

Effectiveness of using Renalof® in the Elimination of Kidney Stones Under 10 mm Located in the Renal-Ureteral Tract

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Abstract

Background

In view of the high recurrence of kidney stones in patients, the search for less aggressive, preventive treatments has become increasingly essential. Renalof® offers a phytotherapy alternative. Due to its diuretic and kidney stone demineralisation properties, it has been widely used in this patient population, disintegrating and eliminating calcium oxalate and struvite kidney stones painlessly in the genitourinary system.

Methods

A Phase II, randomised, prospective, observational, single-blind study with two treatment arms was conducted in order to determine the efficacy of this alternative therapy: a total of 155 patients were enrolled, 120 were assigned to a Renalof® treatment group and 35 to the placebo group.

All were over 18 years of age, of both genders, diagnosed with kidney stones of under 10 mm in diameter, present along the entire renal-ureteral-vesicular tract, diagnosed by ultrasound and renal CAT scan. Divided into two study arms, 120 were administered a dosage of a single 325 mg capsule of the Renalof® product half an hour before the two main meals for 3 months. The presence of kidney stones in any part of the renal-ureteral tract was assessed at monthly consultations using one of the above-mentioned diagnostic tools. The study period ran from August 2019 to July 2020 at the Hospital Universitario Antonio Lenin Fonseca and the Seniors Clinic, in the city of Managua, Nicaragua.

Results

Study results show a high rate of effectiveness with Renalof®, finding up to a 65% expulsion rate (78 patients) in the first 8 weeks of treatment, compared to 11.4% (4 patients) in the placebo control group, $P < 0.001$. It is likely a longer follow-up period would be necessary in patients with kidney stones of 10 mm in diameter or larger than the period applied in the study.

Discussion

We strongly recommend the inclusion of this product in kidney stone disease management protocols, especially for patients with kidney stones under 10 mm in diameter, where high response and effectiveness have been observed. Thus, it should be evaluated to reduce surgical treatment costs, as well as those for possible colic episodes and other associated complications.

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1. Introduction

The formation of kidney stones or nephrolithiasis represents a common medical condition, with a worldwide prevalence of more than 10%, which increases annually [1]. Calcium oxalate (OxCa) stones are the most common type of kidney stone. In fact, approximately 80% of all kidney stones contain OxCa as a major component. One of the main issues with nephrolithiasis concerns its high rate of recurrence, which ranges from 35% to 50% after 5-10 years and increases to 75% after 20 years onwards. Once an individual suffers from nephrolithiasis the rate of relapse increases and the recurrence interval shortens [2].

Calcium oxalate lithiasis tends to increase with age and begins to form via a crystal produced in the renal tubule cavity, in which it adheres to the surface of renal tubular epithelial cells. In healthy people, most crystals formed in the renal tubule cavity are eliminated in the urine, due to the action of macrophages that dissolve the crystals adhering to the surface of the renal tubular epithelial cells or in lysosomes within the cells. However, in individuals with hyperoxaluria or crystalline urine, renal tubular cells become damaged and, thus, crystallise easily [2][3].

Urolithiasis is responsible for 1 in 1000 hospitalisations each year. The recurrence rate is high, ranging from 10 to 20% in the first two years and 40 to 60% ten years after the first episode; and is characterised by recurrences and variable morbidity, depending on the region studied. Kidney stones occur at any age but most frequently between 20 and 50 years of age. It presents more frequently in men than in women in a ratio that varies between 2:1 and 3:1, as well as in all ethnicities; and presents in lesser numbers in African-American individuals, but typically in a more advanced form, such as chorale stones [4][5].

Certain disease presentations have a hereditary factor, such as cystine, uric acid and calcium oxalate stones. Whereas in others, urinary tract infections, climate and the individual's occupation may constitute a risk factor [5]. Most kidney stones encountered in Latin America are radio-opaque due to their calcium content (calcium oxalate monohydrate, calcium oxalate dihydrate, phosphate and calcium carbonate) [6].

Genetic and environmental factors contribute to the development of kidney stones. During the formation of OxCa crystals, osteopontin (OPN) neutralises the initial nuclei, while increasing the recruitment, migration and adhesion of macrophages and modulating the expression of pro-inflammatory cytokines and interleukins. Its deficiency has been associated with a heightened predisposition to develop such kidney stones [7][8]. However, the deposition of this OPN-OxCa complex on the renal tubule cell membrane stimulates the enzyme NADPH oxygenase, generating superoxides at the cytosolic level and oxidative damage at the mitochondrial level due to the activation of cyclophilin D and the consequent opening of MPTP [9]. As a consequence of mitochondrial collapse, the apoptosis process is triggered in the renal tubule cells. The crystals lump into a mass and are excreted into the tubular lumen as nuclei of the urinary stones. Finally, kidney stone nuclei containing crystals, cellular debris and stone matrix substances, such as OPN, are produced in tubular lumen (Figure 1).

