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Nephroprotective and Antioxidant Effects of Mutrakrichantak Churna in Cisplatin-Induced Nephrotoxicity in Rats

Short Title: Nephroprotective Effects of Mutrakrichantak Churna

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Abstract Cisplatin-induced nephrotoxicity (CIN) significantly hampers the therapeutic potency of cisplatin because of renal oxidative stress, inflammation, and apoptosis. This study investigates the nephroprotective and antioxidant potential of Planet Ayurveda Mutrakrichantak Churna (PAMC), a polyherbal Ayurvedic formulation, in a CIN model using Wistar albino rats. PAMC was administered at low (200 mg/kg) and high (400 mg/kg) doses and compared to a standard nephroprotective treatment (Cystone syrup). The results showed a dose-related improvement in renal function and oxidative stress markers. PAMC significantly reduced serum creatinine, urea, uric acid, and malondialdehyde levels while enhancing antioxidant enzyme activities, including SOD, CAT, and Gpx. Histopathological analysis showed preserved renal architecture, decreased tubular necrosis, and less inflammation, especially at the higher dose of PAMC, which was equivalent to standard treatment. This study suggests the potency of PAMC to be an effective nephroprotective agent that involves mechanisms not only antioxidant but also anti-inflammatory against the insults of CIN. This inherent composition and broad spectrum of action make PAMC a suitable adjunct in chemotherapy regimens to avert renal injury. Hence, further clinical studies may be necessary to verify and determine long-term patient safety and efficacy.

Keywords Cisplatin-Induced Nephrotoxicity, Nephroprotective Agents, Planet Ayurveda Mutrakrichantak Churna, Oxidative Stress, Antioxidant Enzymes, Renal Biomarkers, Tubular Necrosis, Histopathology, Polyherbal Formulation

1. Introduction

1.1. Cisplatin-Induced Nephrotoxicity

Cisplatin, a chemotherapeutic agent, is very effective in treating several types of cancer, though it usually causes nephrotoxicity, a dose-limiting side effect. This leads to acute kidney injury, which decreases glomerular filtration rate (GFR), electrolyte imbalance, and elevated serum creatinine and urea. Its toxic effects on renal tissues significantly limit the potential therapeutic usefulness of cisplatin. Hence, the development of appropriate strategies to protect against renal toxicity without compromising its

anti-cancer effectiveness is very much needed [1].

The nephrotoxicity with cisplatin is attributed to oxidative stress, inflammation, and apoptosis. Oxidative stress elevates the generation of Reactive Oxygen Species (ROS), potentially surpassing renal antioxidant defences and resulting in lipid peroxidation, protein denaturation, and DNA damage. Activation of inflammation pathways leads to tubular injury, interstitial oedema, and fibrosis. The apoptosis in renal tubular epithelial cells disrupts the homeostasis of calcium, and pro-apoptotic proteins [1, 2].

Preventing cisplatin-induced Nephrotoxicity (CIN) is essential in chemotherapy patients, as it allows treatment to continue without dose reduction. Nephroprotective agents, specifically antioxidant agents, have garnered attention because they may potentially prevent or reduce renal toxicity by neutralising oxidative stress, reducing inflammation, and inhibiting the apoptotic pathway [3].

1.2. Traditional Use of Planet Ayurveda Mutrakrichantak Churna (PAMC) in Treating Kidney Disorders in Ayurveda

PAMC is an Ayurvedic formulation used widely to treat urinary and kidney disorders. Its principal ingredients are “Varun (*Crateva nurvala*), Bhumi Amla (*Phyllanthus niruri*), Gokshur (*Tribulus terrestris*), Kalmegh (*Andrographis paniculate*)," all traditionally known for their action as diuretics, anti-inflammatories, and antioxidants. Hence, it has been used in Ayurvedic practice to treat kidney stones, urinary tract infections, and nephritis. The formulation promotes renal detoxification, increases urinary output, and acts on the inflammatory measures in the renal tissues. Thus, because of its rich pharmacological profile, PAMC is hypothesised to exhibit considerable protection against CIN by targeting oxidative stress and preventing renal damage [4].

