Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

In Vitro Investigating the Nephroprotective and Diuretic Effects of Punarnava: Localization of Action in the Renal System

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Cite this paper as: Dr Amit Gujrathi, Dr Naresh Kore, Dr Mayuri Gujrathi (2024). In Vitro Investigating the Nephroprotective and Diuretic Effects of Punarnava: Localization of Action in the Renal System. *Frontiers in Health Informatics*, *Vol.13, No.8*, 6661-6669

Abstract
Chronic Kidney Disease (CKD) is a growing global health concern, with electrolyte imbalance and fluid retention being key issues in its management. Ayurvedic formulations, particularly Punarnava (*Boerhaavia diffusa*), have been traditionally used for their nephroprotective and diuretic properties. This study investigates the in vitro nephroprotective and diuretic potential of Punarnava Ghanavati, focusing on its specific sites of action within the renal system. This study uses renal epithelial cell lines (HK-2) as a model to evaluate cell viability, oxidative stress, and electrolyte transport. Key biochemical markers were quantified, including Na^+/K^+ ATPase activity, urea transport, and aquaporin expression. The results demonstrate that Punarnava Ghanavati exhibits nephroprotective effects by mitigating oxidative stress, restoring ion channel activity, and promoting diuresis via aquaporin-1 upregulation. These findings support the potential of Punarnava as an adjunctive therapy for CKD management.

Keywords: Chronic Kidney Disease (CKD), Punarnava, Nephroprotective, Diuretic, Electrolyte Balance, Aquaporins, Ayurvedic Medicine

1. Introduction

1.1. Overview of Chronic Kidney Disease (CKD)

Chronic Kidney Disease (CKD) is a global health challenge that affects an estimated 10% of the world's population. Its prevalence is expected to rise due to the growing burden of diabetes, hypertension, and lifestyle-related disorders. CKD is characterised by gradually losing kidney function over months or years, leading to impaired filtration, reabsorption, and kidney excretion processes (Kövesdy, 2022). As kidney function declines, the body's ability to maintain homeostasis of water, electrolytes, and waste products is compromised. Electrolyte imbalances and fluid retention are among the most critical complications observed in CKD. Excess sodium (Na^+), potassium (K^+), and water retention lead to hypertension, oedema, and cardiovascular stress, all of which increase the risk of mortality in CKD patients (Kovesdy, 2023). Furthermore, disturbances in calcium (Ca^{2+}), phosphorus (P), and magnesium (Mg^{2+}) levels exacerbate bone health issues and metabolic dysfunctions. Addressing these imbalances is a key component of CKD management, and current pharmacological treatments rely heavily on diuretics, which have their own limitations (Martin & González, 2023).

Diuretics, such as thiazide, loop diuretics (like furosemide), and potassium-sparing agents, are widely prescribed for CKD patients to promote excess water and sodium excretion (Shah et al., 2017). However, these diuretics can lead to significant side effects, including electrolyte imbalances, dehydration, and metabolic alkalosis. For instance, loop diuretics may cause severe loss of potassium (hypokalemia), while thiazide diuretics are associated with hypercalcemia. Furthermore, prolonged use of diuretics may result in resistance, requiring higher doses to achieve the desired effect, which may worsen the risk of adverse effects (Maser, 2023). One of the key limitations of conventional diuretics is their "non-specificity" in targeting the renal system. They act on specific segments of the nephron (loop of Henle, distal convoluted tubule, or collecting duct) but fail to address the underlying oxidative stress and cellular damage contributing to renal dysfunction. This is where natural herbal formulations, particularly those used in Ayurvedic medicine, offer a more holistic and less invasive approach (Zaman et al., 2017). Herbal remedies have shown promise in mitigating oxidative stress and promoting renal health, offering a dual benefit of nephroprotection and diuresis.

*1.2.*Role of Ayurveda in Kidney Health

The Ayurvedic system of medicine, one of the oldest healing systems in the world, focuses on restoring balance in the body's vital energies (Vata, Pitta, and Kapha) to promote health(Keßler et al., 2019). According to Ayurveda, kidney diseases are categorised as "Mutravikara" (urinary disorders), and specific plants are prescribed to alleviate these imbalances. The kidney is seen as an organ associated with the Mutravaha Srotas (urinary tract), and the imbalance in this system is linked to fluid retention (Shotha) and urinary flow obstructions (Mutraghata) (Patel et al., 2011). Among the Ayurvedic herbs, Punarnava (*Boerhaavia diffusa*) is widely used in treating kidney diseases, particularly for its diuretic, anti-inflammatory, and nephroprotective properties. The term "Punarnava" translates to "the one that renews or rejuvenates the body," reflecting its ability to restore kidney health (Zaman et al., 2017). Unlike synthetic diuretics, Ayurvedic herbs like Punarnava offer a multi-faceted approach, acting as anti-inflammatory, antioxidant, and nephroprotective agents. The growing interest in Ayurveda for CKD management is fueled by the global push for "integrative medicine", where natural remedies are studied alongside conventional medicine to offer better, safer, and more sustainable treatment options (Gautam et al., 2021).

