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A Novel Insight into the Anti-Urolithiatic Effect of Manjistha and Triphala Syrup Formulation

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ABSTRACT

Urolithiasis is a common, painful renal disorder of calcium oxalate crystal precipitation. Some traditional medicines have been found to have anti-urolithiatic actions, including Manjistha (*Rubia cordifolia*) and Triphala. This study investigates the litholytic potential of a polyherbal syrup made from these plants. Hydroalcoholic extracts of R. cordifolia roots and Triphala were prepared by Soxhlet extraction. The herbal syrup was then subjected to anti-urolithiatic testing in vitro, through methodologies such as egg membrane assay and sedimentary crystal formation model. Cystone was taken as a reference standard. TLC was adopted for phytochemical profiling of the extracts. TLC helped identify the main phytoconstituents such as gallic and ellagic acids. Using the egg membrane assay method, the dissolution of calcium oxalate crystals was found to be 48% by the herbal syrup, while for Cystone this value was 54%. The sedimentary crystal model revealed a significant reduction in calcium oxalate crystals under treatment as compared with the control. The preparation showed promising effects of anti-urolithiatic activity in vitro, which could be due to synergistic phytoconstituent effects. These findings support its traditional usage and its potential for the natural treatment of urolithiasis. It is further suggested that investigating the activity in vivo, followed by clinical experiments, would be recommended for its confirmation.

Keywords: *Rubia cordifolia, Triphala, Anti-urolithiatic, Herbal syrup, Calcium oxalate, Litholytic activity.*

1. INTRODUCTION

Urolithiasis, commonly known as kidney stones, is a widespread health issue that affects millions of people around the globe each year. It primarily targets the urinary system and stands as the third most common condition affecting this part of the body.[1] It is estimated that 2% of the population may experience kidney stone disease at some stage in their lives, with a male to female ratio of 2:1.[2] It roughly affects 10% to 15% of the worldwide population and half of recurrences happen within five years after the first incident.[3] The process of stone formation include Supersaturation, Nucleation, aggregation and then retains in the urinary system. Urinary stones develop when dehydrated or acidic urine allows crystals to form and grow due to missing natural blockers like citrate. Over time, these crystals stick in the kidneys or urinary tract, becoming painful stones.[4] The risk factors associated with urolithiasis are inadequate fluid intake which leads to concentrated urine or Excessive intake of animal protein and salt. The common symptoms include Severe pain, burning sensation during urination, nausea and vomiting, blood in urine, frequent urination.[5]

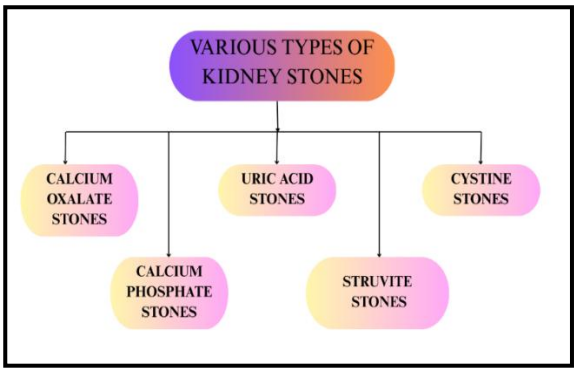


Figure 1: Types of kidney stones

The recent treatment procedures like surgical removal, percutaneous techniques and extracorporeal shock wave lithotripsy (ESWL), Ureteroscopy (URS): laser stone removal using a scope, Percutaneous Nephrolithotomy (PCNL), retrograde intrarenal surgery (RIRS) and laparoscopic ureterolithotomy are prohibitively costly for the common man and with these procedure, recurrence is quite common.[6][7]. Looking to this various herbal drugs are mentioned which helps in reducing Urolithiasis. The herbal drug is Manjistha (*Rubia cordifolia*) as it has anti-urolithiatic activity. Along with it Triphala is included with manjistha as it improves gut health.[8][9] In present study an attempt has been made to explore the potent indigenous plant and emphasis on its use in prevention and treatment of urolithiasis. Therefore, the aim of this study is to explore and confirm the potential of a hydro-alcoholic extract made from Manjistha and Triphala in preventing or treating kidney stones.

