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NETWORK PHARMACOLOGY-GUIDED EVALUATION OF PUNARNAVA GHANAVATI IN CHRONIC KIDNEY DISEASE: AN IN SILICO AND PRECLINICAL PILOT INVESTIGATION

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ABSTRACT

Background: Chronic Kidney Disease (CKD) is a progressive renal disorder associated with oxidative stress, inflammation, altered tubular transport and cardiovascular risk. Punarnava Ghanavati, prepared from Punarnava, is traditionally described as *Mutrala*, *Shothahara* and *Rasayana*. Objective: To evaluate the nephroprotective potential of Punarnava Ghanavati using network pharmacology, molecular docking and a preclinical urinary excretion pilot study. Materials and Methods: Punarnavoside targets were predicted through BindingDB, STITCH, SuperPred and DigiPred, annotated through UniProt and analyzed with CKD-related targets using STRING, WikiPathways and Cytoscape. Molecular docking was performed against SGLT2 with dapagliflozin as the reference compound. Male Wistar rats received Punarnava Ghanavati orally for five days, and urinary sodium, chloride and glucose were estimated. Results: SLC5A2/SGLT2 and NRF2-related signaling emerged as relevant computational findings. Punarnavoside showed a docking score of -7.062 against SGLT2 compared with -9.074 for dapagliflozin. Punarnava Ghanavati increased urinary sodium excretion, while urinary glucose remained nearly unchanged. Conclusion: The findings suggest renal activity through natriuresis and multi-target pathway modulation. Classical SGLT2 inhibition is unlikely to be the main mechanism, and further controlled studies are required.

KEYWORDS: Punarnava Ghanavati; Chronic Kidney Disease; Network Pharmacology; SGLT2; NRF2; Ayurveda

INTRODUCTION

Chronic Kidney Disease (CKD) is a long-term reduction in renal structure or function and is increasingly recognized as a systemic disorder rather than an isolated kidney condition. As renal function declines, disturbances in fluid balance, electrolyte handling, acid-base regulation, endocrine function and cardiovascular stability develop progressively. Conventional management focuses on controlling blood pressure, glycemic burden, proteinuria, fluid overload and cardiovascular risk. However, despite modern therapeutic advances, many patients continue to progress toward advanced renal dysfunction, creating a need for additional nephroprotective strategies that are mechanistically plausible and experimentally verifiable.

CKD progression involves repeated injury to glomerular, tubular, vascular and interstitial compartments. Tubular epithelial stress, activation of inflammatory mediators, oxidative injury and maladaptive repair can lead to fibrosis and nephron loss. The progression of CKD is not always linear; periods of stability can be followed by decline when additional insults such as hypertension, diabetes, infection, nephrotoxic exposure or dehydration occur.

Sodium handling has special relevance in CKD because sodium retention contributes to volume expansion, hypertension, edema and increased cardiovascular load. Interventions that modify sodium excretion may therefore influence renal hemodynamics and systemic blood pressure. However, increased natriuresis must be interpreted carefully because excessive diuresis in vulnerable patients may worsen dehydration or electrolyte imbalance. In a preclinical pilot setting, urinary sodium output provides an early clue regarding tubular or hemodynamic effects.

Punarnava, botanically identified as *Boerhavia diffusa Linn.*, is one of the most frequently cited Ayurvedic herbs for urinary and renal disorders. The name Punarnava suggests renewal or restoration, and traditional descriptions emphasize its use in conditions associated with swelling, fluid accumulation, altered urination and systemic debility. Pharmacological reports have attributed diuretic, anti-inflammatory, antioxidant, hepatoprotective and nephroprotective properties to Punarnava and its constituents. Among its reported phytoconstituents, Punarnavoside has attracted attention because it can be studied computationally through target prediction and molecular docking.

In Ayurveda, Punarnava is traditionally described with actions such as *Mutrala* (promoting urine output), *Shothahara* (reducing swelling) and *Rasayana* (supporting restoration and vitality). These properties are clinically relevant to renal conditions involving edema, fluid retention and systemic weakness. Punarnava Ghanavati is a tablet formulation that offers a standardized and practical dosage form for experimental and clinical evaluation.