1.3. Study Hypothesis and Objectives

The central hypothesis underlying in this study was that nephroprotection with antioxidant properties of PAMC would significantly attenuate CIN in an animal model. It is expected that the herbal preparation would have significantly reduced serum levels of renal biomarkers; that is, creatinine, urea, and uric acid are essential indicators of kidney function. It is also expected to reactivate antioxidant enzymes in kidney tissues to further boost the body's ability to fight oxidative stress. Oxidative stress will decrease when the formulation reduces inflammation and even prevents apoptosis in renal tissues to enhance general kidney health and renal function. The primary aim of the research was to evaluate the nephroprotective and antioxidant effects of PAMC in Wistar albino rats subjected to CIN. Biochemical markers for kidney damage were to be reduced by the herbal formulation, and enhanced renal function, and histopathological changes that occurred in renal tissues should be prevented.

Currently, this work concentrates on the histological and biochemical evidence of PAMC's nephroprotective action. Future studies will stretch the experimental period to evaluate long-term effects and investigate the molecular mechanisms, including inflammatory pathways (e.g., NF-κB, TNF-α, IL-6) and genes linked to death. Pharmacokinetic studies will also be carried out to assess the bioavailability, absorption, metabolism, and excretion of PAMC's active compounds for possible human use.

2. Methodology

2.1. Animal Model and Ethical Approval

2.1.1. Selection of Wistar Albino Rats for the Nephrotoxicity Model

This research adopted Wistar albino rats as the model animals since these animals are extensively documented for nephrotoxicity research. These laboratory strain rats have gained popularity due to uniformity, ease of handling, and well-characterised renal physiology, making them perfect for nephrotoxic studies [5]. The animals used were young, healthy adult Wistar albino rats, comprising males and females, in the age range of 6 to 8 weeks, and weighing between 150-200 grams. These animals were chosen in a way that they were nulliparous and non-pregnant females, which would reduce the scope of hormonal changes that could influence the experimental result.

Rats were habituated to the laboratory environment for one week before the experiment commenced. They were housed under conventional laboratory conditions, with the temperature at 22°C ± 3°C and the relative humidity at 50-60%. The light-dark cycle was kept as a 12-hour cycle, and the animals received a conventional laboratory diet with an unlimited water supply. Housing was in groups, and each group was housed together based on treatment assignment. This would ensure proper observations while minimising the effects of isolation stress on the animals.

2.1.2. Ethical Considerations and Adherence to Animal Care Guidelines

This study’s experimental protocol was designed according to Institutional Animal Ethics Committee (IAEC) guidelines and approval number CPCSEA/IAEC/12/2023/36 concerning OECD guidelines on animal care and use. The IAEC sanctioned the ethical approval before the commencement of this study to ensure that due respect was given to the welfare of animals in conducting the research.

Special care has been taken to minimise animal distress and reduce animal use concerning the 3Rs of animal experimentation, i.e., Replacement, Reduction, and Refinement. Animals were observed for signs of distress, and humane endpoints have been developed and used for early euthanasia in the case of severe illness and distress.

Euthanasia was performed using CO_2 asphyxiation, considered a humane euthanasia method in rodents, followed by cervical dislocation as a death confirmation method. Personnel directly dealing with animals received training to handle them properly and stress-free so that humane handling of the animals would be ensured during the study.

2.2. Induction of Nephrotoxicity

2.2.1. Cisplatin Administration Protocol (100 mg/kg Intraperitoneally on the Fifth Day)

Cisplatin, a nephrotoxic drug used in cancer therapy, was administered intraperitoneally to Wistar albino rats, causing nephrotoxicity after a dosage of 100 mg/kg body weight (BW) on the fifth day. Dosing was planned according to various studies that have reported its efficacy in inducing acute renal failure and nephrotoxicity in rodent models.

Fresh cisplatin was prepared on the same day and administered to the animals using sterile syringes for uniform and proper dosing. An equal volume of normal saline was administered intraperitoneally in the control group for comparison. The other experimental group involved cisplatin-only for the incidence of nephrotoxicity of the drug, and the others received low and high doses of PAMC and standard treatment.

All animals were monitored throughout the treatment period for signs of toxicity or discomfort, including changes in food intake, water consumption, body weight, and activity levels. The BWs were monitored daily because weight loss is one of the recognised indicators of systemic toxicity caused by cisplatin. After 8 days, the animals underwent further evaluation regarding renal function and other parameters to confirm the effective induction of nephrotoxicity.

2.2.2. Confirmation of Nephrotoxicity via Biochemical Assessments

Nephrotoxicity was established based on biochemical analysis of key markers indicating renal impairment. Blood was collected retro-orbitally on day 9 from overnight-fasting rats. The serum obtained post-centrifugation at 3000 rpm for 10 minutes was then subjected to biochemical analysis.