SR.NO	DRUG	GEOGRAPHICAL SOURCE	PART USED	MECHANISM OF ACTION
1.	Manjistha (*Rubia cordifolia)*	India, China, Japan, Vietnam and Malaysia. In India, it typically grows in hilly regions such as Dehradun, Kashmir, Nagpur, and Malvan[10]	Root	Hydroalcoholic liquid root extracts of *Rubia cordifolia* significantly reduced the urine oxalate, calcium and phosphates dose dependently and effectively lowered the renal tissue excess calcium and oxalates in addition. Also, it successfully works by reducing and preventing the formation of kidney stones as it has been shown to affect renal stone development at an initial stage.[8]
2.	Amla (*Emblica Officinalis*)	Tropical and subtropical regions, thriving in coastal areas and it grows abundantly in the deciduous forests of Madhya Pradesh and can be found in Burma as well.[11]	Fruit	Improve digestion, alleviate chronic constipation, and lessen intestinal cramps. Prolonged usage of triphala, has been associated with enhancements in stool frequency, consistency, and general bowel health, while also contributing to the reduction of abdominal discomfort.[9]
3.	Bibhitaki (*Terminalia belerica)*	It thrives in various parts of the country, particularly in the forests of Assam, Gujarat, Odisha, Andhra Pradesh, Kerala, and Tamil Nadu. [12]		
4.	Haritaki (*Terminalia chebula)*	India, Sri Lanka, Bhutan, Nepal, Bangladesh, and Pakistan. Additionally, it is found in Myanmar, Cambodia, Laos, Vietnam, Indonesia, Malaysia, and the Philippines[13]		

Table 1: Overview of various herbs

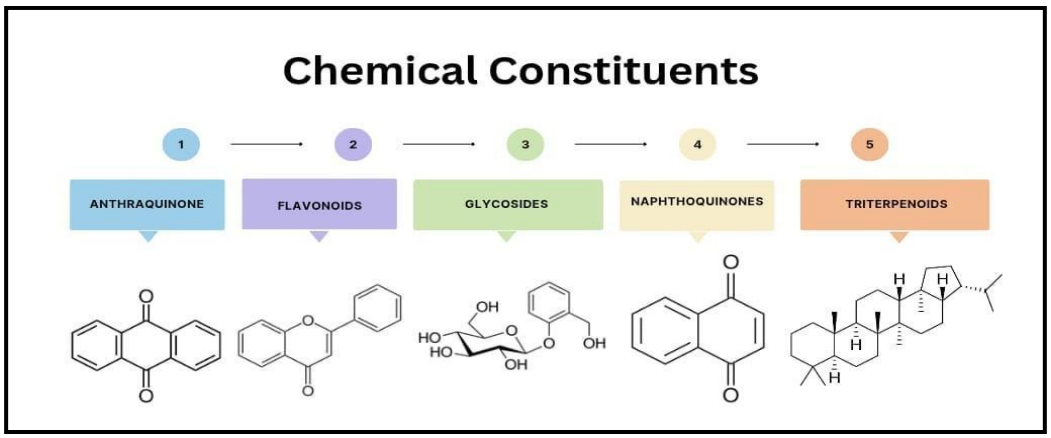


Figure 2: Chemical Constituents of *Rubia cordifolia*[14]

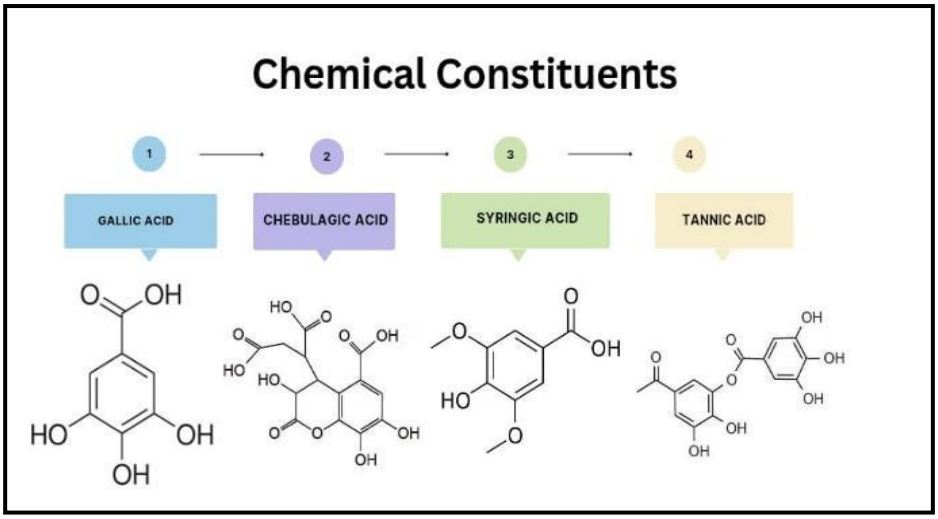


Figure 3: Chemical Constituents of Triphala[15]