The Ayurvedic concept of *Shotha* includes swelling and inflammatory accumulation, while *Mutrala* action indicates an effect on urinary elimination. From a contemporary pharmacological perspective, these descriptions can be explored through natriuretic, diuretic, anti-inflammatory, antioxidant and renal tubular mechanisms. The current study attempts to bridge these explanatory systems without reducing the Ayurvedic action to a single molecular target.

Network pharmacology is particularly suitable for Ayurvedic formulations because it does not assume a single-drug, single-target mechanism. Instead, it allows the investigator to examine how a phytoconstituent may interact with multiple proteins and pathways. This approach is valuable for CKD, where oxidative stress, inflammation, hemodynamic stress, tubular dysfunction and metabolic abnormalities interact in a network rather than in isolation.

The original pilot observations showed a specific experimental pattern: Punarnava

Ghanavati increased urinary sodium excretion while urinary glucose was almost unchanged. This finding is important because the computational analysis predicted a possible relationship with SGLT2, a renal transporter targeted by drugs such as dapagliflozin. If Punarnava Ghanavati had acted mainly as a classical SGLT2 inhibitor, a stronger increase in urinary glucose would have been expected. Therefore, the combined evidence supports a more nuanced interpretation in which SGLT2 binding may exist but may not dominate the biological activity of the formulation.

AIM AND OBJECTIVES

Aim: To evaluate the potential nephroprotective mechanisms of Punarnava Ghanavati in Chronic Kidney Disease by integrating network pharmacology, molecular docking and preclinical urinary excretion findings.

-To identify predicted protein targets of Punarnavoside using multiple in silico databases.

-To identify CKD-associated molecular targets and determine overlap with Punarnavoside-related targets.

-To construct compound-target-pathway networks using Cytoscape and analyze the relationship between Punarnavoside, SLC5A2/SGLT2, NRF2 signaling and renal disease pathways.

-To compare the molecular docking interaction of Punarnavoside with SGLT2 against dapagliflozin as a standard SGLT2 inhibitor.

-To evaluate the short-term effect of Punarnava Ghanavati on urinary sodium, chloride and glucose excretion in male Wistar rats.

-To interpret whether the experimental urinary profile supports or challenges the predicted SGLT2-mediated mechanism.

Working hypothesis

The working hypothesis was that Punarnavoside, a phytochemical constituent of Punarnava Ghanavati, may interact with CKD-associated molecular targets including SGLT2 and may influence renal function through pathways associated with oxidative stress regulation and tubular transport. The pilot animal study was designed to observe whether this predicted interaction translated into measurable changes in urinary glucose and electrolyte excretion.

Contemporary background of CKD and renal tubular dysfunction

The kidney maintains internal homeostasis by filtering plasma and selectively reabsorbing or excreting water, electrolytes, glucose, organic solutes and metabolic waste products. In CKD, both glomerular filtration and tubular adaptive responses are disturbed. Tubular cells remain metabolically active even when glomerular filtration declines, and they are exposed to hypoxia, oxidative stress, inflammatory mediators and altered solute loads. Therefore, tubular transporters and tubular stress pathways are important in understanding disease progression and possible renal-supportive interventions.

In the context of renal therapeutics, sodium transport and glucose transport are closely connected. Proximal tubular reabsorption of glucose through sodium-glucose cotransport is coupled with sodium movement. Distal nephron sodium handling then influences

extracellular volume, blood pressure and edema formation. A formulation that changes urinary sodium excretion without provoking marked glucosuria may indicate a renal action that differs from the pharmacological profile of modern SGLT2 inhibitors. This point is central to the interpretation of the present investigation.

The study was planned as an integrated hypothesis-generating investigation rather than as a definitive efficacy trial. The computational component was used to identify biologically plausible molecular targets, while the preclinical component was used to determine whether one predicted functional outcome, urinary glucose excretion, was actually seen. This design is appropriate at an early stage because network pharmacology can generate several possible mechanisms, but physiological confirmation is necessary before proposing a dominant mechanism.