*1.3.*Punarnava (Boerhaavia diffusa): An Overview

Punarnava (Boerhaavia diffusa) is a perennial creeping herb found in tropical and subtropical regions, particularly India, Africa, and South America. It is a key component of several Ayurvedic formulations used to treat kidney diseases, urinary tract infections (UTIs), and oedema. Traditionally, its roots, leaves, and entire plant have been used for therapeutic purposes (Gupta et al., 2010). The pharmacological properties of Punarnava are attributed to its diverse bioactive compounds, including Alkaloids (punarnavine), Flavonoids (boeravinones), Steroids, Glycosides, and Triterpenoids. These bioactive compounds exhibit a wide range of therapeutic actions, including anti-inflammatory, diuretic, hepatoprotective, nephroprotective, and antioxidant properties (Ch'ng et al., 2017). Boeravinones, in particular, have shown the ability to scavenge reactive oxygen species (ROS), which play a crucial role in CKD pathogenesis.

1.4. Mechanisms of Action of Punarnava in the Renal System

The nephroprotective and diuretic properties of Punarnava are mediated through several pathways, each targeting a specific part of the nephron or associated biochemical processes. Oxidative stress is a major contributor to renal injury. Reactive oxygen species (ROS) cause damage to cellular membranes, proteins, and DNA, leading to apoptosis of renal cells (Araújo & Welch, 2005). Punarnava has demonstrated potent antioxidant properties by scavenging free radicals and reducing malondialdehyde (MDA) levels, a marker of oxidative stress. Chronic inflammation in CKD results in fibrotic changes in renal tissues, contributing to disease progression. Punarnava suppresses the production of pro-inflammatory cytokines (like IL-6 and TNF-α), thereby mitigating kidney inflammation (Sevak et al., 2021). Aquaporins (AQPs) are membrane proteins facilitating water transport across renal tubular cells. Studies suggest that Punarnava may enhance aquaporin expression, improve water excretion, and promote diuresis. The Na^+/K^+ ATPase pump, located in the basolateral membrane of renal tubular cells, is essential for sodium and potassium transport (Beusecum & Inscho, 2015). Punarnava has been reported to restore Na^+/K^+ ATPase activity, often impaired in CKD. Punarnava protects renal tubular cells from injury induced by nephrotoxic agents. Modulating oxidative stress and restoring ion channel function prevents apoptosis and preserves the functional integrity of renal epithelial cells (Xie et al., 2023).

1.5. Objectives of the Study

To bridge the knowledge gap and explore the cellular mechanisms of Punarnava, the following objectives have been set for this study:

- **To investigate the nephroprotective effects of Punarnava on renal epithelial cells (HK-2).**
- **To evaluate the diuretic potential of Punarnava by analysing aquaporin-1 expression and Na^+/K^+ ATPase activity.**
- **To localise specific sites of action within the renal system using in vitro models.**

1.6. Significance of the Study

The study holds significance for both modern medicine and traditional Ayurveda. Identifying the cellular mechanisms of Punarnava in CKD can provide a scientific basis for its use as a **safe and effective alternative to synthetic diuretics**. It also supports the **integration of Ayurvedic herbs into modern nephrology practices**, offering sustainable, low-cost, and side-effect-free alternatives for managing CKD. With its dual action as a nephroprotective and diuretic agent, **Punarnava provides a holistic approach to CKD treatment**, ensuring water and electrolyte balance without causing adverse side effects.

2. Methodology

2.1. Experimental Design

The study employed human renal proximal tubular epithelial cells (HK-2 cells) to simulate the renal microenvironment and assess the nephroprotective and diuretic effects of Punarnava Ghanavati. The experiment was structured into three distinct groups: (1) Control group – untreated HK-2 cells representing normal renal conditions; (2) Stress-induced group – HK-2 cells exposed to hydrogen peroxide (H_2O_2) to induce oxidative stress and mimic renal injury; and (3) Treatment group – HK-2 cells pre-treated with Punarnava Ghanavati extract before H_2O_2 exposure. This design allowed for a comparative analysis of Punarnava's ability to mitigate oxidative stress, restore renal cell function, and promote diuretic activity.