Renal tubule

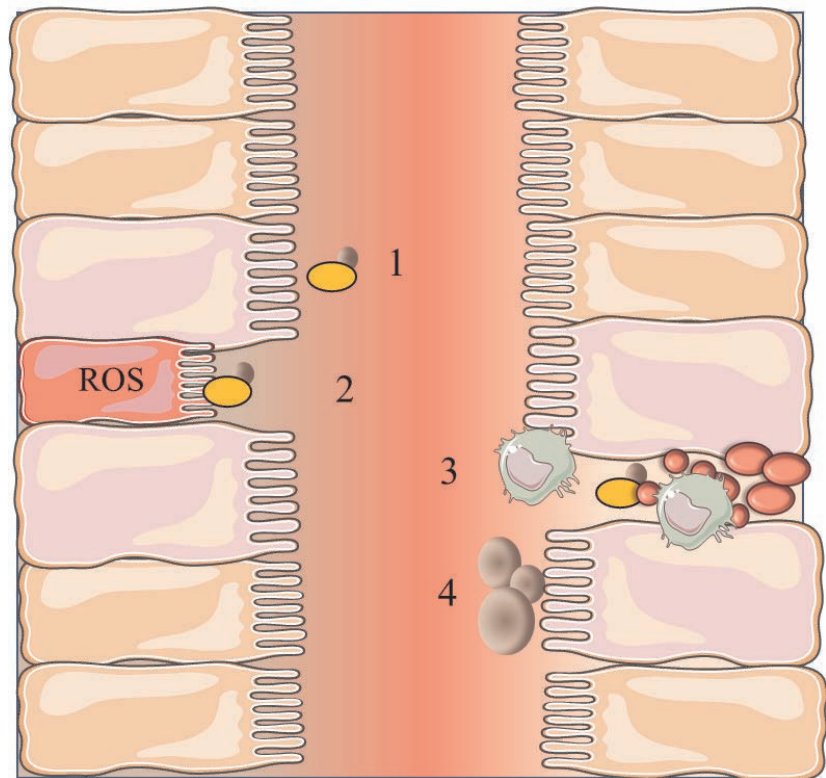


Figure 1. Diagram of cell damage in the renal tubule with the presence of calcium oxalate crystals. Neutralisation of OxCa crystals by OPN (1); adhesion of the OPN-OxCa complex to the plasma membrane of the renal tubule cells, increase in ROS (2); initiation of cell apoptosis in cells under ROS and stimulation of inflammation via macrophages (3); formation of OxCa aggregates, OPN and cell debris that lead to kidney stones (4).

Clinical diagnosis remains non-specific due to the diverse clinical presentations that may be observed in each patient. Furthermore, a metabolic assessment becomes necessary in order to identify the metabolic and physical/chemical risk factors that may influence the formation of the kidney stone, given the multiple causes that contribute to its occurrence [10][11].

Though the last two decades have seen considerable improvement in the treatment and prevention of kidney stones via a combination of diet, surgical and pharmaceutical treatments, the side effects of these treatments and the high recurrence rate necessitate alternative strategies for the prevention and treatment of nephrolithiasis. In view of the higher probability of chronic kidney damage due to the repetitive incidence of kidney stone disease, prompt diagnosis and treatment measures are essential to prevent kidney vulnerability.

The impact that extracorporeal lithotripsy, ureterolithotripsy and percutaneous nephrolithotomy have had on the destruction and elimination of urinary stones, as interventional procedures, have virtually replaced open surgery methods. However, it clearly emerges that the recurrence of kidney stones can be reduced with medical treatment, which selectively targets those patients with recurrent kidney stones, under 10 mm in diameter, supported by therapy not only for physiological, metabolic or physicochemical abnormalities that affect stone formation but also with the use of remedies such as Renalof[®], which painlessly and progressively destroys and breaks down stones in the genitourinary system (Figure 2), thereby achieving metabolic inactivity [12][13].

Kidney damage caused by stones reaches a point of no return since injury to renal epithelial cells promotes the adhesion of OxCa crystals and their retention in the renal tubules, processes which are pivotal in the perpetuation and formation of new kidney stones. In addition to the potential for renal damage from lithotripsy in recurrent patients, prevention provides a new approach [14].

For this reason, plants have been used in traditional medicine as a popular treatment for a multitude of pathologies, including kidney stone therapy. Findings drawn from a limited number of scientific studies so far suggest that phytotherapy could serve as an alternative or adjuvant therapy in the treatment of nephrolithiasis [15][16][17].