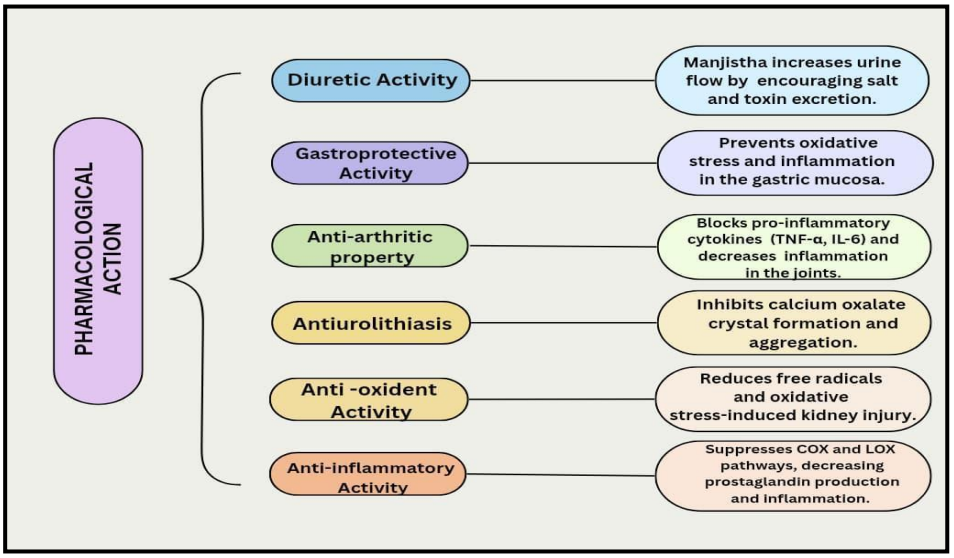


Figure 4: Pharmacological actions of *Rubia cordifolia*[16]

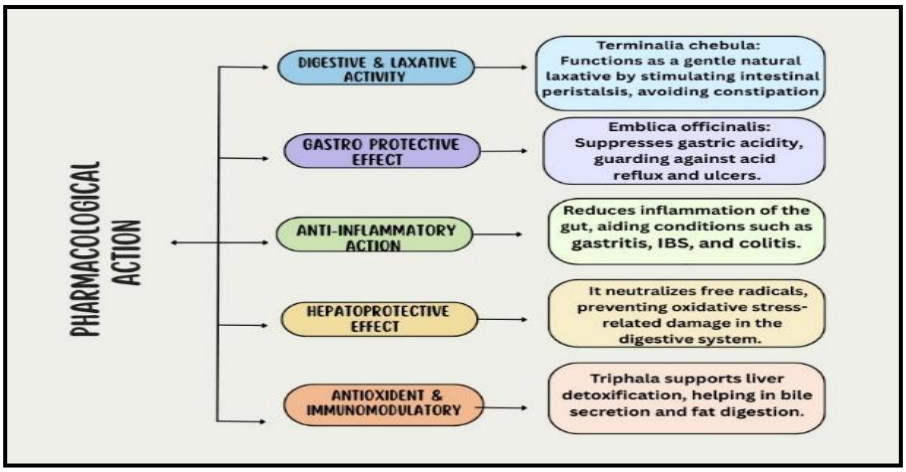


Figure 5: Pharmacological actions of Triphala[17]

2. MATERIALS AND METHODS

Chemicals:

Cystone (Himalaya Health Care, India) was procured from the local market. Ethanol, Ammonia Solution (25%), Sodium oxalate, Sodium CMC, Sodium Benzoate was obtained from Research-Lab Fine Chem Industries, Mumbai, India. All other chemicals and reagents used were of analytical grade.