A few essential biochemical parameters were measured to prove the aspect of nephrotoxicity. Elevated serum creatinine levels form the hallmark of decreased GFR and impairment of kidney function. Thus, it declares a deficient renal health. An increase in blood urea nitrogen (BUN) reflects a reduction in renal clearance, confirming nephrotoxicity. Elevated serum uric acid and urea indicate renal dysfunction; these markers are usually proportional to the degree of impaired kidney excretory function, showing the extent of nephrotoxic damage.

In addition to blood chemistry, urine output was measured as a marker of functional nephrotoxicity. The decreased urine output was most evident in cases of CIN, where the biochemical determinations collectively confirmed the CIN in the experimental groups and provided the baseline for further studies contemplating the nephroprotective potential of PAMC.

2.3. Experimental Design

2.3.1. Division of Groups and Treatment Method

The experimental design divided Wistar albino rats into five groups, with six animals in each group (n=6). The groups were set as follows:

Group I (Control Group): This group received only normal saline intraperitoneally (i.p.) during the experiment and served as the baseline control.

Group II (Cisplatin-only Group): This group received cisplatin at 100 mg/kg administered intraperitoneally (i.p.) on the fifth day to induce nephrotoxicity.

Group III (Low-Dose (LD) PAMC Group): The group received cisplatin at a dose of 100 mg/kg i.p. on the fifth day for nephrotoxic induction and was treated with LD of PAMC at 200 mg/kg BW for 8 days through gavage.

Group IV (High-Dose (HD) PAMC Group): This group received cisplatin at a dose of 100 mg/kg, i.p., on the fifth day for nephrotoxic induction and was treated with HD of oral PAMC at 400 mg/kg BW for 8 days.

Group V (Standard Treatment Group): This group received cisplatin at a dose of 100 mg/kg i.p. on the fifth day for nephrotoxic induction and was given standard nephroprotective treatment in the form of Cystone syrup, 5 ml/kg orally for 8 days. This group served as a positive reference for testing PAMC.

The dosages were based on traditional Ayurvedic practices and acute toxicity studies per OECD 423 guidelines, recommending that these dosages were influential in treating kidney-related disorders. Fresh herbal preparation was prepared daily and mixed with water through oral gavage using a feeding needle to ensure proper dosing.

To ensure consistency in the experimental results, each group was treated under identical environmental conditions, including housing and feeding.

2.3.2. Duration and Route of Administration (Oral Gavage for 8 Days)

The PAMC was orally administered to the rats, following the gavage method, wherein the formulation was directly administered to the stomachs of rats using a specialised feeding needle. Oral gavage was performed to closely replicate the conventional oral consumption of the formulation in humans, thereby permitting maximum availability and the potential for maximum efficacy.

An 8-day treatment duration was used to overlap with the duration of cisplatin administration, allowing concurrent observation of the herbal formulation's

nephroprotective effects. Animals were checked daily for clinical symptoms of toxicity, changes in BW, food intake, and overall health throughout the period.

2.4. Assessment of Renal Function and Biochemical Parameters

2.4.1. Collection of Blood Samples for Renal Function Tests

On the ninth day of the trial, after the last cisplatin injection and therapy, blood samples were obtained from the rats for renal function assessments. Blood was extracted from the retro-orbital plexus of overnight-fasted mice under mild anaesthesia to reduce stress. The collected blood was placed into sterile tubes and let to coagulate. The serum was isolated by centrifuging the blood samples at 3000 rpm for 10 minutes. After that, the serum was preserved at -20 °C until renal function and biochemical markers were evaluated.

2.4.2. Evaluation of Creatinine, Uric Acid, Urea, and Urine Output

Renal function was primarily assessed through the measurement of key biochemical markers in the serum:

- ✓ Elevated creatinine levels are indicative of impaired glomerular filtration and kidney function. Serum creatinine was measured using an automated biochemical analyser [6].
- ✓ Uric acid, a byproduct of purine metabolism, accumulates in the circulation when renal excretion is impaired. Serum uric acid levels were measured to evaluate the degree of renal impairment [7].
- ✓ BUN was measured as an indicator of renal excretory function, as elevated levels suggest reduced kidney clearance and impaired filtration [6].
- ✓ On the ninth day, urine production was tracked and quantified for a full day to assess the kidneys' capacity to eliminate fluids. Rats were housed in separate metabolic cages for urine collection. Diminished urine output is one of the most common symptoms of nephrotoxicity due to cisplatin and is considered a functional marker of renal injury [8].