Renalof[®], whose main ingredient is an extract of *Agropyrum repens* enhanced with Molecular Activation Technology (Catalysis S.L., Table 1).

Agropyron repens is a very common species of plant native to Europe. Its rhizome contains numerous roots that extend over a long distance and at a shallow depth, and is considered an invasive species and weed. It has spread to Central Asia and can now also be found in Africa. It is traditionally used as a diuretic and to relieve pain and urinary tract spasms. It also serves as a demulcent and tonic. The plant contains different carbohydrates, mucilaginous substances, pectin, tritacin, cyanogenetic glucosides, phenol compounds, flavonoids, saponins, volatile oils, essential oils, vanillin glucoside, salicylic acid, iron and other minerals and large amounts of silica such as magnesium silicate [18][19].

Among the possible diuretic action mechanisms of *Agropyron repens*, tritacin has been reported to possess antibiotic, pH regulating and diuretic properties [26], while mannitol, saponins and flavonoids serve as osmotic regulators at the nephron level [27][28][29][30], salicylic acid as an analgesic and anti-inflammatory agent [31,32], and magnesium silicate as an ionic competitor in the formation of calcium oxalate (Figure 1).

Therefore, the purpose of this study was to determine the effectiveness of therapy with Renalof[®] as an alternative therapy in kidney stones of less than 10 mm in diameter. For that purpose, the main metabolic and physicochemical risk factors were described, as well as the patients' behaviour according to kidney stone activity pre- and post-treatment. Moreover, the main adverse effects of the treatment were monitored.

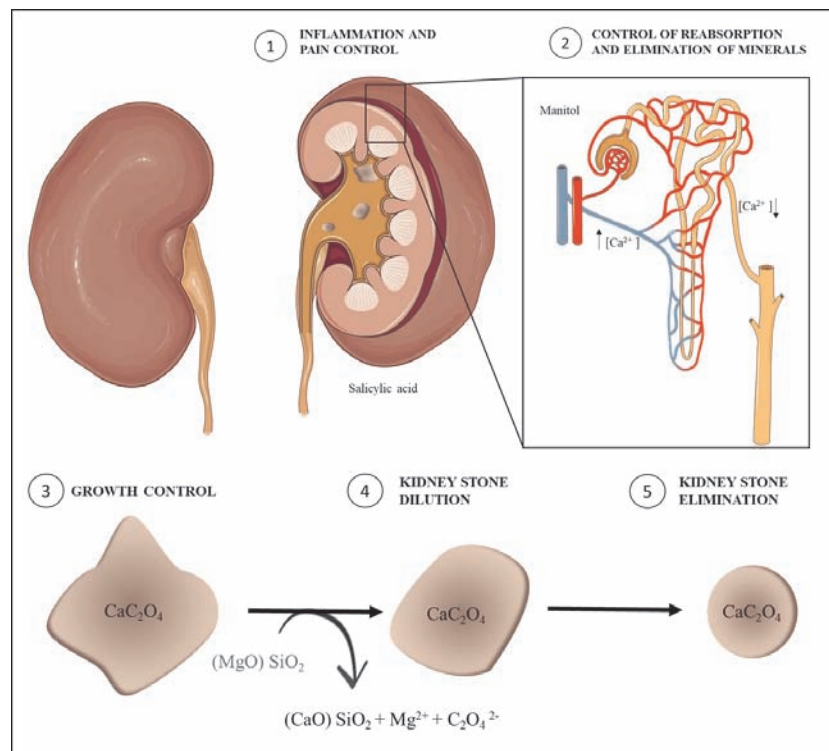


Figure 2. Diagram of potential mechanism of action of Renalof®. Analgesic and anti-inflammatory effect mediated by salicylic acid (1); diuretic effect and osmotic regulation of mannitol at the level of the nephron (2); control of kidney stone growth by reabsorption of calcium (3); dilution of the stone mediated by magnesium silicate as an ion exchanger (4); elimination of the stone via the urinary tract (5).

Without a doubt, nephrolithiasis is a growing public health problem, often due to insufficient water consumption, exposure to high ambient temperatures or water contamination, without neglecting possible anatomical malformations that can contribute to the development of lithiasis. Faced with this situation, the ideal is to opt for drugs that prevent the evolution of stones that at the time are small in millimetres and that are not obstructive. Renalof® is undoubtedly a harmless option, easily accessible, with high expulsion levels, with the presence of scarce adverse reactions which can be managed satisfactorily, obtaining a much higher risk / benefit ratio in favor of the patient.

Thus, the main endpoint of this study was to determine the time to expulsion or elimination, and secondary endpoints were changes in symptomatology or quality of life associated with renal-ureteral kidney disease and evaluation of study medication adverse events.