Preparation of Herb Extract:

1.Roots of *Rubia cordifolia* were washed with distilled water, shade-dried, and further dried in an oven at 30°C to preserve heat-sensitive constituents. The dried roots were coarsely powdered, passed through sieve no. 20, and stored in an airtight container at room temperature.[18]

2.Coarsely powdered *Rubia cordifolia* roots (255 g) and Triphala (93 g) were separately extracted using Soxhlet apparatus with 70:30 ethanol-water as solvent. The extracts were concentrated by evaporating the solvent at 70°C using a water bath and stored at 2–8°C for further analysis.[19]

Figure 6: Extraction of *Rubia cordifolia* and Triphala

3. EVALUATION OF HERBAL EXTRACTS OF MANJISTHA AND TRIPHALA:

Thin Layer Chromatography (TLC) Analysis:

The hydroalcoholic extracts were analyzed using Thin Layer Chromatography (TLC). This technique involves the separation of compounds based on their distribution between two phases: a stationary phase, which functions through adsorption, and a mobile phase, which consists of a liquid solvent that moves across the stationary phase. A solvent mixture of Toluene, Ethyl Acetate, and Formic Acid in a ratio of 7:3:1 for Manjistha and A solvent mixture of Toluene: Ethyl Acetate: Formic Acid (3:2:0.4) for Triphala were prepared and used as a mobile phase.[20]

4. EXPERIMENTAL SETUP

1. Preparation of herbal syrup formulation:

A herbal syrup was formulated using *Rubia cordifolia* (Manjistha) and Triphala extracts. The composition of the syrup is outlined in Table 2.

Table 2: Formulation of herbal syrup

Ingredients	Function	Formulation
Rubia Cordifolia	Active drug	25.2 g
Triphala	Active drug	5 g
Sodium benzoate	Preservative	0.15 g
Sodium CMC	Stabilizer	2 g
Simple syrup	Base/Smoothing agent	Up to 100 ml (q.s. base)

2. Dose calculation:

Manjistha was calculated as approximately 2 g/day and for Triphala as 0.5 g/day for a 60 kg individual using standard Km factor conversion and Human equivalent Dose calculation.[21]

3. Method to prepare Herbal syrup:

To prepare the syrup, the required amount of each ingredient was carefully measured. The extract was first blended with a small quantity of water using a mortar and pestle. Next, the preservative (sodium benzoate) and stabilizer (sodium CMC) were added and mixed thoroughly with the extract. Simple syrup was then incorporated, ensuring even distribution. Finally, the mixture was diluted with distilled water to reach a total volume of 100 ml, resulting in a uniform solution. The clarity of the solution was visually assessed to confirm its solubility. Once prepared, the syrup was transferred into a properly labelled bottle and stored for future use.[22]

5. ASSESSMENT OF HERBAL SYRUP FORMULATION FOR ANTI-UROLITHIATIC ACTIVITY

To conduct in-vitro antiurolithiatic activity, Egg membrane assay and Simulation of the sedimentary crystal formation model were selected

1. Egg Membrane Assay:

Preparation of Semi-Permeable Membrane: Eggshells were emptied and decalcified overnight in diluted HCl (4 mL conc. HCl in 200 mL distilled water). The resulting membranes were washed, neutralized with ammonia, and stored in a moist state at pH 7–7.4.

Preparation of Standard Drug: Cystone tablets were de-coated with absolute ethanol, crushed, and 400 mg of powder was dissolved in 100 mL distilled water. The solution was filtered and used as a standard for in vitro anti-urolithiatic activity.[23]

Preparation of Calcium Oxalate Crystals: Calcium oxalate was prepared by mixing 100 mL of 1.34 g sodium oxalate (in 2 N H_2SO_4) with 100 mL of 1.47 g calcium chloride solution. The precipitate was neutralized with ammonia, washed with distilled water, and dried at 60 °C for 4 hours.[24]

Estimation of Calcium Oxalate Using Dissolution Model: Four semi-permeable egg membranes were filled with different drug samples and suspended in 100 mL of 0.1 M Tris buffer at 37 °C for 2 hours. Post-incubation, the contents were treated with 1 N H_2SO_4 and titrated against 0.9494 N $KMnO_4$, where 1 mL $KMnO_4$ = 0.1898 mg Ca. Dissolved calcium oxalate was calculated by subtracting the undissolved amount from the initial quantity, and percentage dissolution was determined accordingly.[25]