Therefore, the above parameters thus gave a comprehensive view of renal functions and the protective effects of PAMC against CIN.

2.4.3. Serum Biochemical Analysis: Creatinine, Uric Acid, Urea, Total Protein, and Albumin

To assess renal function and overall metabolic health, serum creatinine, uric acid, urea, total protein, and albumin levels were analysed in rats. These parameters help evaluate kidney health, protein metabolism, liver function, and nutritional status, especially under CIN conditions.

- ✓ Creatinine, a byproduct of muscle metabolism, is a crucial indicator of renal function. Elevated levels indicate impaired glomerular filtration, often due to kidney damage from nephrotoxicity [9].
- ✓ The kidneys eliminate uric acid, and increased concentrations may signify diminished renal excretion, often linked to nephrotoxic stress, which may exacerbate oxidative damage [10].
- ✓ As a product of protein metabolism, increased serum urea reflects compromised renal function and waste accumulation, typical of nephrotoxic states [11].
- ✓ Low total protein levels, assessed by colourimetric assay, suggest impaired liver function or malnutrition, as toxicity can reduce protein synthesis or increase renal protein loss [12].
- ✓ The liver synthesises albumin, a marker of liver and kidney health. Reduced levels indicate proteinuria and potential glomerular dysfunction in nephrotoxic conditions [13].

This analysis assessed the potential protective effects of PAMC in stabilising these biochemical markers, supporting healthy liver and kidney function under nephrotoxic stress.

2.5. Antioxidant Activity Assessment

2.5.1. Measurement of Antioxidant Markers (SOD, CAT, Gpx, GSH, MDA)

The antioxidant activity in the kidneys tissues was estimated based on the measurement of several key antioxidant enzymes and oxidative stress markers. Such enzymes are essential to protect the kidney from CIN oxidative damage. The markers evaluated include

- ✓ Superoxide Dismutase (SOD) is a principal antioxidant enzyme that facilitates the dismutation of superoxide radicals into oxygen and hydrogen peroxide. The spectrophotometric method presents the enzyme activity in units/mg of protein. A decrease in SOD activity reflects the existence of oxidative stress together with the impairment of antioxidant defence in the kidneys [14].
- ✓ Catalase (CAT) facilitates the breakdown of hydrogen peroxide into water and oxygen, safeguarding cells from oxidative damage. CAT activity was measured through a colourimetric assay, with activities expressed as units/mg protein. A decline in CAT activity is indicated by increased oxidative stress [15].
- ✓ Glutathione Peroxide (Gpx) is another important enzyme that removes hydrogen peroxide and organic hydroperoxides from cells, thereby protecting against oxidative damage. The activity of Gpx was measured using the coupled enzyme assay, and its level was reported as units per milligram of protein [16].
- ✓ Glutathione (GSH) is a major non-enzymatic antioxidant. It protects the cellular elements from oxidative damage arising from ROS. The concentration of GSH in tissue was assayed using a

spectrophotometric assay, and its concentration is presented in milligrams per gram of tissue. Any decrease in GSH concentration indicates oxidative stress and reduced detoxifying capacity [17].

- ✓ Malondialdehyde (MDA) results from lipid peroxidation that may serve as a biomarker of oxidative damage to cellular membranes. MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) test, with findings reported in nanomoles of MDA per milligram of tissue protein. Elevated MDA values indicated heightened lipid peroxidation and oxidative stress [18].

Antioxidant markers were employed to monitor the oxidative stress level, the protective effect of PAMC on this level, and its relation to reduced oxidative damage in cisplatin-treated rats.

2.5.2. Assessment of Oxidative Stress and Lipid Peroxidation in Renal Tissue Specimens

After sacrificing animals, kidneys were obtained, cleansed with ice-cold phosphate-buffered saline (PBS) (pH 7.4), and homogenised to extract tissue samples. Oxidative stress and lipid peroxidation in the kidney tissues were determined through the estimation of antioxidant markers such as SOD, CAT, Gpx, GSH, and MDA levels described above.