1. Materials and Methods

1.1. Product under study

In this trial, Renalof® (Catalysis S.L., Spain) was the study product used, with a dosage of one 325-mg capsule twice a day before meals (Table 1).

Table 1. Qualitative-quantitative composition of the Renalof® capsules product.

COMPOSITION	CONCENTRATION (mg/capsule)
Corn starch	200 mg
Mannito	188 mg
Talc	25 mg
Grass extract (<i>agropyron repens (L.) P. Beauv.</i>)	12 mg

1.2. Study population

A total of 155 patients were enrolled in the trial, randomly assigned to two groups as per a 4:1 ratio Renalof® treatment group and placebo control group.

Inclusion criteria:

1. Patients over 18 years old, all of whom were mentally and physically able to agree to be part of this study.
2. No existing associated comorbidities or diseases endangering the patient's stability.
3. No diagnosis of Chronic Kidney Disease, in any stage.
4. Existence of non-obstructive stones under 10 mm located in the renal-ureteral tract.
5. No impairment of renal viability.

Exclusion criteria:

1. Patients under 18 years of age.
2. Existence of associated comorbidities or diseases endangering the patient's stability.
3. Existence of a diagnosis of Chronic Kidney Disease, in any stage.
4. Occurrence of non-obstructive or obstructive stones over 10 mm located in the renal-ureteral tract.

5. Impairment of renal viability.
6. In the placebo group, patients with a creatinine equal to or greater than 3 ng/dl were excluded to prevent further renal damage.

1.3. Ethics committee

This study was evaluated and approved by the ethics committee of the Seniors Clinical. Likewise, the patient volunteers gave their consent to enter the study based on the above criteria ([Annex I](#)).

1.4. Clinical trial design

Randomised, controlled, single-blind, prospective study with two patient groups: (1) Renalof® and (2) placebo, with the same physical characteristics as the experimental product, whereby the patient would not be aware of whether they were receiving one form of treatment or another.

For this purpose, a database was compiled and the descriptive analysis conducted with frequency determination. In all, the study followed 120 patients treated with Renalof® and 35 with a placebo, at a dose of 650 mg BID, for 4 months. A preliminary analysis of the kidney stone types present in the study patients, based on the main compound, was conducted using a biophysical profile via biochemical analysis and the clinical characteristics, medical history and biochemical profile of the creatinine values of the test patients ([Table 2](#)). During the trial, systematic monthly imaging tests were performed on all patients by the on-site radiologist and evaluated by the primary researchers. Quality of life tests were performed using specific bimonthly urolithiasis tests to check the efficacy of the treatment ([Annex II](#)) and the possible adverse effects of the test product on patients with kidney stones.

The main variable for this trial was kidney stone clearance with Renalof® treatment in comparison to the placebo, with monthly evaluation during 4 months. Secondary variables included quality of life with established bimonthly measurements and the number of colic episodes during the 3-month follow-up.

1.5. Test analysis

All patients in the trial were systematically assessed applying the same techniques and procedures. The assessment conducted in the trial comprised ultrasound imaging tests (Philips Clear Vue 650) and initial tomography (16-channel Philips Tomography), and each month during the 4-month follow-up; initial analysis of kidney stone composition by 24-hour clinical urine tests.

Quality of life was established in regard to the presence or absence of symptoms associated with the disease by means of a bimonthly personal questionnaire ([Annex II](#)): dysuria or burning on urination, urinary urgency, renal colic, difficulty in carrying out daily activities and periodicity of urinary infections (mild, moderate, severe, yes or no).

Possible adverse effects were evaluated on a bimonthly basis according to the presence or absence of possible adverse effects associated with the product to be evaluated: stomach discomfort, gastric reflux, nausea (mild, moderate, severe, yes or no).

The intensity level of the symptoms referred by the patient was assessed every two months, as well as the level of disability that these could generate. It should be noted that the level of limitation in the patient was minimal, so no dose reduction or suspension of the treatment scheme is warranted.

1.5. Statistic

The baseline characteristics were summarized in number and percentage for categorical variables. The difference of study endpoints between experimental and placebo groups were calculated using Fisher's exact chi-square test. Endpoints measurements included all patients who were randomized and received at least one dose of study medication (intention-to-treat analysis). We estimated an average of 60% of stone expulsion rate in experimental group. The study was designed to have a statistical power of 95% to detect an absolute difference of 40% in the rates of stone elimination (60% in the group with renalof vs. 20% in the placebo group). Considering a type I error of 0.01 and a type II error of 0.05, 155 patients (120 in Renalof[®] vs 35 in placebo) were needed to reach statistical significance. The level of significance was defined as $P \leq 0.05$ (two tailed). All statistical analyses were performed using SPSS software for windows, version 26 (SPSS Inc., USA).

