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A COMPREHENSIVE STUDY ON COW URINE POWDER: PHYTOCHEMICAL ANALYSIS, PHYSICOCHEMICAL PROPERTIES, AND CAPSULE DEVELOPMENT

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ABSTRACT

Objective: This study aims to evaluate the phytochemical composition and physicochemical properties of cow urine powder to determine its suitability for pharmaceutical formulations. It also focuses on developing and comparing two herbal diuretic capsule formulations, one without excipients and another with excipients, to assess their pharmaceutical viability and effectiveness. **Background:** Herbal formulations have gained increasing attention for their therapeutic potential. Cow urine, a component of traditional medicine, is known for its bioactive properties, including antimicrobial, antioxidant, and diuretic effects. This study focuses on the phytochemical and physicochemical evaluation of cow urine powder and its formulation into herbal diuretic capsules containing synergistic herbal ingredients. **Methods:** Phytochemical screening was conducted using standard qualitative tests to identify the presence of alkaloids, flavonoids, tannins, carbohydrates, proteins, amino and amino acids. Physicochemical properties, including angle of repose, bulk density, tapped density, solubility, pH, and foaming index, were analysed to determine the stability and formulation feasibility. Two capsule formulations were prepared—one without excipients and another with excipients to enhance flow, dissolution, and bioavailability. **Results:** Phytochemical analysis confirmed the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, and tannins, while glycosides were absent. Physicochemical evaluations indicated good flow properties, moderate bulk density, high water solubility,

slightly alkaline pH (8.5), and a foaming index of 100, suggesting the presence of surfactant-like compounds. The formulated capsules met standard pharmaceutical parameters, with the excipient-based formulation demonstrating improved characteristics. **Conclusion:** The study highlights the potential of cow urine powder in herbal diuretic formulations, supported by its bioactive composition and favourable physicochemical properties. The findings suggest its suitability for nutraceutical and pharmaceutical applications, warranting further research into its stability, pharmacological efficacy, and clinical relevance.

KEYWORDS: Cow urine powder, phytochemical screening, physicochemical analysis, diuretic capsules, herbal formulation.

1. INTRODUCTION

Diuretics are essential in managing conditions such as hypertension, edema, and renal disorders by promoting urine excretion. While synthetic diuretics are widely used, their long-term use often leads to adverse effects such as electrolyte imbalance and dehydration. To address this issue, the exploration of natural diuretics has gained significant attention. Traditional medicine has long utilized plant-based remedies for their diuretic properties, offering a safer and more holistic alternative.

In this study, we focus on formulating a novel diuretic capsule using cow **urine powder** in combination with medicinal herbs like **Punarnava (Boerhavia diffusa), Guggulu (Commiphora mukul), Amla (Emblica officinalis), Gokhru(Tribulus terrestris), Fennel(Foeniculum vulgare), Black pepper(Piper nigrum**). Cow urine has been traditionally recognized in Ayurveda for its therapeutic potential, including antimicrobial, Anti-urolithiatic[1], detoxifying, and diuretic properties.[2] By boiling and processing cow urine into a powder, we aim to create a stable and concentrated form suitable for pharmaceutical applications. The selected herbal ingredients are well-documented in traditional and modern pharmacology for their diuretic effects, further enhancing the efficacy of the formulation.

The uniqueness of this formulation lies in the synergistic combination of cow urine powder with diuretic herbs, which may offer enhanced efficacy compared to individual herbal extracts. Additionally, the development of a capsule dosage form ensures ease of administration, standardized dosing, and improved patient compliance. This study aims to

explore the diuretic potential of this novel formulation, paving the way for its potential application in managing fluid retention disorders naturally and effectively.