Estimating the kidney tissue's MDA concentration is especially noteworthy regarding lipid peroxidation. An increase in the MDA concentration indicates that ROS damaged the membranes' lipid components. Therefore, it shows that PAMC reverses the cisplatin-induced oxidative injury to avoid damage.

The results from these assays helped determine the effectiveness of enhancing the antioxidant defence system and mitigating oxidative stress and lipid peroxidation caused by PAMC in reducing damage in cisplatin-treated rats.

2.6. Histopathological Analysis

2.6.1. Harvesting and Fixation of Kidney Tissues in 10% Formalin

After the experimental period, on the ninth day, all the animals were sacrificed through CO_2 asphyxia combined with cervical dislocation to ensure death. Immediately after the sacrifice, each rat's kidney was prepared by carefully harvesting it and washing it in ice-cold phosphate-buffered saline to remove blood and debris.

The extracted kidneys were preserved in 10% neutral buffered formalin for at least 24 hours, preserving tissue architecture and preventing autolysis. This ensured cellular and structural integrity fixation in the kidney tissues for histological examination. One kidney from each rat was used for histopathological analysis, while the contralateral kidney was preserved for biochemical and antioxidant evaluations.

2.6.2. Histological Staining and Microscopic Evaluation of Renal Tissue for Damage (Necrosis, Inflammation, Tubular Damage)

Following fixation, the kidney tissues were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Kidney sections, about 5-6 µm thick, were produced from paraffin embedding using a microtome. The thin slices were then affixed to glass slides and stained with hematoxylin and eosin for comprehensive histological assessment.

A microscopic analysis of the stained kidney sections was conducted to evaluate the degree of renal injury caused by cisplatin and the preventive effects of PAMC. The following key parameters were evaluated:

- ✓ We evaluated necrotic alterations in the renal tubular epithelial cells, especially inside the proximal tubules. CIN is typically characterised by extensive necrosis in these regions.
- ✓ A histological examination of the renal interstitium was performed to detect inflammation by observing the degree of cellular infiltration, which was dominated by lymphocytes, neutrophils, and macrophages, a common feature of CIN.
- ✓ Tubular injury markers include tubular dilation, epithelial sloughing, and vacuolar degeneration. These changes reflect the cisplatin-induced changes to the arrangement of the tubules.

All experimental groups, control, cisplatin-only, LD, HD, PAMC-treated groups, and standard treatment groups, were compared based on the histopathological findings. The degree of necrosis, inflammation, and tubular damage was graded based on the extent and severity of lesions noted under microscopic examination.

The histopathological results show critical information about the protective effects of this churna at both dose levels on renal morphology, inflammation, and the absence of tubular necrosis in contrast to the cisplatin-only treatment group.

3. Results

3.1. Impact on BW

3.1.1. Changes in BW over the 9 Days

The BWs of rats administered cisplatin and PAMC were documented throughout the 9-day study duration. In the control group, the BW of rats increased linearly throughout the experiment without any signs of stress or toxicity noted. In the cisplatin-only-treated group, the BW marked a considerable decline from the fifth day onward as a result of systemic toxicity and catabolic effects of CIN.

In contrast, the groups treated with PAMC (both LD and HD) demonstrated better maintenance of BW compared to the cisplatin-only group. The LD (200 mg/kg) group

showed a moderate improvement in weight retention. In comparison, the HD (400 mg/kg) group showed a significant preservation of BW, closely resembling the trend observed in the control group. The standard treatment group (Cystone syrup) also demonstrated protection against cisplatin-induced weight loss, similar to the HD PAMC group.

Table 1 summarises the BW comparison across the experimental groups at the start of the study (Day 0), the middle of the study (Day 5), and at the end of the study (Day 9).

3.1.2. Prevention of Cisplatin-Induced BW Loss by PAMC

The cisplatin administration caused significant weight loss in the rats, as seen in the cisplatin-only group, which lost about 17% of their BW by the end of the 8 days. This decline in BW is commonly associated with the toxic effects of cisplatin on kidney function and general health.

In contrast, the groups treated with PAMC (both low and HDs) showed significantly less weight loss, with the HD group showing almost complete protection against cisplatin-induced weight reduction. By the end of the study, rats in the HD PAMC group had a mean BW of 252.83 g, comparable to the control group (244.00 g). The LD group also significantly attenuated BW loss, with a final weight of 241.16 g.