2. Results

A total of 155 patients were enrolled in the clinical trial. A descriptive baseline analysis (Table 2) was performed comparing the population of the Renalof[®] group with the placebo group. In the trial group, 65.0% of the patients had been diagnosed with kidney stone disease for the first time, while 80% of the placebo group had experienced recurrent kidney stone disease previously. Most of the stones diagnosed in both groups were calcium-based and smaller than 7 mm in diameter. Likewise, the majority of the two groups did not suffer renal damage (76.60% of the Renalof[®] group and 97.14% in Placebo) and the most frequent metabolic alteration was hyperuricemia (29.0% and 11.4% respectively).

Table 2. Baseline characteristics of study patients enrolled in the trial for the Renalof[®] and Placebo[®] groups.

Variable		Renalof [®] Group N=120	Placebo [®] Group N=35	P value
		n (%)	n (%)	
Kidney Stone Recurrence	First recurrence	78 (65.0)	7 (20.0)	<0.001
	More than once	42 (35.0)	28 (80.0)	
Previous comorbidities	No	82 (68.3)	12 (34.29)	<0.001
	Yes	38 (31.7)	23 (65.71)	
Size of the kidney stones	Ø < 5 mm	67 (55.83)	18 (51.43)	0.175
	5 > Ø < 7 mm	35 (29.17)	15 (42.86)	
	8 > Ø < 9 mm	18 (15.00)	2 (5.71)	
Existence of kidney damage	Yes	28 (23.3)	1 (2.86)	0.006
	No	92 (76.6)	34 (97.14)	
Composition of kidney stones	Calcium-based	65 (54.17)	26 (75.00)	0.040
	Uric acid-based	32 (26.67)	9 (25.00)	
	Mixed origin	23 (19.16)	0 (0.00)	
Previous metabolic disorders	Yes	39 (32.4)	5 (14.3)	0.006
	No	81 (67.6)	30 (85.7)	
Type of Metabolic Alteration ^a	Hyperuricemia	35 (29.0)	4 (11.4)	0.210
	Hypercalcemia	3 (2.5)	1 (2.9)	
	Hypermagnesemia	1 (0.8)	0 (0.0)	
Serum Creatinine Levels (ng/dl)	<1.4	102 (85.00)	33 (94.29)	0.264
	1.4 - 3.0	17 (14.10)	2 ^b (5.71)	
	>3.0	1 (0.90)	0 (0.00)	

^a Type of metabolic alteration in the event of previous metabolic alterations.

^b In the placebo group, patients with creatinine equal to or greater than 3 ng/dl were excluded to prevent further renal damage.

The results obtained in the study (depicted in Table 3) show a 65% remission of kidney stones in the Renalof® group in the first 8 weeks, compared to only 11.4% elimination in the Placebo group (P<0.001). Over 12 weeks, until the completion of follow-up, 97.5% of patients in the Renalof® group remitted, compared to 11.4% with persistent stones in the Placebo group (P<0.001).

Table 3. Evolution of the kidney stone clearance period in the study patients.

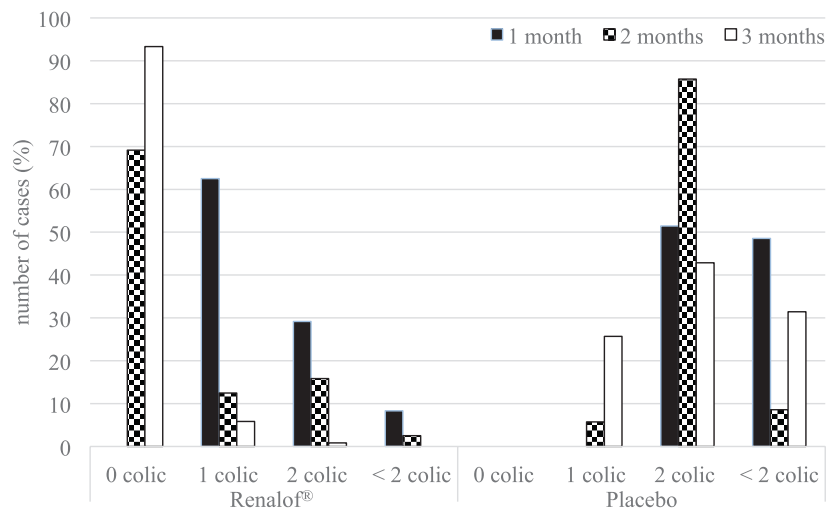
Variable		Renalof® Group N=120	Placebo® Group N=35	P value
		n (%)	n (%)	
Time of elimination (weeks of treatment)	< 8 weeks	78 (65.0)	4 (11.4)	<0.001
	8 - 10 weeks	93 (78)	0 (0.0)	<0.001
	10 - 12 weeks	117 (98)	0 (0.0)	<0.001
	> 12 weeks	120 (100)	31 (88.6)	<0.001

