ secretion in the cortical collecting ducts by producing high luminal electronegativity (17,18).

The means obtained from groups administrated with Chuchuwasi with doses of 250, 500, 750 and 1000 mg/kg also indicate an effect of urinary acidification. The mechanism of this effect may be related to the inhibitory property of Maytenus macrocarpa on prostaglandins (19), given it has been described to be many receptors for these located in kidneys (20). However, until now there are no direct studies that show the effect of Maytenus macrocarpa on urinary pH.

We suggest that this scientific evidence can be taken into account and applied to medical praxis, this is explained by the fact that a large amount of population consumes phytodrugs believing they are free of adverse effects or drug interaction, thus exposing themselves to renal insufficiency and hypotension due to sodium depletion (7) and the consequent hypovolemia, caused by ACE inhibitor interaction, digitalis intoxication by digoxin interaction, ototoxicity and nephrotoxicity by the use of amphotericin B and other otologic drugs which would have a potentiated effect, amidst others (21, 22).

From our results we suggest to continue the preclinic research of this plant. More studies aimed to determine the mechanisms of action, the interactions of this plant with commonly used drugs and the isolation of active principles of Maytenus macrocarpa(13,14) are required.

At the end of the investigation we could conclude that the diuretic effect from the ethanolic extract of Maytenus macrocarpa is evidenced in this research with positive diuretic outcome in all levels of doses applied: 250, 500, 750 and 1000mg/kg in comparison to the control and furosemide groups.

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