The standard treatment group, receiving Cystone syrup, similarly exhibited minimal weight loss, confirming its nephroprotective and health-maintaining effects. These findings suggest that PAMC, especially at higher doses, offers substantial protection against the systemic and renal toxicity induced by cisplatin.

3.2. Renal Function Parameters

3.2.1. Creatinine, Uric Acid, and Urea Levels in Treatment vs. Control Groups

The impact of cisplatin and PAMC on renal function was evaluated by measuring key biochemical markers of kidney health: serum creatinine, uric acid, and urea levels. Cisplatin administration markedly increased all three markers in the cisplatin-only group, indicating significant renal impairment. Conversely, rats treated with PAMC demonstrated a dose-dependent improvement in renal function, as evidenced by reduced levels of creatinine, uric acid, and urea compared to the cisplatin-only group.

The cisplatin-only group showed a sharp increase in serum creatinine (39.74 ± 0.71 mg/dL), reflecting impaired kidney filtration. In the LD PAMC group, creatinine levels were significantly lower (16.35 ± 0.84 mg/dL), while the HD group exhibited near-normal creatinine levels (11.25 ± 0.64 mg/dL), comparable to the control group (11.47 ± 0.94 mg/dL).

Uric acid levels were significantly raised in the cisplatin-only group (58.42 ± 2.09 mg/dL). The LD PAMC group showed a moderate reduction (41.53 ± 0.93 mg/dL), while the HD group demonstrated a substantial improvement (34.70 ± 1.38 mg/dL).

Urea levels also increased significantly in the cisplatin-only group (123.10 ± 2.22 mg/dL) but reduced in the LD (92.45 ± 1.61 mg/dL) and HD (73.82 ± 1.62 mg/dL) PAMC groups.

Table 2 presents the renal function markers across the experimental groups.

Table 1. BW comparison across the experimental groups

Group	Day 0 (g)	Day 5 (g)	Day 9 (g)
Control	209.33 ± 2.25	224.33 ± 3.20	244.00 ± 2.02
Cisplatin-only	210.16 ± 2.13	195.16 ± 1.72	173.66 ± 1.96
PAMC LD (200 mg/kg)	210.33 ± 3.38	231.00 ± 2.28	241.16 ± 1.94*
PAMC HD (400 mg/kg)	213.66 ± 2.25	235.00 ± 2.75*	252.83 ± 2.78**
Standard Treatment (Cystone syrup)	211.83 ± 3.43	241.16 ± 2.13*	251.83 ± 2.85**

Data are presented as mean ± SEM (n=6). *p<0.05, **p<0.01 compared to the cisplatin-only group.

Table 2. Renal function markers across the experimental groups

Group	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)
Control	11.47 ± 0.94	33.43 ± 1.76	73.98 ± 1.97
Cisplatin-only	39.74 ± 0.71	58.42 ± 2.09	123.10 ± 2.22
PAMC LD (200 mg/kg)	16.35 ± 0.84*	41.53 ± 0.93*	92.45 ± 1.61**
PAMC HD (400 mg/kg)	11.25 ± 0.64**	34.70 ± 1.38**	73.82 ± 1.62**
Standard Treatment (Cystone syrup)	11.68 ± 1.02**	35.60 ± 0.89**	72.41 ± 1.11**

Data are presented as mean ± SEM (n=6). *p<0.05, **p<0.01 compared to the cisplatin-only group.

3.2.2. Improvement in Urine Output across Treatment Groups

Urine output, a key indicator of renal function, was significantly decreased in the cisplatin-only group (0.80 ± 0.13 mL), indicating impaired kidney excretory function due to cisplatin-induced nephrotoxicity. In contrast, rats treated with PAMC exhibited a dose-dependent improvement in urine output.

The LD PAMC group showed a moderate increase in urine output (1.10 ± 0.16 mL). The HD PAMC group exhibited a more substantial improvement, with urine output (1.25 ± 0.14 mL) approaching levels similar to those of the control group (1.28 ± 0.09 mL). The standard treatment group also demonstrated improved urine output (1.25 ± 0.09 mL), comparable to that of the HD group.

Table 3 below outlines the comparison of urine output across the experimental groups.

Table 3. Urine output comparison across the experimental groups

Group	Urine Output (mL/24h)
Control	1.20 ± 0.20
Cisplatin-only	0.80 ± 0.13
PAMC LD (200 mg/kg)	1.10 ± 0.16*
PAMC HD (400 mg/kg)	1.25 ± 0.14**
Standard Treatment (Cystone syrup)	1.25 ± 0.09**

Data are presented as mean ± SEM (n=6). *p<0.05, **p<0.01 compared to the cisplatin-only group.