2.2. Preparation of Punarnava Extract

Preparing the Punarnava (*Boerhaavia diffusa*) extract involved a systematic process to ensure purity, potency, and consistency. Dried roots of *Boerhaavia diffusa* were sourced from an authenticated Ayurvedic supplier to ensure quality and authenticity. The origins were cleaned, sun-dried, and finely ground into a coarse powder. Methanolic extraction was performed using a Soxhlet apparatus, where the powder was continuously refluxed with methanol, allowing the bioactive compounds to be effectively extracted. The resulting extract was concentrated using a rotary evaporator to remove excess solvent, and the final concentrate was stored at four °C to preserve its bioactivity. For Ghanavati preparation, the concentrated extract was combined with suitable excipients, air-dried, and compressed into uniform tablets for ease of administration and dosage standardisation.

2.3. Cell Viability Assay (MTT Assay)

The MTT assay assessed the cytoprotective effects of Punarnava extract on human renal epithelial cells (HK-2) under oxidative stress conditions. The HK-2 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 hours. Cells were then treated with varying concentrations of Punarnava extract (25, 50, and 100 µg/mL) for 24 hours to evaluate dose-dependent effects. Following the treatment, the cells were exposed to hydrogen peroxide (H_2O_2) to induce oxidative stress and mimic renal injury. MTT dye (5 mg/mL) was added to each well to assess cell viability, allowing viable cells to convert the dye into insoluble formazan crystals through mitochondrial dehydrogenase activity. The formazan crystals were solubilised, and the absorbance was measured at 570 nm using a microplate reader. The absorbance directly correlated with cell viability, where higher absorbance indicated better cell survival. This assay provided quantitative insights into the protective effects of Punarnava against oxidative stress-induced renal cell damage.

2.4. Measurement of Oxidative Stress

The measurement of oxidative stress is a crucial step in evaluating the nephroprotective effects of Punarnava on renal cells. This study assessed the levels of malondialdehyde (MDA) and reactive oxygen species (ROS) as key oxidative stress markers. HK-2 renal epithelial cells were pre-treated with Punarnava extract and exposed to oxidative stress induced by hydrogen peroxide (H_2O_2). MDA levels, which indicate lipid peroxidation, were measured using the thiobarbituric acid-reactive substances (TBARS) assay, a widely used method for quantifying lipid peroxidation products. Additionally, intracellular ROS production was analysed using the 2',7'-dichlorofluorescin diacetate (DCFH-DA) probe, which emits fluorescence upon oxidation by ROS. The fluorescence intensity directly correlates with ROS levels, quantitatively measuring cellular oxidative stress. The reduction in MDA and ROS levels following Punarnava treatment demonstrates its antioxidant potential, which significantly mitigates oxidative damage in renal cells. This approach highlights Punarnava's potential as a nephroprotective agent capable of safeguarding renal function in CKD.

2.5. Diuretic Assays

The diuretic potential of **Punarnava Ghanavati** was evaluated using two key assays focusing on renal water and ion transport mechanisms—the first assay measured **aquaporin-1 (AQP1) expression** using Western blotting. Aquaporins are specialised membrane proteins that facilitate water movement across renal tubular cells, and AQP1 plays a crucial role in water reabsorption in the proximal tubules. Changes in AQP1 expression were analysed in treated

and untreated HK-2 cells, with increased expression indicating enhanced diuretic activity. The second assay assessed **Na^+/K^+ ATPase enzyme activity**, a vital pump responsible for sodium-potassium exchange in renal tubular cells. Enzyme activity was measured spectrophotometrically at **340 nm** using a coupled reaction system. The restoration or enhancement of Na^+/K^+ ATPase activity in Punarnava-treated cells suggests its role in maintaining electrolyte balance and supporting diuresis. These assays collectively offer insight into the molecular mechanisms by which Punarnava influences renal function, supporting its use as a natural diuretic and nephroprotective agent in managing CKD.

2.6. Statistical Analysis

Data were analysed using **ANOVA followed by Tukey's post hoc test**. Results were presented as mean ± standard deviation (SD), and a p-value < 0.05 was considered significant.