Relevance of Ayurvedic terminology to experimental endpoints

The traditional terms *Mutrala*, *Shothahara* and *Rasayana* were interpreted carefully during study planning. *Mutrala* was linked to urinary elimination and electrolyte excretion, *Shothahara* was considered relevant to edema, inflammation and fluid accumulation, and *Rasayana* was viewed as a broader concept that may correspond to tissue protection, antioxidant activity and restoration of function. The present work does not claim that these traditional concepts are identical to modern pharmacological mechanisms; rather, it uses measurable laboratory endpoints to explore possible correlations.

Such a translational approach is important for Ayurveda research because many classical actions are multidimensional. A drug described as *Mutrala* may influence urine volume, sodium output, renal blood flow or tubular transporter function. Similarly, a drug described as *Rasayana* may act through antioxidant, anti-inflammatory, immunomodulatory or cytoprotective pathways. Network pharmacology offers one way to map these broader actions into testable biological hypotheses.

MATERIALS AND METHODS

In silico network pharmacology workflow

The network pharmacology workflow began with collection of chemical information for Punarnavoside. The molecular formula, molecular weight and canonical SMILES were obtained from public chemical databases. The SMILES string was used as the structural input for target prediction platforms. Multiple target-prediction resources were used to reduce dependence on a single algorithm and to improve biological coverage.

BindingDB, STITCH, SuperPred and DigiPred were used for target identification. The predicted protein targets were converted into standardized gene identifiers using UniProt. CKD-related targets were collected from disease and therapeutic target resources, including a therapeutic target database and disease gene resources. A relevance threshold was applied for CKD-associated genes to focus on targets with stronger disease association.

After target collection, the predicted Punarnavoside targets and CKD-associated targets were compared to identify common

genes. These overlapping genes were considered candidate targets through which Punarnavoside could potentially influence CKD-related biological processes. This intersection-based approach links phytochemical prediction with disease relevance.

Protein-protein interaction and pathway enrichment

Common targets were uploaded to STRING version 12.0 to explore protein-protein interactions. The STRING output was used to understand whether the candidate targets formed biologically meaningful clusters. Pathway enrichment was performed using WikiPathways and related pathway resources to identify renal and oxidative stress pathways associated with the target set.

Cytoscape version 3.10.2 was used to construct compound-target, target-pathway and compound-target-pathway networks. Node size and color were interpreted according to edge count and degree of interaction, with highly connected nodes considered potentially important within the network. Particular attention was given to SLC5A2/SGLT2 and NRF2 signaling because they emerged as mechanistically relevant to renal tubular function and oxidative stress.

Molecular docking

Molecular docking was performed to evaluate the binding interaction between Punarnavoside and SGLT2. Dapagliflozin, a known SGLT2 inhibitor, was used as the reference compound. The SGLT2 protein structure was represented by PDB ID 8HEZ in the source document. Ligand preparation, receptor preparation, grid generation and docking were performed to obtain docking and Glide scores.

The docking analysis aimed to determine whether Punarnavoside could occupy or interact with the SGLT2 binding environment in a manner comparable to dapagliflozin. Docking score was interpreted as an estimate of binding favorability; more negative scores generally indicate stronger predicted affinity. However, docking is not a direct measure of in vivo inhibition. It is a structural prediction that must be interpreted together with biochemical and animal data.

The docking comparison also examined interacting amino acid residues. The analysis indicated that Punarnavoside and dapagliflozin shared several residues within the SGLT2 binding environment, including GlnA457, HisA525, TyrA526, LeuA274, AspA454, LeuA84, ValA95, PheA98 and PheA453. Shared interacting residues support the possibility of a common or overlapping binding region, but they do not prove equivalent pharmacodynamic activity.

TABLE 1. Comparative Docking Scores of Dapagliflozin and Punarnavoside Against SGLT2

Ligand-protein complex	Docking score	Glide score	Interpretation
SGLT2 + Dapagliflozin (PDB ID: 8HEZ)	-9.074	-9.074	Reference SGLT2 inhibitor with stronger predicted binding

SGLT2 + Punarnavoside	-7.062	-7.062	Favorable predicted interaction, weaker than dapagliflozin

Experimental animals and ethical approval

Male Wistar rats weighing 250-300 g were used for the preclinical pilot experiment. The animals were obtained from the animal house facility of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra. Before the experiment, rats were acclimatized to metabolic cages for three days. This acclimatization period minimized stress-related variation in urine collection.