1.1 Need of study

a. Limitations of Synthetic Diuretics

Synthetic diuretics are widely used to manage conditions like hypertension and edema, but they often lead to side effects such as electrolyte imbalances, dehydration, and kidney dysfunction. A safer, natural alternative with fewer adverse effects is needed.

b. Potential of Herbal and Cow Urine-Based Formulations

Traditional medicine has long utilized herbs and cow urine for their therapeutic benefits, including diuretic activity. However, there is a lack of scientific exploration and standardization of such formulations for pharmaceutical applications.

c. Synergistic Action of Herbal Ingredients

Individual herbs like Punarnava[3] [4], Guggulu[5], Amla[6], Gokhru[7], Fennel[8], etc are known for their diuretic, anti-inflammatory, and detoxifying properties. Combining them with cow urine powder may enhance their efficacy, offering a more potent natural diuretic solution.

d. Need for a Standardized and Convenient Dosage Form

Most herbal diuretics are consumed as decoctions or crude extracts, making dosage control difficult. Developing a capsule formulation ensures precise dosing, ease of administration, and improved patient compliance.

e. Bridging the Research Gap

While cow urine and diuretic herbs have been studied individually, there is limited research on their combined effects in a standardized pharmaceutical formulation. This study aims to fill this gap by scientifically formulating and evaluating a novel herbal diuretic capsule.

f. Potential for Safe and Affordable Diuretic Therapy

Many synthetic diuretics are expensive and may not be accessible to all populations. A natural, cost-effective formulation can provide an alternative, especially in resource-limited settings where herbal medicine is widely accepted.

2. MATERIAL AND METHODS

2.1 Materials

The formulation of the diuretic capsule consisted of cow urine extract and a blend of herbal powders, each selected for their traditional medicinal properties. The ingredients used in the formulation, along with their respective quantities, are as follows**.**

Cow Urine Extract (Khillari Breed)
Amla Powder (Phyllanthus emblica)
Fennel Powder (Foeniculum vulgare)
Punarnava Powder (Boerhavia diffusa)
Gokhru Powder (Tribulus terrestris)
Black Pepper Powder (Piper nigrum)
Guggul Powder (Commiphora mukul)

The cow urine used in the formulation was collected over a period of seven days from the **Khillari** breed of cows. The powdered herbal ingredients were procured from a local authenticated supplier(Gopal Govind Lokhande & Sons, Pune) and were visually inspected for purity before use. The formulation was encapsulated using size 0 gelatin capsules, filled manually with a manual capsule filling machine.

2.2 METHODOLOGY

A. Collection and Processing of Cow Urine Extract

Collection: Fresh cow urine was collected over seven days from Khillari breed cow.[9] A total of 5 liters was collected and stored in clean, airtight glass containers to prevent microbial contamination and evaporation of volatile components.

Storage: The urine was stored in a cool, dark place to maintain its integrity. No preservatives or stabilizers were added.

Boiling Process: The stored urine was boiled using traditional firewood methods in a stainless steel vessel. Boiling continued until a solid residue (cow urine powder) was obtained.[10]

Drying & Sieving: The obtained residue was air-dried at room temperature for 24 hours, then finely ground using a mechanical grinder. The powdered extract was sieved using a 60-mesh sieve to ensure uniformity.

Storage of Powder: The sieved cow urine powder was stored in an airtight amber-coloured container to prevent moisture absorption and degradation.[11]

C. Preparation of Herbal Powder Mixture

Procurement & Authentication: The herbal ingredients Amla, Fennel, Punarnava, Gokhru, Black Pepper, and Guggul were procured from a local authenticated supplier. The raw materials were visually inspected for purity, colour, and texture.

Drying & Grinding: The herbs were dried at 40°C in a hot air oven for 24 hours to remove moisture. Dried herbs were coarsely ground using a mechanical grinder and then sieved using a 40-mesh sieve to obtain a uniform particle size.

Storage of Powders: Each powdered ingredient was stored separately in airtight containers and labelled appropriately.

C. Capsule Formulation

Selection of Capsule Type: Size 0 gelatin capsules were selected based on standard pharmaceutical dosage considerations.

Weighing & Mixing of Ingredients: Each ingredient was accurately weighed using a digital balance. The powders were blended using geometric dilution and trituration techniques to ensure homogeneity.

Formulation Composition: -The formulation of cow urine powder capsules was designed to provide a synergistic diuretic effect with other herbal ingredients. Two formulations were prepared: one without excipients and another with excipients to enhance stability and bioavailability. The capsules were formulated in size 0, with a total weight of 550 mg per capsule.