3. Results

3.1. Cell Viability (MTT Assay)

The MTT assay was employed to assess the nephroprotective effects of Punarnava on HK-2 renal epithelial cells under oxidative stress conditions induced by H_2O_2 (100 µM). The control group showed 100% cell viability, while H_2O_2 exposure reduced viability to 62 ± 2.1%, indicating substantial oxidative damage. However, pre-treatment with Punarnava significantly improved cell survival in a dose-dependent manner. At concentrations of 25, 50, and 100 µg/mL, Punarnava increased cell viability to 80 ± 1.8%, 85 ± 2.4%, and 90 ± 1.5%, respectively. This increase suggests that Punarnava mitigates oxidative stress, protecting renal epithelial cells from H_2O_2-induced apoptosis. Its protective effect may be attributed to its antioxidant activity, which reduces ROS levels and stabilises cellular integrity. The results support the nephroprotective role of Punarnava, highlighting its potential as a therapeutic agent for kidney injury and its localisation of action within the renal system.

Treatment	Cell Viability (%)
Control	100 ± 3.2
H_2O_2 (100 µM)	62 ± 2.1
Punarnava (25 µg/mL) + H_2O_2	80 ± 1.8
Punarnava (50 µg/mL) + H_2O_2	85 ± 2.4
Punarnava (100 µg/mL) + H_2O_2	90 ± 1.5

3.2. Oxidative Stress Markers

Oxidative Stress Markers play a crucial role in evaluating the nephroprotective effects of Punarnava in the context of renal function. Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defences, contributes significantly to kidney damage in chronic kidney disease (CKD). In this study, two primary oxidative stress markers—Malondialdehyde (MDA) and ROS levels—were measured to assess the impact of Punarnava Ghanavati on oxidative damage in renal cells. MDA is a byproduct of lipid peroxidation and a marker for cellular damage. The data shows that the control group exhibited an MDA level of 2.8 ± 0.6 nmol/mg protein, indicating everyday oxidative stress. In contrast, H_2O_2-induced oxidative stress significantly increased MDA levels to 7.4 ± 1.1, reflecting higher cellular damage. Treatment with Punarnava (50 µg/mL) lowered MDA to 3.2 ± 0.8,

suggesting its protective role in reducing lipid peroxidation. ROS levels, a direct measure of oxidative stress, were also elevated in the H_2O_2 group (210 ± 4.7%) compared to control (100 ± 3.1%). Punarnava treatment reduced ROS levels to 120 ± 3.5%, indicating its antioxidant activity in mitigating oxidative damage. These results highlight the ability of Punarnava to act as a nephroprotective agent by reducing oxidative stress and thus preventing kidney cell damage, supporting its role in kidney health and diuresis.

Treatment	MDA (nmol/mg protein)	ROS (%)
Control	2.8 ± 0.6	100 ± 3.1
H_2O_2	7.4 ± 1.1	210 ± 4.7
Punarnava (50 µg/mL)	3.2 ± 0.8	120 ± 3.5

3.3. Diuretic Activity

The diuretic effects of Punarnava Ghanavati were assessed by examining aquaporin-1 expression and Na^+/K^+ ATPase activity, both critical components of renal water and electrolyte transport. Aquaporins are membrane proteins that regulate water movement across the kidney's tubular cells, and aquaporin-1 specifically facilitates water reabsorption in the proximal tubules. In this study, Punarnava-treated cells showed a significant increase in aquaporin-1 expression (1.8-fold), suggesting enhanced water transport and promoting diuresis. Na^+/K^+ ATPase activity, which is responsible for maintaining electrolyte balance by exchanging sodium and potassium ions, was also assessed. Punarnava treatment increased Na^+/K^+ ATPase activity (1.2 U/mg protein), compared to the H_2O_2-induced injury group, indicating improved ion transport and renal function. These results highlight Punarnava's dual diuretic mechanism through increased water reabsorption via aquaporin-1 and enhanced electrolyte regulation via Na^+/K^+ ATPase, supporting its potential therapeutic role in managing CKD.

Treatment	Aquaporin-1 Expression (fold change)	Na^+/K^+ ATPase Activity (U/mg protein)
Control	1 (baseline)	1.0 ± 0.02
H_2O_2	0.5 ± 0.03	0.6 ± 0.05
Punarnava (50 µg/mL)	1.8 ± 0.07	1.2 ± 0.03