All study procedures were approved by the Institutional Animal Ethics Committee registered under CCSEA, Government of India. The protocol approval number was IAEC/2025/II R26. Animals were maintained under standard laboratory conditions, and urine was collected using individual metabolic cages to avoid contamination and to allow separate measurement of urinary parameters.

Dose calculation and drug administration

The human dose of Punarnava Ghanavati was considered as 500 mg twice daily, equivalent to 1000 mg per day. The rat equivalent dose was calculated using body surface area conversion based on Km factors. With a rat Km of 6 and a human Km of 37, the calculated rat dose was 162.16 mg/kg. For a 250 g rat, the dose corresponded to approximately 40.54 mg per animal.

Punarnava Ghanavati was administered orally for five consecutive days. On the fifth day, after the last dose, animals were placed individually in metabolic cages and urine was collected for five hours. Urine samples were then analyzed for sodium, chloride and glucose using commercial kits. These parameters were selected because they directly reflect renal tubular handling of electrolytes and glucose.

Detailed approach to target prediction

The use of more than one target-prediction database was intended to improve confidence in the in-silico observations. Each database applies a different algorithmic logic and may emphasize different types of chemical-protein relationships. For example, a database may rely on known binding data, chemical similarity, pharmacophore comparison or integrated machine-learning predictions. When a target appears across or after comparison of multiple resources, it becomes more suitable for further pathway analysis than a target predicted from a single source alone.

Standardization of protein and gene identifiers was a necessary step before network construction. Target names obtained from chemical databases may appear as protein names, gene symbols, accession numbers or synonyms. UniProt annotation was therefore used to harmonize identifiers before overlap analysis. This reduces duplication and prevents biologically identical targets from being treated as separate nodes in Cytoscape.

Selection of CKD-associated targets was performed to connect the phytochemical prediction with disease relevance. Targets with stronger relevance to CKD were prioritized so that the final network would not become too broad or non-specific. This

step is especially important for phytoconstituents because they may have predicted interactions with a wide range of proteins that are not necessarily related to the disease under study.

Network construction and interpretation

The compound-target-pathway network was not interpreted as a direct proof of clinical activity. It was used as a visual and analytical map to identify probable relationships among Punarnavoside, predicted proteins and CKD-relevant pathways. Nodes with greater connectivity were considered potentially important because they linked more components of the network. However, biological importance also depends on renal expression, tissue exposure, concentration at the target site and functional relevance in living systems.

Pathway enrichment was interpreted at the level of biological themes rather than as a single isolated pathway. NRF2 signaling was considered relevant because oxidative stress is a recurring mechanism in CKD progression and renal tubular injury. This pathway-level interpretation fits the multi-target logic of Ayurvedic pharmacology and avoids overemphasis on one transporter alone.

Docking precautions and interpretation

Molecular docking results were used to support structural plausibility, not to establish in vivo efficacy. Docking scores can be influenced by receptor conformation, ligand protonation, grid selection and scoring function. Therefore, a favorable score for Punarnavoside was treated as evidence that the compound may fit within or near the SGLT2 binding environment, while the rat urinary data were used to judge whether this predicted interaction produced a functional pattern resembling SGLT2 inhibition.

The inclusion of dapagliflozin as a reference compound strengthened the docking comparison because it provided a known SGLT2 inhibitor within the same docking environment. The more negative score for dapagliflozin indicated stronger predicted binding. The weaker but favorable score for Punarnavoside suggested possible interaction, but the difference in scores supported caution in claiming competitive inhibition as the principal biological mechanism.

Preclinical endpoint selection

The urinary endpoints were selected to connect the computational prediction with renal physiology. Urinary glucose was included because classical SGLT2 inhibition should increase glucosuria. Urinary sodium and chloride were included because Punarnava is traditionally used in conditions involving altered fluid balance, and because sodium handling is relevant to edema and hypertension in CKD. The five-hour urine collection period provided a short-term functional snapshot after repeated oral administration for five days.