Formulation A: Without Excipients

Ingredient	Quantity (mg)	Function
Cow urine extract	275	Active ingredient, diuretic
Amla	80	Antioxidant, diuretic
Fennel	25	Digestive aid, diuretic
Punarnava	80	Diuretic, anti-inflammatory
Gokhru	60	Diuretic, kidney tonic
Blackpepper	10	Bioavailability enhancer
Guggul	20	Anti-inflammatory, lipid-lowering

Formulation B: With Excipients

Ingredient	Quantity (mg)	Function
Cow urine extract	260	Active ingredient, diuretic
Amla	70	Antioxidant, diuretic
Fennel	20	Digestive aid, diuretic
Punarnava	70	Diuretic, anti-inflammatory
Gokhru	50	Diuretic, kidney tonic
Blackpepper	10	Bioavailability enhancer
Guggul	20	Anti-inflammatory, lipid-lowering
Sodium Starch Glycolate	22	Disintegrant
Sodium Lauryl Sulphate	10	Surfactant, solubility enhancer
Paraben	5	Preservative
Titanium Dioxide	5	Opacifier
Talc	8	Glidant, improves powder flow

The two formulations were successfully prepared, one containing only active herbal ingredients and the other incorporating excipients to improve stability and performance. The addition of excipients in **Formulation B** enhances the disintegration, solubility, and overall manufacturability of the capsules, making them more suitable for large-scale production.

Capsule Filling and Encapsulation

- Filling Method: A manual capsule filling machine was used. The calibrated weights of powder were filled into empty gelatin capsules to ensure uniformity.
- Sealing & Storage: The filled capsules were sealed, weighed individually, and stored in airtight containers under controlled conditions

3. Physiochemical Properties of Cow Urine Powder

The physicochemical characterization of substances is essential to determine their stability, quality, and potential applications in various industries, including pharmaceuticals and nutraceuticals. Evaluating parameters such as flow properties, density, solubility, pH, and foaming index provides insights into the material's suitability for formulation and therapeutic use. This study aims to analyse the physicochemical attributes of cow urine powder to assess its potential for further applications.[12]

A. Flow Properties

(i) Angle of Repose

Definition: The angle of repose represents the maximum angle at which a pile of powder remains stable without collapsing. It indicates the powder’s ability to flow freely.

Formula: $\theta = \tan^{-1}(h/r)$

Procedure

- A funnel is fixed at a specific height above a flat surface.
- The powder is allowed to flow freely through the funnel, forming a heap.
- The height (h) and radius (r) of the heap are measured**.**
- The angle of repose (θ) is calculated using the formula.

(ii) Hausner's Ratio

Definition: This ratio is an indicator of powder cohesiveness, which impacts its flow ability and compressibility.

Formula: Hausner's Ratio = Tapped Density / Bulk Density

(iii) Carr's Index (Compressibility Index)

Definition: This parameter evaluates the ability of the powder to settle and determines its compressibility.

Formula: Carr's Index = ((Tapped Density - Bulk Density) / Tapped Density) × 100

B. Density Parameters

(i) Bulk Density

Definition: Bulk density is the mass of a powder per unit volume, considering the void spaces between particles.

Procedure (As per IP 2022)

- Weigh approximately 50 g of the sample (adjust weight based on powder characteristics).
- Pour the powder gently into a 100 mL graduated cylinder without tapping or compacting.
- Level the surface of the powder without disturbing it and record the initial volume (V_bulk) in milliliters (mL).
- Calculate the bulk density using the formula:

Formula: Bulk Density = Mass of Powder / Bulk Volume

(ii) Tapped Density

Definition: Tapped density refers to the density of the powder after repeated tapping, reducing air gaps between particles.

Procedure (As per IP 2022)

- Weigh approximately 50 g of the sample (adjust weight based on powder characteristics).

- Pour the powder gently into a 100 mL graduated cylinder with 50 tapping or compacting.
- Level the surface of the powder without disturbing it and record the initial volume (V_tap) in milliliters (mL).
- Calculate the bulk density using the formula:

Formula: Tapped Density = Mass of Powder / Tapped Volume

C. Solubility

Definition: Solubility determines the ability of the powder to dissolve in a solvent, which directly impacts its absorption and effectiveness.