Focusing on the symptoms of renal-ureteral kidney stone disease, over the 4-month follow-up period, 62.5% of patients in the Renalof® group suffered from associated symptoms such as dysuria, pollakiuria and urinary urgency for 2 weeks, 23.3% for 4 weeks and only 14.2% for 6 weeks or more. However, in the Placebo group, a higher percentage of patients were observed with unaltered persistent symptoms (51.4%) or for 6 or more weeks (42.9%).

Table 4. Variation in the symptomatology associated with renal-ureteral kidney disease in trial patients.

Variable		Renalof® Group N=120	Placebo® Group N=35	P value
		n (%)	n (%)	
Duration of dysuria, pollakiurina, urinary urgency	2 weeks	75 (62.5)	0 (0.0)	<0.001
	4 weeks	103 (23.3)	2 (5.7)	<0.001
	6 weeks or more	120 (14.2)	17 (42.9)	<0.001
	No variations	0 (0.0)	18 (51.4)	<0.001

With regard to colic (Graph 1), a significant decrease in colic was observed in the Renalof® group during the treatment period, dropping from 62.6%, 29.17% and 8.33% in the first month with 1, 2 or more colic episodes respectively, to 5.83%, 0.83% and 0% after 3 months of treatment. In the placebo group, an increase in the number of colic episodes was observed, with most patients suffering at least 2 colic episodes per month (51.43% in the first month; 85.71% in the second month and 42.86% in the third month).



Graph 1. Evolution of the number of colic attacks caused by kidney stones per month of treatment in trial patients in the Renalof® treatment group compared to the control group.

With regard to symptoms and colic, patients with renal-ureteral stones treated with Renalof® experienced an improvement in their quality of life (95.0%) compared to the control group (11.4%).

Table 5. Assessment of the improvement in patients' quality of life with kidney stones in the Renalof® treatment trial.

Variable		Renalof® Group N=120	Placebo® Group N=35	P value
		n (%)	n (%)	
Improved quality of life	YES	114 (95.0)	4 (11.4)	<0.001
	NO	6 (5.0)	31 (88.6)	<0.001

Finally, only 3.33% of patients in the Renalof® group developed an adverse reaction related to the treatment product (Table 6).

Table 6. Adverse reactions during the treatment.

Variable		Renalof® Group N=120	Placebo® Group N=35	P value
		n (%)	n (%)	
Adverse reactions	YES	4 (3.33)	0 (0.0)	0.848
	NO	116 (96.67)	35 (100.0)	

3. Discussion

Kidney stones, especially in terms of small elements, have a high incidence, particularly in tropical countries where they occur frequently owing to the lack of water consumption. The techniques for its treatment approach have changed, initially involving open surgery. Currently, multiple minimally invasive surgery techniques exist, which allow for a rapid recovery of the patient with an excellent rate of lithiasic mass removal.

Prevention of stone formation remains essential, i.e. sufficient water consumption and the correction of metabolic disorders that render patients susceptible to the development of new stones. In this study, most stones presented a calcium aetiology, predominantly in male individuals. The size of the stone was less than 5 mm in 55.83% of cases, without causing renal damage. Most of the patients were managed via ultrasound.

Results shown in this study corroborate the high rate of effectiveness of Renalof®, within phytotherapy, finding up to 65% clearance in the first 8 weeks of treatment and 97.5% after 12 weeks of treatment in kidney stones of less than 10 mm in diameter.

Likewise, an improvement in the quality of life of these patients was observed, with a reduction in the symptoms associated with renal-ureteral kidney stones such as dysuria, pollakiuria, urinary urgency, as well as a drop in the number of colic episodes throughout the treatment, as shown in the results. This presents a key to controlling such commonplace complications of kidney stones, as well as to simplifying lithotripsy surgical treatment itself.

The placebo group had minimal response to the treatment, with poor stone clearance (only 11.4%), with stones being less than 5 mm in diameter. Larger stones did not vary throughout the trial. However, while the size

of the stones remained unaltered, the number of colic episodes and associated symptoms rose, especially towards the second half of the trial's follow-up, a very common occurrence that increases the average cost of this type of treatment.

Minimal adverse drug reactions occurred. Only 3.33% were associated with nausea when using said product, and only possibly due to the rejection of the capsule format. This indicates good product acceptability and safety.