The pilot study was not designed to establish long-term nephroprotection. It did not include a disease-induced CKD model, histopathological assessment or broad biochemical profiling. Its purpose was more focused: to observe whether Punarnava Ghanavati produced a urinary pattern consistent with predicted SGLT2 inhibition or whether the observed renal activity

suggested another direction for future mechanistic studies.

Table 2. Preclinical Pilot Study Design

Parameter	Details
Species/strain	Male Wistar rats
Body weight	250-300 g
Acclimatization	3 days in metabolic cages
Treatment duration	5 consecutive days
Dose	40.5 mg per 250 g rat / approximately 162.16 mg/kg
Urine collection	5 hours after final dose
Measured parameters	Sodium, chloride and glucose

RESULTS / OBSERVATIONS

Network pharmacology analysis

The predicted biological target analysis identified SLC5A2 among the important targets of Punarnavoside. SLC5A2 encodes sodium-glucose cotransporter 2, commonly known as SGLT2. SGLT2 is a renal proximal tubular transporter responsible for reabsorption of a major fraction of filtered glucose. The identification of SLC5A2 therefore provided a mechanistic bridge between Punarnava phytochemistry and renal tubular physiology.

Pathway mapping showed that the Punarnavoside target network intersected with CKD-associated pathways, especially NRF2 signaling. NRF2 signaling is a central antioxidant response pathway that regulates cellular defense against oxidative stress. Since oxidative stress contributes to tubular injury, inflammation, fibrosis and CKD progression, involvement of NRF2 signaling is biologically plausible for a nephroprotective phytoconstituent.

The Cytoscape network conceptually linked Punarnavoside to protein targets and renal disease pathways. In such a network, highly connected nodes may represent mechanistic hubs. The presence of SLC5A2 in the target network does not imply that SGLT2 inhibition is the only mechanism; rather, it identifies one possible target among several disease-relevant interactions. A multi-target interpretation is particularly appropriate for herbal formulations.

Table 3. Summary of Network Pharmacology Findings

Network component	Observation	Biological meaning
Compound	Punarnavoside	Key phytochemical component considered from Punarnava Ghanavati
Predicted target	SLC5A2/SGLT2	Renal tubular glucose and sodium-glucose cotransport relevance
Pathway signal	NRF2 signaling	Oxidative stress regulation and CKD relevance
Network tool	Cytoscape 3.10.2	Visualization of compound-

		target-pathway relations

Molecular docking observations

Docking analysis showed that dapagliflozin had a docking score of -9.074 against SGLT2, while Punarnavoside had a docking score of -7.062. The more negative score for dapagliflozin is consistent with its role as a standard SGLT2 inhibitor. Punarnavoside showed favorable binding, although weaker than the standard drug. This suggests a possible but less potent interaction with SGLT2.

The identification of common interacting residues between dapagliflozin and Punarnavoside strengthened the structural plausibility of SGLT2 binding. However, molecular docking alone cannot establish whether Punarnavoside produces functional SGLT2 inhibition in biological systems. Ligand solubility, permeability, metabolism, plasma protein binding, tissue exposure, transporter dynamics and formulation characteristics can all modify whether predicted binding becomes measurable activity in vivo.

Therefore, the docking result was treated as hypothesis-generating. It justified a pilot urinary study focused on glucose and sodium excretion. If Punarnava Ghanavati had produced robust SGLT2 inhibition, increased urinary glucose excretion would have been expected. The animal data were therefore essential for interpreting the computational predictions.

Urinary excretion observations

The preclinical pilot study compared urinary sodium, chloride and glucose values between control animals and animals receiving Punarnava Ghanavati. The most notable observation was an increase in urinary sodium from 118.25 in the control condition to 146.53 after Punarnava treatment. This pattern indicates a natriuretic effect during the five-hour collection period.

Urinary chloride did not increase in parallel with sodium. The mean chloride value was 528.20 in the control condition and 495.07 after Punarnava treatment. Urinary glucose also remained almost unchanged, with a control value of 16.42 and a Punarnava value of 16.62. The absence of increased glucose excretion is important because SGLT2 inhibition is typically expected to cause glucosuria.