Procedure

- Weigh 1 g of the sample (powder).
- Add the weighed sample to a test tube or conical flask.
- Gradually add the solvent (Water, Ethanol, Methanol, Chloroform, or other prescribed solvent) while stirring at room temperature (25°C ± 2°C).
- Observe the extent of solubility (clear solution, partial dissolution, or insoluble particles).
- If required, heat gently (below boiling point) to assess solubility in warm conditions

D. pH Measurement

Definition: The pH value indicates the acidity or alkalinity of an aqueous solution of the powder, influencing stability and biological interactions.

Procedure

- Prepare a 1% w/v solution of the powder in distilled water.
- Stir well and allow the solution to stand for 5 minutes.
- Measure the pH using a calibrated pH meter.

E. Foaming Index

Definition: The foaming index is used to assess the presence of saponins or other surfactant-like compounds that generate foam in aqueous solutions.

Procedure

- Prepare a 1 g/100 mL aqueous extract of the sample.
- Transfer 1 mL of this extract into a 10 mL graduated test tube.
- Shake the tube vigorously for 15 seconds and let it stand for 1 minute.

- Measure the foam height (a in mm) formed at the surface.
- Calculate the foaming index using the given formula.

Formula: Foaming Index=1000×a/V

where: a = Height of the foam in mm after shaking

V = Volume of the decoction (mL) used in the test

4. Phytochemical screening

The following results were obtained by performing qualitative phytochemical tests on cow urine powder. The presence or absence of different phytochemicals was determined using standard procedures.[13], [14]

A. Carbohydrates

Test	Procedure
Molisch Test	Add Molisch reagent and conc. H_2SO_4 along the sides of the test tube. Observe for a purple ring.
Iodine Test	Add iodine solution to the extract. A blue-black color indicates the presence of starch.
Seliwanoff's Test	Add Seliwanoff's reagent (resorcinol in HCl) to the extract and heat. A red color indicates ketose sugars.
Fehling's Test	Mix Fehling's A & B solutions with the extract and heat. A brick-red precipitate confirms reducing sugars.

B. Proteins

Test	Procedure
Xanthoprotein Test	Add conc. HNO_3 to the extract and heat. A yellow color appears, which turns orange with NaOH.
Lead Acetate Test	Add lead acetate solution to the extract. A white precipitate confirms proteins.

C. Amino Acids

Test	Procedure
Cysteine Test	Add lead acetate to the extract and heat. A black precipitate confirms cysteine (sulfur-containing amino acids).

D. Alkaloids

Test	Procedure
Dragendorff's Test	Add Dragendorff's reagent to the extract. A reddish-brown precipitate confirms alkaloids.
Hager's Test	Add Hager's reagent (picric acid solution) to the extract. A yellow precipitate indicates alkaloids.
Wagner's Test	Add Wagner's reagent (iodine in potassium iodide) to the extract. A reddish-brown precipitate confirms alkaloids.
Mayer's Test	Add Mayer's reagent (potassium mercuric iodide solution) to the extract. A white or creamy precipitate confirms alkaloids.

E. Glycosides

Test	Procedure
Fehling's Test for Glycosides	Hydrolyze the extract with dilute HCl, neutralize, then add Fehling's A & B solutions and heat. A brick-red precipitate confirms glycosides.

F. Flavonoids

Test	**Procedure**
Sulfuric Acid Test	Add conc. H_2SO_4 to the extract. A yellow to orange color appears.
Lead Acetate Test	Add lead acetate solution to the extract. A yellow precipitate confirms flavonoids.
Alkali Test	Add sodium hydroxide (NaOH) solution to the extract. A yellow color that disappears with acid confirms flavonoids.

G. Tannins

Test	**Procedure**
Ferric Chloride Test	Add $FeCl_3$ solution (5%) to the extract. A blue-black or green color confirms tannins.
Lead Acetate Test	Add lead acetate solution to the extract. A white precipitate confirms tannins.
Dilute Iodine Test	Add dilute iodine solution to the extract. A transient red color confirms tannins.
Dilute HNO_3 Test	Add dilute nitric acid to the extract. A reddish-brown color indicates tannins.

5. Evaluation of Capsule

A. Appearance

Capsules were visually inspected for colour, texture, and uniformity.