Group	Group Name	Test Parameters
Group I	Negative Control	5 mg of Calcium oxalate + 2 ml of distilled water
Group II	Standard (Positive Control)	5 mg of Calcium oxalate + 2 ml of Cystone solution (10 mg/ml)
Group III	Test Group	5 mg of Calcium oxalate + 2 ml of polyherbal syrup formulation

Table 3: Egg membrane assay

Dissolved Calcium Oxalate = Total quantity used in the experiment in the beginning - undissolved calcium oxalate.

2. Sedimentary Crystal Formation Model:

A modified method with continuous agitation was used to induce crystal formation and prevent sedimentation. At 10 µg/mL concentration, crystal growth was monitored using light microscopy (10X) by observing a drop of the sample on a clean slide.[26]

6. RESULTS & DISCUSSION

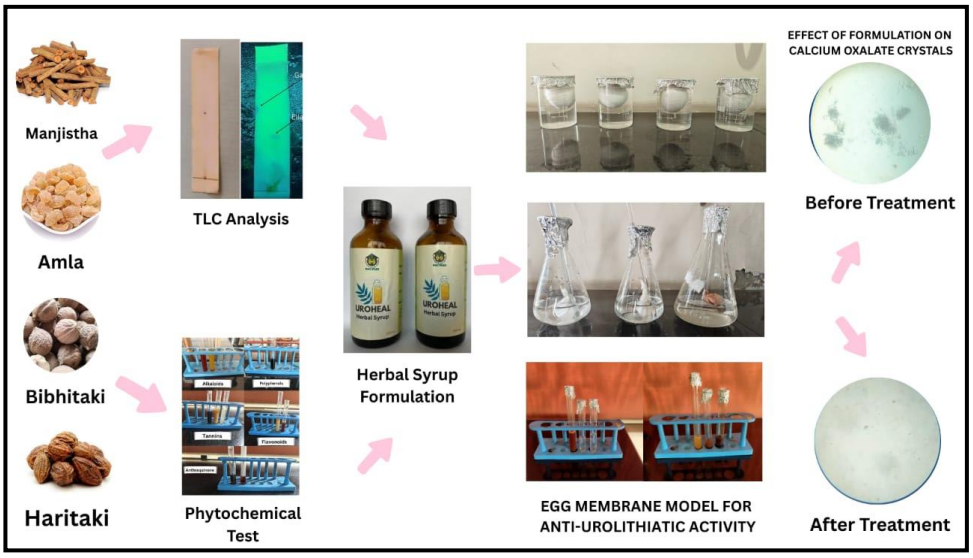


Figure 7: Plan of action

1. Thin Layer Chromatography (TLC) Analysis:

TLC of *Rubia cordifolia* revealed consistent Rf values ranging from 0.63 to 0.65, closely matching the standard range of 0.64–0.74, indicating the presence of its characteristic phytoconstituents. In the TLC analysis of Triphala, two distinct spots corresponding to standard Gallic acid and Ellagic acid were observed. The Rf value for Gallic acid was 0.60, comparable to the standard value of 0.56. Ellagic acid showed an Rf value of 0.43, aligning with its standard of 0.47.