The final profile of the patients treated in the Renalof® group was that of outpatients, in which a prompt remission of the symptoms occurs, which are usually incapacitating and cause the patient to receive medical care. Therefore, the time spent by the patient corresponds to the time spent in the follow-up medical consultation, and the average time spent performing follow-up examinations, such as imaging, or providing a urine sample to the laboratory, without being subject to medical leave or hospitalisation.

For larger stones, a longer treatment time of up to 12 weeks would be required. This would necessitate a study with increased sampling and follow-up time to test the effectiveness of this product in these cases.

For the above reasons, considering that the average time spent in hospital for renal colic averages 3 days, it costs approximately 200 dollars for the use of the bed, food and daily administration of medication. If such a condition is resolved surgically, the length of stay increases to 5 days and the cost rises to 3,000 dollars, including the surgical procedure (endourological procedure, with stent placement generally). Furthermore, the average length of medical leave lasts from 15 to 21 days. In the group treated with non-invasive Renalof®, the average cost per patient with renal-ureteral kidney stones would be greatly reduced, diminishing the number of interventions including treatment of kidney stones smaller than 10 mm in diameter and prevention of relapses in recurrent patients.

Therefore, we strongly recommend the inclusion of this product in kidney stone disease management protocols, especially for small-sized kidney stones, where high response and effectiveness have been observed.

4. Conclusions

In this study, the effectiveness of the Renalof® treatment demonstrated a 97.5% effectiveness in the elimination of renal-ureteral stones of less than 10 mm in diameter. Additionally, it improved the associated symptomatology and decreased the number of colic episodes per patient without any noticeable side effects, thereby improving the quality of life and the average cost per patient.

5. Bibliography

- [1]. Campuzano S, Bousoño C, Málaga S, Díaz JJ, Rodríguez M, Campos C. Riesgo de urolitiasis en pacientes con fibrosis quística. *Nefrología*. 2009; 29:61-6.
- [2]. McDougal, W., 2012. *Campbell-Walsh Urology Tenth Edition Review*. 10th ed. Philadelphia: Elsevier/Saunders, pp.112-125.
- [3]. Tsujihata, M., 2007. Mechanism of calcium oxalate renal stone formation and renal tubular cell injury. *International Journal of Urology*, 15(2), pp.115-120.
- [4]. Johnson CM, Wilson DM. Renal stone epidemiology: a 25-year study in Rochester, Minesotta. *Kidney Int*. 2007; 16(5): 624-31.
- [5]. Soucie JM, Coastes RJ, McClellan W. Relation between geographic variability in kidney stone prevalence and risk factors for stones. *Am. J. Epidemiol*. 2006; 143(5): 487-95.