B. Weight Variation (as per IP)

Procedure

- Select 10 random capsules from the batch.
- Weigh each capsule individually and record the weight.
- For hard gelatin capsules, weigh the empty shell separately and determine the net content weight.
- Calculate the average weight of the 10 capsules.
- Determine the permissible weight variation (Indian Pharmacopoeia Commission, 2022):
- - > 300 mg → ±7.5%
- - 130–300 mg → ±10%
- - < 130 mg → ±15%

- Check compliance:
- - If ≤ 2 capsules deviate, the batch passes.
- - If > 2 capsules deviate, the batch fails.[15]

C. Disintegration Test (as per IP)

Procedure

- Use the disintegration test apparatus with 6 glass tubes, each 77.5 mm long and having an internal diameter of 21.5 mm, with a 10-mesh sieve at the bottom.
- Fill the beaker with the specified liquid medium, maintaining a temperature of 37 ± 2°C.
- Place one capsule in each tube and suspend the basket in the beaker.
- Operate the apparatus at 30 cycles per minute.
- Observe the capsules. They must disintegrate completely, leaving no residue except fragments of the insoluble capsule shell.
- For hard gelatin capsules, the disintegration time should not exceed 30 minutes.
- For soft gelatin capsules, the disintegration time should not exceed 60 minutes.[15]

D. Stability Studies

Stability studies are conducted to determine the shelf life, storage conditions, and Formulation integrity of pharmaceutical products over time. The objective of this study is to assess the physical, chemical, microbiological[16], and dissolution stability of the formulated cow urine-based diuretic capsules (Formulation A & Formulation B) under different storage condition.

The formulated capsules were stored under three different environmental conditions for One month, mimicking real-life conditions.

Storage Condition	Temperature & Humidity	Purpose
Room Temperature (Controlled Condition)	25°C ± 2°C, 60% RH ± 5%	Long-term stability
Accelerated Condition	40°C ± 2°C, 75% RH ± 5%	Stress testing for degradation
Cold Storage Condition	4°C ± 2°C	Evaluation of stability under refrigeration

5.RESULT

5.1 Phytochemical Screening

- These formulations retained essential phytochemicals, including alkaloids, flavonoids, tannins, and proteins.

- Glycosides were absent in all formulations, indicating their non-existence in cow urine powder.
- Formulation B (With Excipients) maintained the phytochemical profile while enhancing capsule stability and flow properties.

Test Name	Cow Urine Powder	Formulation B (Without Excipients)	Formulation B (With Excipients)
1.Carbohydrates Tests			
Molisch Test	+	+	+
Iodine Test	-	-	-
Seliwanoff's Test	-	-	-
Fehling's Test	+	+	+
2.Protein Tests			
Xanthoprotein Test	+	+	+
Lead Acetate Test	+	+	+
3.Amino Acid Test			
Cysteine Test	+	+	+
4.Alkaloid Tests			
Dragendorff's Reagent	+	+	+
Hager's Reagent	+	+	+
Wagner's Reagent	+	+	+
Mayer's Reagent	+	+	+
5.Glycoside Test			
Fehling's Test	-	-	-
6.Flavonoids Tests			
Sulfuric Acid Test	+	+	+
Lead Acetate Test	+	+	+
Alkali Test	+	+	+
7.Tannins Tests			
Ferric Chloride Test	+	+	+
Lead Acetate Test	+	+	+
Dilute Iodine Test	+	+	+
Dilute HNO_3 Test	+	+	+

(Abbreviation:- + indicates positive , - indicates negative)

5.1 Physiochemical Properties

Formulation B (With Excipients) exhibited the best physicochemical properties**,** including Enhanced flowability, compressibility, solubility, and stability**,** making it the preferred formulation for diuretic capsule preparation**.**

Physiochemical Property	Cow Urine Powder	Formulation A (Without Excipients)	Formulation B (With Excipients)
Angle of Repose (°)	16	14	13
Hausner Ratio	1.30	1.23	1.12
Carr's Index (%)	21	18.75	16
Tap Density (g/mL)	0.70	0.64	0.55
Bulk Density (g/mL)	0.58	0.52	0.55
Solubility	Soluble in water	Soluble in water	Soluble in water
pH	8	7.8	7.5
Foaming Index	210	190	142