The urinary profile therefore suggests that the principal short-term renal effect of Punarnava Ghanavati in this pilot experiment was selective sodium excretion rather than glucose wasting. This observation does not invalidate the in-silico prediction, but it changes its interpretation. Punarnava may interact with SGLT2 structurally while producing its dominant renal action through other tubular, hemodynamic or antioxidant mechanisms.

Integrated interpretation of network and urine findings

The network pharmacology findings and the urinary observations should be interpreted together. The computational analysis indicated that SLC5A2/SGLT2 is a plausible predicted target of Punarnavoside. However, the in vivo urinary profile did not show a meaningful rise in glucose excretion. This combination suggests that target binding predicted in silico may not necessarily translate into functionally significant

transporter inhibition under the tested conditions.

Several explanations are possible for this observation. Punarnavoside may bind to SGLT2 weakly or at a site that does not strongly inhibit glucose transport. The compound may not reach adequate renal tubular concentrations after oral administration of the formulation. Other constituents in the formulation may dominate the acute renal response. It is also possible that the sodium effect is mediated through pathways unrelated to SGLT2, such as altered renal hemodynamics or effects on other sodium transport mechanisms.

The sodium finding is nevertheless important because it provides an experimental signal consistent with the traditional *Mutrala* description of Punarnava. The rise from 118.25 to 146.53 suggests increased sodium elimination during the observation window. Although the sample size was limited and statistical testing was not emphasized, the direction of change is mechanistically useful for planning future-controlled work.

The chloride observation requires cautious interpretation. Chloride did not increase with sodium and showed a modest decrease. In renal physiology, sodium and chloride may move together in many tubular segments, but they are not always altered in identical directions depending on transporter activity, urine flow, bicarbonate handling and dietary or hydration status. Because this was a pilot study, chloride should be considered an exploratory observation rather than a definitive mechanistic endpoint.

The absence of glucosuria is one of the most useful negative findings in the study. Negative results should not be hidden because they refine the mechanistic hypothesis. Here, the negative glucose finding prevented overinterpretation of the docking result and guided the discussion toward natriuresis, antioxidant signaling and broader renal-supportive mechanisms rather than a narrow SGLT2 inhibitor claim.

Table 4. Pilot Urinary Excretion Observations After Oral Administration of Punarnava Ghanavati

Urinary parameter	Control	Punarnava Ghanavati	Direction of change
Sodium	118.25	146.53	Increased
Chloride	528.20	495.07	Slightly decreased
Glucose	16.42	16.62	No meaningful change

DISCUSSION

The present manuscript demonstrates the value of combining computational and experimental methods in the evaluation of Ayurvedic formulations. The in-silico findings identified SLC5A2/SGLT2 and NRF2 signaling as biologically meaningful components of the Punarnavoside-CKD network. These findings are relevant because SGLT2 and NRF2 represent two different but kidney-relevant domains: tubular transport and oxidative stress response.

SGLT2 has become an important renal target because pharmacological SGLT2 inhibitors provide kidney and cardiovascular benefits

beyond glucose lowering. However, the mechanism of established SGLT2 inhibitors includes increased urinary glucose excretion. In the present pilot experiment, Punarnava Ghanavati did not increase urinary glucose. Therefore, the available evidence does not support a conclusion that the formulation primarily acts as a classical SGLT2 inhibitor. The increased urinary sodium excretion is consistent with the traditional *Mutrala* description of Punarnava. Natriuresis may contribute to reduction in fluid retention and may indirectly support renal and cardiovascular balance. Nevertheless, sodium excretion alone cannot establish nephroprotection. Future studies should include serum creatinine, blood urea nitrogen, estimated creatinine clearance, urine protein, kidney histopathology, oxidative stress markers, inflammatory cytokines and renal transporter expression.

The NRF2 signal offers a complementary explanation. Activation or modulation of NRF2-related antioxidant defenses could reduce oxidative injury and inflammation in renal tissue. Such a mechanism would be consistent with the broader pharmacological profile reported for Punarnava. A multi-target model in which Punarnava Ghanavati produces mild natriuresis while also influencing oxidative stress and inflammatory pathways is more consistent with the combined data than a single-target SGLT2 inhibition model.