- [6]. Pack C.Y. Etiology and treatment of urolithiasis. *Am. J. Kidney Dis.* 1991; 18: 624.
- [7]. Wesson JA, Johnson RJ, Mazzali M, Beshensky AM, Stietz S, Giachelli C, Liaw L, Alpers CE, Couser WG, Kleinman JG, Hughes J. Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. *J Am Soc Nephrol.* 2003 Jan;14(1):139-47.
- [8]. González Fajardo, J., Zamora González, N., Aguirre Gervás, B., Marcos Sánchez, H., Vidriales Vicente, I., Calvo Nieves, M. and Vaquero Puerta, C., 2016. La osteopontina como biomarcador de riesgo neurológico en la enfermedad carotídea. *Angiología*, 68(6), pp.459-464.
- [9]. Kohri, K., Yasui, T., Okada, A., Hirose, M., Hamamoto, S., Fujii, Y., Niimi, K. and Taguchi, K., 2012. Biomolecular mechanism of urinary stone formation involving osteopontin. *Urological Research*, 40(6), pp.623-637.
- [10]. Song L, Maalouf NM. Nephrolithiasis. [Updated 2020 Mar 9]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. *Endotext* [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-.
- [11]. Worcester, Elaine M, and Fredric L Coe. "Nephrolithiasis." *Primary care vol. 35,2* (2008): 369-91, vii.
- [12]. Kristyantoro B, Alif S, Tarmono, Budiono. The effectiveness of Renalof compared to kalkurenal and placebo. *Indonesian Journal of Urology*, Vol.19, No.2, July 2012 :73-78.
- [13]. Aties Sanchez, M.C., Edighill Villanueva, V., Abreu Vázquez R. Randomized double-blind study with Renalof in patients with calcium oxalate renal lithiasis. *Revista Cubana de Plantas Medicinales.* 2012;31(1):87-100.
- [14]. Mykoniatis I, Sarafidis P, Memmos D, Anastasiadis A, Dimitriadis G, Hatzichristou D. Are endourological procedures for nephrolithiasis treatment associated with renal injury? A review of potential mechanisms and novel diagnostic indexes. *Clin Kidney J.* 2020 May 22;13(4):531-541.
- [15]. Grases, F., Ramis, M., Costa-Bauzá, A. and March, J., 1995. Effect of *Herniaria hirsuta* and *Agropyron repens* on calcium oxalate urolithiasis risk in rats. *Journal of Ethnopharmacology*, 45(3), pp.211-214.
- [16]. Schulz, V., Hänsel, R. and Tyler, V., 2001. *Rational Phytotherapy – Guide to herbal medicine.* 4th ed. Berlin: Springer.
- [17]. Beydokthi, S., Sendker, J., Brandt, S. and Hensel, A., 2017. Traditionally used medicinal plants against uncomplicated urinary tract infections: Hexadecyl coumaric acid ester from the rhizomes of *Agropyron repens* (L.) P. Beauv. with antiadhesive activity against uropathogenic *E. coli*. *Fitoterapia*, 117, pp.22-27.
- [18]. Paslawska S, Piekos R. Studies on the optimum conditions of extraction of silicon species from plants with water. IV. *Agropyron repens*. *Planta Med.* 1976 Nov;30(3):216-22.
- [19]. Al-Snafi, Ali. Chemical constituents and pharmacological importance of *Agropyron repens* – A review. *Research Journal of Pharmacology and Toxicology.* (2015). 1. 37-41.
- [20]. Bragadottir, G., Redfors, B. and Ricksten, S., 2020. Mannitol Increases Renal Blood Flow And Maintains Filtration Fraction And Oxygenation In Postoperative Acute Kidney Injury: A Prospective Interventional Study.
- [21]. Hägerström, E., Lindberg, L., Bentzen, J., Brødbæk, K., Zerahn, B. and Kristensen, B. The Nephroprotective Effect of Mannitol in Head and Neck Cancer Patients Receiving Cisplatin Therapy. *Clinical Medicine Insights: Oncology*, 2019, 13, p.117955491882132.
- [22]. Kiss, K., Molnár, M., Söndergaard, S., Molnár, G. and Ricksten, S. Mannitol clearance for the determination of glomerular filtration rate—a validation against clearance of ⁵¹Cr-EDTA. *Clinical Physiology and Functional Imaging*, 2016. 38(1), pp.10-16.

- [23]. Shawkat, H., Westwood, M. and Mortimer, A. Mannitol: a review of its clinical uses. *Continuing Education in Anaesthesia Critical Care & Pain*, 2012. 12(2), pp.82-85.
- [24]. Riley, J., Kim, H., Averch, T. and Kim, H. Effect of Magnesium on Calcium and Oxalate Ion Binding. *Journal of Endourology*, 2013, 27(12), pp.1487-1492.
- [25]. Grases, F., Rodriguez, A. and Costa-Bauza, A. Efficacy Of Mixtures Of Magnesium, Citrate And Phytate As Calcium Oxalate Crystallization Inhibitors In Urine. 2015 Sep;194(3):812-9.
- [26]. Punitha, S.M.J., Babu, M.M., Sivaram, V. et al. Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper *Epinephelus tauvina* juvenile against *Vibrio harveyi* infection. *Aquacult Int* 16, 511–523 (2008).
- [27]. Better OS, Rubinstein I, Winaver JM, Knochel JP. Mannitol therapy revisited (1940-1997). *Kidney Int.* 1997 Oct;52(4):886-94.
- [28]. Bipat R, Steels P, Cuypers Y, Toelsie JR. Mannitol Reduces the Hydrostatic Pressure in the Proximal Tubule of the Isolated Blood-Perfused Rabbit Kidney during Hypoxic Stress and Improves Its Function. *Nephron Extra.* 2011 Jan;1(1):201-11.
- [29]. Damasceno-Ferreira JA, Abreu LAS, Bechara GR, Costa WS, Pereira-Sampaio MA, Sampaio FJB, De Souza DB. Mannitol reduces nephron loss after warm renal ischemia in a porcine model. *BMC Urol.* 2018 Mar 6;18(1):16.
- [30]. Shi J, Qian J, Li H, Luo H, Luo W, Lin Z. Renal tubular epithelial cells injury induced by mannitol and its potential mechanism. *Ren Fail.* 2018 Nov;40(1):85-91.
- [31]. Ekinci D, Senturk M, Kufreviolu O. Salicylic acid derivatives: synthesis, features and usage as therapeutic tools. *Expert Opin Ther Pat.* 2011 Dec;21(12):1831-41.
- [32]. Mahmud S, Rosen N. History of NSAID Use in the Treatment of Headaches Pre and Post-industrial Revolution in the United States: the Rise and Fall of Antipyrine, Salicylic Acid, and Acetanilide. *Curr Pain Headache Rep.* 2019 Jan 23;23(1):6.