5.3 Capsule Evaluation

5.3.1 Weight Variation

Sr. No.	Before Stability (g)	After Stability (g)
1	0.6471	0.6425
2	0.6679	0.6632
3	0.6652	0.6608
4	0.59	0.5856
5	0.648	0.6442
6	0.6503	0.6481
7	0.6558	0.6519
8	0.6534	0.6493
9	0.6556	0.6525
10	0.5653	0.5609

Before Stability:

- Average Capsule Weight: 0.6399 g
- Standard Deviation: 0.0340 g
- Weight Variation Range: 0.1026 g
- Compliance with IP Standards: Acceptable

After Stability

- Average Capsule Weight: 0.6359 g
- Standard Deviation: 0.0337 g
- Weight Variation Range: 0.1023 g
- Compliance with IP Standards: Acceptable

5.3.2 Disintegration Test

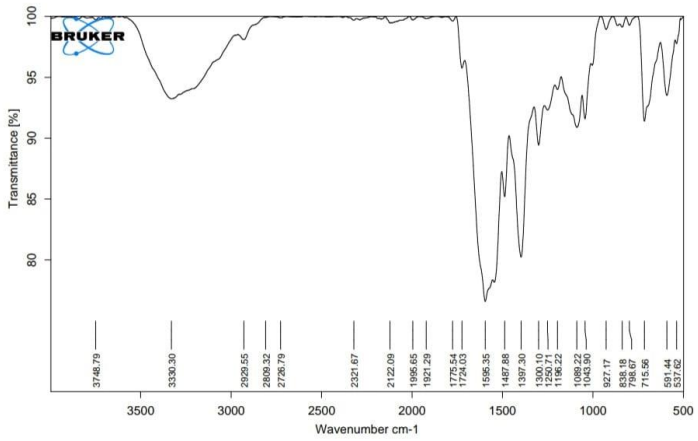
The disintegration time increased slightly after the stability study, indicating a minor effect of storage conditions on capsule integrity. However, all capsules remained within the acceptable limits as per the Indian Pharmacopoeia (IP) standards.

Capsule Number	Disintegration Time (Before Stability Studies) (min)	Disintegration Time (After Stability Studies) (min)
Capsule 1	12.5	15.0
Capsule 2	13.0	15.2

Capsule 3	12.8	14.8
Capsule 4	13.2	15.1
Capsule 5	12.7	15.0
Capsule 6	13.1	15.3

5.3.3 FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) was performed on the cow urine powder sample to identify the presence of functional groups associated with diuretic compounds.[17] Since cow urine is known to contain bioactive nitrogenous compounds, this analysis aids in confirming the presence of urea and other potential diuretic agents that contribute to increased urine production and kidney function regulation.



FTIR Peak Interpretation

Wavenumber (cm^{-1})	Functional Group	Possible Compound
3340-3300	N-H Stretch	Urea, Amides
2925-2855	C-H Stretch	Alkanes, Lipids
1655-1620	C=O Stretch (Amide)	Urea, Proteins
1585-1515	N-H Bending	Amides, Proteins
1455-1400	C-N Stretch	Urea, Creatinine
1100-1000	C-O Stretch	Sugars, Organic Acids

Presence of Urea and Nitrogenous Compounds with Diuretic Activity

One of the most significant peaks observed is around 1655-1620 cm^{-1}, which corresponds to the C=O stretching of amide groups. This strongly indicates the presence of urea, an important nitrogenous compound in cow urine. Urea acts as an osmotic diuretic, meaning it promotes water excretion by drawing water into the renal tubules, leading to increased urine output.

Additionally, the presence of C-N stretching (1455-1400 cm^{-1}) suggests other nitrogen-containing compounds such as creatinine, uric acid, and ammonia derivatives, which are known to influence kidney function. These compounds may aid in detoxification and electrolyte balance, further supporting their role in diuresis.

6. CONCLUSION

This study successfully developed and evaluated cow urine-based herbal diuretic capsules using a synergistic combination of herbal diuretics**.** Formulation B (with excipients) demonstrated superior flow properties, stability, dissolution, making it the preferred formulation over Formulation A (without excipients)**.**

Overall, this research supports the therapeutic potential of cow urine-based herbal formulations and provides a scientific basis for their use in diuretic therapy. Future studies should focus on clinical validation and long-term stability assessment for commercial application.

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Conflicts of Interest

The authors declare no conflicts of interest.

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