Proposed mechanistic model

The proposed model begins with Punarnava Ghanavati delivering phytoconstituents such as Punarnavoside. In silico target prediction suggests that these constituents may interact with proteins involved in renal tubular transport and stress response. Among these, SLC5A2/SGLT2 is structurally plausible based on docking, while NRF2 signaling provides a pathway-level explanation for antioxidant nephroprotection.

At the physiological level, the observed increase in urinary sodium suggests that the formulation may influence sodium handling in the kidney. This may occur through tubular transport modulation, changes in renal blood flow or indirect effects on fluid balance. Because urinary glucose did not increase, the sodium effect is unlikely to be fully explained by classical SGLT2 inhibition.

A reasonable integrated hypothesis is that Punarnava Ghanavati acts through a combination of natriuretic, antioxidant, anti-inflammatory and renal hemodynamic effects. SGLT2 interaction may represent one component of the molecular network, but it is probably not the dominant acute functional mechanism in the tested rat model. This interpretation preserves the value of the docking result while remaining faithful to the wet-lab data.

Textual pathway diagram: Punarnava Ghanavati -> Punarnavoside and related phytoconstituents -> predicted targets including SLC5A2/SGLT2 and oxidative stress regulators -> NRF2-associated pathway modulation -> reduced oxidative stress and altered tubular handling -> increased urinary sodium excretion with minimal change in urinary glucose -> potential renal-supportive effect requiring further validation.

Translational relevance

This study has translational relevance because it uses a workflow that can be applied to Ayurvedic formulations without ignoring their multi-component nature. Instead of assuming that Punarnava Ghanavati must behave like a single modern drug, the study identifies probable molecular interactions and then evaluates whether a predicted target produces a measurable physiological signal.

For CKD research, such integration is useful because renal protection is multifactorial. A formulation may be clinically meaningful if it reduces edema, improves sodium balance, reduces oxidative stress or protects tubular structures, even if it does not behave as a potent SGLT2 inhibitor. The present pilot findings support further investigation of Punarnava Ghanavati as a renal-supportive intervention, especially in models where edema, oxidative stress or inflammatory injury are prominent.

At the same time, translation to human CKD requires caution. The pilot study was short, used a small number of animals and measured only urinary electrolytes and glucose. It did not evaluate glomerular filtration, histological injury, proteinuria, fibrosis or long-term renal outcomes. Therefore, the current evidence is preliminary and should be used to design stronger studies rather than to make therapeutic claims.

Limitations

The study has several limitations. First, the animal experiment was a pilot study with limited sample size, reducing statistical power. Second, the treatment duration was only five days, which is insufficient to evaluate chronic nephroprotection in progressive kidney disease. Third, the experiment measured urinary sodium, chloride and glucose but did not include serum biochemical markers such as creatinine, urea, electrolytes or cystatin C.

Fourth, histopathological assessment of kidney tissue was not included. Without histology, it is not possible to determine whether Punarnava Ghanavati reduced glomerular, tubular, vascular or interstitial injury. Fifth, transporter expression studies were not performed. Therefore, the relationship between Punarnava and SGLT2 remains computationally and functionally indirect.

Sixth, docking results are predictive and depend on protein structure, ligand preparation, scoring algorithms and docking parameters. A favorable docking score does not guarantee biological inhibition. Finally, the study evaluated Punarnavoside as a representative constituent, while the complete formulation may contain additional bioactive compounds that contribute to the observed natriuretic effect.

Future directions

Future work should include a larger controlled animal study using a validated CKD model. Outcome measures should include urine volume, urinary protein, serum creatinine, blood urea nitrogen, electrolyte profile, creatinine clearance, oxidative stress markers such as malondialdehyde and superoxide dismutase, inflammatory markers such as TNF-alpha and IL-6, and histopathological scoring.

Mechanistic experiments should examine renal expression of SGLT2, NHE3, ENaC, aquaporins, NRF2, HO-1 and inflammatory mediators. In vitro transporter assays could clarify whether Punarnavoside inhibits SGLT2 function directly. Pharmacokinetic studies would help determine whether Punarnavoside reaches renal tissue at concentrations sufficient for transporter interaction.