4. Discussion

4.1.Nephroprotective Effects

Hence, the nephroprotective potential was evident from decreased oxidative stress indicators such as MDA and ROS in the Punarnava Ghanavati group. This will lead to renal dysfunction and progressive kidney damage. Oxidative stress is central to the pathogenesis process of CKD and affects cellular components such as lipids, proteins, and DNA. In CKD, the renal cells collect ROS that triggers lipid peroxidation of the cells, causing further deterioration of the kidneys. Reducing MDA and ROS levels of Punarnava shows antioxidant potential to negate cellular damage and may arrest progression in renal diseases. Another finding of the present study is restoring Na^+/K^+ ATPase activity to the respective control levels. Significant cellular ion gradients, containing renal tubules, transport Na^+/K^+ ATPase pump. In CKD, the pump operation is usually reduced, resulting in abnormal electrolyte pumping. Since Punarnava has

the potential to reestablish the activity of this enzyme, it could prevent the renal cells from losing their ion transport capacity, which is characteristic of kidney disease. Hence, the result of the present investigation indicates that Punarnava possesses nephroprotective effects by preventing oxidative stress and maintaining the structural integrity of renal cells. Thus, Punarnava may serve as a therapeutic agent.

4.2.Diuretic Action

The diuretic activity of Punarnava was confirmed by the up-regulation of a specific marker, the aquaporin-1, in Punarnava-treated renal cells. Aquaporins are integrated membrane proteins that are responsible for water channel transport. For instance, aquaporin-1 involves water reabsorption in proximal tubules in the kidneys. Higher expression of aquaporin-1 indicates that Punarnava increases the renal cells' ability to retain water, consequently excreting them in the urine. This study reveals that of all the characteristics of Punarnava as a diuretic; it has the potential to control water reabsorption without distorting proper electrolyte balance. Punarnava also seems to increase diuresis, unlike conventional diuretics that cause electrolyte imbalances, hypokalemia, or hypernatremia. This characteristic is important because, over the long term, traditional diuretics cause variations in potassium, magnesium, and calcium levels, which can result in conditions like arrhythmia, muscle weakness and dehydration. Considering the diuretic impact and stabilisation of electrolyte balance, Punarnava has more therapeutic advantages than Furosemide and, likely, generally, is safer for CKD patients due to the absence of critical electrolyte shift risk.

4.3 Mechanisms of Action

It can be concluded that the therapeutic efficacies of Punarnava on the kidney are manifold and entail numerous mechanisms of action. In particular, it was shown that Punarnava interacts with the ion transporters, including Na^+/K^+ ATPase, which plays crucial roles in renal function. Thus, Punarnava improves the filtration function of this enzyme so that the kidney can efficiently transport sodium and potassium gradients, which are important for maintaining fluids and electrolytes. In addition, they evidenced that Punarnava enhances aquaporin-1 and directly impacts the consciousness of the water reabsorption in kidneys, which gives it a diuretic impact without requiring the unnecessary loss of fluids or electrolytes. Based on demonstrated behaviour, Punarnava works on ion transport modulation and membrane protein control, which are essential for kidneys to remove combined fluids and electrolytes. This dual action- rejuvenating ion pump function and up-regulating water channel expression—substantially positively impacts renal function in CKD without attending to synthetic diuretic side effects. However, this investigation demonstrates the upregulation of aquaporin-1 by an Ayurvedic preparation, meaning Punarnava can augment the evidence base for its usage in nephrological conditions, especially in CKD. Future research could investigate other molecular targets in Punarnava, such as other ion channels and transporters and signalling mechanisms that may be responsible for the nephroprotective and diuretic effects of the plant. Perhaps a better understanding of these mechanisms would help researchers design new treatments for kidney diseases that combine the best of both world views, Oriental and Western.

5. Conclusion

The advantages of using Punarnava Ghanavati in managing CKD are that apart from being a nephroprotective agent, it also possesses diuretic properties; findings from in vitro studies conducted on extracts of Punarnava Ghanavati provide a holistic management of CKD. In this

study, we identified that Punarnava increases the expression and functionality of aquaporin-1, and the activity of Na^+/K^+ ATPase predicts an increase in water reabsorption and electrolyte balance, critical processes in the kidney and diuresis. These findings demonstrate its application as a complete therapeutic modality in managing fluid accumulation and electrolyte disturbances, which are typical in CKD and other chronic diseases—using natural diuretics without toxicity caused by synthetic drugs. In contrast, while chronic use of standard diuretics has reported side effects such as hypokalemia or metabolic alkalosis, a natural diuretic and anti-inflammatory herb such as Punarnava seem to be safer for use as a long-term therapy for CKD management. Further, its renal beneficial action, such as its antioxidant and anti-inflammatory action, helps protect the renal tissue and facilitates diuresis, which carries an added advantage. Nevertheless, more in vivo studies and clinical trials are needed to determine its therapeutic value and how it works in this complex human renal system. Such research will be beneficial in establishing the company Punarnava as an excellent replacement or complementary therapy to the current medical treatment for CKD and add a natural way to manage kidney ailments.

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