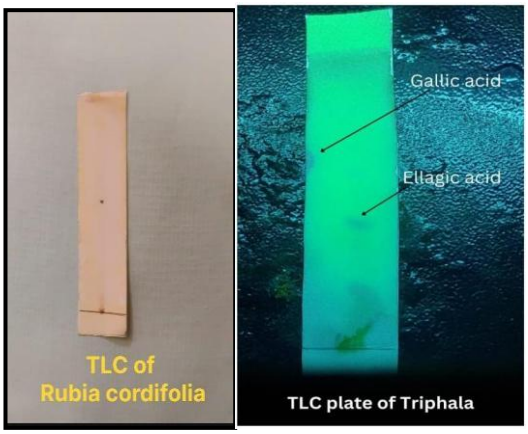


Figure 8: TLC of Hydroalcoholic Extracts of *Rubia cordifolia* and Triphala

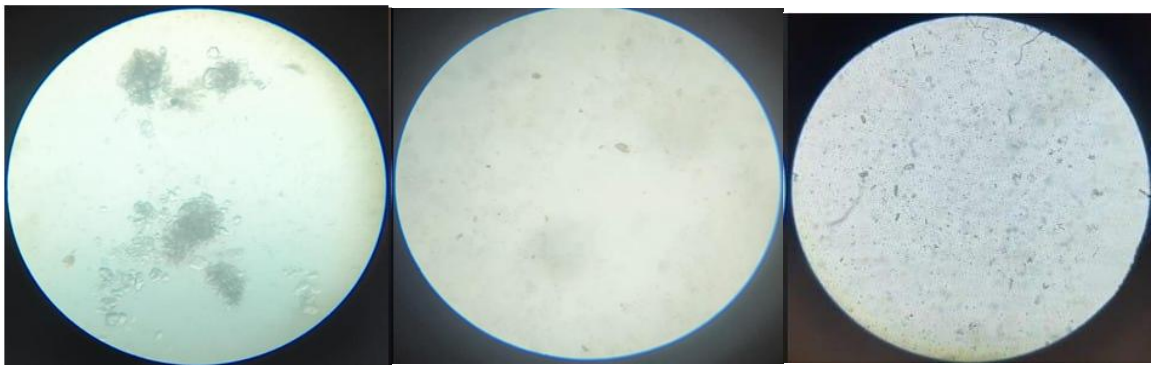
2. Egg Membrane Assay:

Groups	Vol. of KMnO4 (ml)	Weight of Calcium oxalate Estimated (mg)	Mean Weight of Calcium Reduced (mg)	Percentage Dissolution
Negative Control	28.2	5	0	0%
Standard (Cystone)	15.2	5	2.7	54%
Test group (Syrup formulation)	13.5	5	2.4	48%

Table 4: Estimation of Percentage Dissolution by Titrimetric Method

The percentage dissolution of calcium oxalate was calculated based on titrimetric studies with potassium permanganate ($KMnO_4$). The negative control group showed no dissolution (0%), confirming the stability of calcium oxalate in distilled water. The standard group (Cystone) showed a 54% dissolution, indicating effective litholytic activity. The test group exhibited 48% dissolution, demonstrating considerable anti-urolithiatic potential.

3. **Sedimentary Crystal Formation Model:**



(a) Negative Control Group (b) Standard Cystone (c) Herbal Syrup formulation

Figure 9: Sedimentary Crystal Formation Model Slides

We conducted a sedimentary crystal formation test to assess the effectiveness of our herbal syrup in preventing kidney stone formation. Under a microscope, we compared two slides—one from the untreated control group and another treated with our formulation. The control slide showed a high number of calcium oxalate crystals, which are the main culprits behind kidney stones. In contrast, the treated slide had noticeably fewer crystals, indicating that the syrup may help reduce their formation. These results highlight the potential of our herbal formulation as a natural remedy for kidney stone prevention.

7. CONCLUSION

The current research effectively formulated and tested a new hydroalcoholic herbal syrup with *Rubia cordifolia* (Manjistha) and Triphala extract for in vitro anti-urolithiatic activity. Phytochemical confirmation through Thin Layer Chromatography established the presence of active phytoconstituents gallic acid and ellagic acid. The syrup exhibited significant 48% dissolution of calcium oxalate crystals in the egg membrane model, which is nearly comparable to the reference drug Cystone (54%). Microscopic examination in the sediment crystal model additionally corroborated its effectiveness by depicting a clear decrease in crystal growth. These results indicate that anthraquinones in Manjistha and polyphenols in Triphala in the formulation effectively interfere with nucleation and aggregation of calcium oxalate, the main reason behind the development of kidney stones.

The synergistic activity of anthraquinones in Manjistha and polyphenols in Triphala might also help in decreasing oxidative stress and inflammation, thereby maintaining kidney health. In addition, the syrup's liquid state makes it easy to use, especially for elderly patients, and its herbal nature limits the danger of drug side effects. Despite these promising findings, these are restricted to in vitro data. Thus, additional in vivo studies and clinical trials are suggested in order to evaluate its pharmacokinetics, bioavailability, and long-term therapeutic effects in using it as a natural, safer treatment in the prevention and treatment of urolithiasis.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this article.

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