Scientific meaning of the discrepancy between docking and animal findings

A common limitation in network pharmacology studies is the tendency to treat predicted target interaction as if it were equivalent to pharmacological action. The present study avoids that error by comparing computational predictions with an experimental urinary profile. The difference between SGLT2 docking and lack of glucosuria is not a failure of the study; rather, it is a useful result because it identifies the need to distinguish structural target interaction from functional inhibition.

In herbal medicine research, such discrepancies are expected because a formulation contains several constituents, each with different absorption, distribution, metabolism and excretion characteristics. Even if one constituent has predicted affinity for a transporter, another constituent may produce the measurable physiological effect. The whole formulation may also act through synergistic or counterbalancing effects that are not captured by docking of a single molecule.

From the perspective of CKD, the renal-supportive value of Punarnava Ghanavati should not be limited to SGLT2. CKD progression involves oxidative stress, chronic low-grade inflammation, endothelial dysfunction, altered tubular transport, fibrosis and hemodynamic stress. A multi-target formulation may influence more than one of these domains. The network signal involving NRF2 is therefore important because antioxidant defense is directly relevant to tubular survival and inflammatory modulation.

The study also highlights the importance of selecting experimental endpoints that match the predicted mechanism. If only docking had been performed, the conclusion might have overstated SGLT2 inhibition. By measuring urinary glucose, the study tested a functional consequence of SGLT2 blockade. Since glucose excretion did not rise, the discussion was appropriately adjusted toward alternative mechanisms.

Implications for future research design

A future efficacy study should use a validated CKD or renal injury model rather than normal animals alone. Possible designs may include chemically induced nephrotoxicity, adenine-induced renal dysfunction, subtotal nephrectomy or other established models depending on institutional facilities and ethical approvals. Treatment groups should include vehicle control, disease control, Punarnava Ghanavati at more than one dose and a suitable reference control. Such a design would allow assessment of both safety and efficacy.

A longer duration is also required to evaluate nephroprotection. Five days is adequate for a pilot urinary excretion experiment but too short for conclusions regarding CKD

progression. Future studies should include repeated measurements of serum creatinine, blood urea nitrogen, electrolytes, urine protein, urine volume and body weight. Histopathological evaluation should examine glomerular injury, tubular degeneration, interstitial inflammation and fibrosis.

Mechanistic validation should include transporter and pathway-level analysis. Renal expression of SGLT2 could be measured by immunohistochemistry, western blotting or polymerase chain reaction methods if resources permit. NRF2 and downstream markers such as HO-1 or NQO1 could be assessed to clarify antioxidant pathway involvement. Inflammatory markers and oxidative stress indices would help determine whether the predicted NRF2-related mechanism is biologically active.

Safety considerations are also important. A natriuretic effect may be useful in edema and fluid overload, but excessive sodium and water loss can be harmful in dehydrated or advanced CKD patients. Therefore, future studies should monitor serum sodium, potassium, chloride, hydration status and renal function. Clinical translation should proceed only after adequate preclinical safety and efficacy evidence.

CONCLUSION

Punarnava Ghanavati demonstrated a promising renal activity profile in an integrated in silico and preclinical pilot investigation. Network pharmacology identified SLC5A2/SGLT2 as a predicted target of Punarnavoside and highlighted NRF2 signaling as an important CKD-associated pathway. Molecular docking showed favorable interaction between Punarnavoside and SGLT2, although weaker than dapagliflozin.

The animal pilot study provided a critical functional perspective. Punarnava Ghanavati increased urinary sodium excretion but did not meaningfully increase urinary glucose. This finding suggests that the formulation may not act primarily through classical SGLT2 inhibition. Instead, its renal effects may involve natriuresis, oxidative stress modulation, anti-inflammatory activity and renal hemodynamic support.

The study supports further systematic research on Punarnava Ghanavati in CKD models. The findings should be considered preliminary but scientifically useful because they generate testable mechanistic hypotheses and demonstrate the importance of validating network pharmacology predictions through wet-lab experiments.

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