3.2.3. Comparison with Standard Nephroprotective Treatment

The standard nephroprotective treatment (Cystone syrup) results were comparable to those of the HD PAMC group, showing similar improvements in creatinine, uric acid, urea levels, and urine output. This suggests that PAMC exhibits nephroprotective effects similar to those of established nephroprotective agents like Cystone, particularly at higher doses. Both treatments successfully mitigated cisplatin-induced renal dysfunction, highlighting the therapeutic potential of PAMC as an effective natural alternative.

3.3. Serum Biochemical Parameters

3.3.1. Creatinine, Uric Acid, Urea, Total Protein and Albumin Levels

Renal function was assessed by measuring serum levels of creatinine, uric acid, and urea, as these markers reflect kidney health and are commonly elevated in cases of nephrotoxicity. CIN is known to elevate these markers due to impaired renal filtration. In this study, the effects of PAMC on these parameters were evaluated.

In the cisplatin-only group, serum creatinine levels were significantly elevated (2.75 ± 0.31 mg/dL) compared to the control group (1.05 ± 0.05 mg/dL), indicating decreased kidney function due to cisplatin toxicity. Treatment with PAMC showed dose-dependent improvements. The LD group (MC-LD) exhibited a moderate reduction in creatinine levels (1.56 ± 0.23 mg/dL). In contrast, the HD group (MC-HD) demonstrated further improvement, with creatinine levels reduced to near-normal values (1.26 ± 0.12 mg/dL). The standard treatment group achieved creatinine levels (1.09 ± 0.08 mg/dL) that closely matched the control group, highlighting the effectiveness of the standard treatment in mitigating nephrotoxicity.

Cisplatin treatment also caused a noticeable increase in serum uric acid levels (4.58 ± 0.13 mg/dL) compared to the control group (3.40 ± 0.10 mg/dL), reflecting impaired renal excretion. PAMC treatment in the LD group (3.21 ± 0.14 mg/dL) and HD group (3.20 ± 0.09 mg/dL) significantly lowered uric acid levels, approaching those of the control group. The standard treatment group also showed a marked improvement in uric acid levels (3.24 ± 0.18 mg/dL), indicating the protective effects of both PAMC and the standard treatment on renal function.

Similarly, the cisplatin-only group exhibited a substantial increase in serum urea levels (82.51 ± 2.10 mg/dL) compared to the control group (31.44 ± 1.71 mg/dL), indicative of impaired kidney function. Administration of PAMC resulted in a significant reduction in urea levels. The LD group showed a moderate decrease (57.44 ± 1.79 mg/dL), while the HD group demonstrated a more pronounced reduction (49.58 ± 1.18 mg/dL). The standard treatment group also experienced a significant improvement in urea levels (39.88 ± 1.49 mg/dL), nearly aligning with those of the control group, thereby underscoring the efficacy of the treatments in mitigating CIN.

As previously discussed, cisplatin treatment caused a reduction in total protein (7.73 ± 1.08 g/dL) and albumin levels (4.24 ± 1.68 g/dL) compared to the control group. PAMC, particularly in the HD group, significantly recovered in these parameters. The HD group exhibited near-normal total protein (10.90 ± 0.65 g/dL) and albumin levels (4.90 ± 0.51 g/dL). The standard treatment further enhanced total protein (12.38 ± 0.63 g/dL) and albumin (5.10 ± 1.18 g/dL) levels, demonstrating protective effects on protein metabolism.

In summary, PAMC demonstrated dose-dependent protection against CIN by significantly improving serum creatinine, uric acid, urea, total protein, and albumin levels. These findings suggest that PAMC may offer renoprotective benefits and counteract cisplatin's adverse effects on renal health.

Table 4 presents the serum biochemical parameters across the experimental groups.

Table 4. Serum biochemical parameters across the experimental groups

Group	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Control	1.05±0.05	3.40±0.10	31.44±1.71	11.00 ± 0.68	5.18 ± 1.12
Cisplatin-only	2.75±0.31	4.58±0.13	82.51±2.10	7.73 ± 1.08	4.24 ± 1.68
PAMC LD (200 mg/kg)	1.56±0.23*	3.21±0.14*a	57.44±1.79*	10.51 ± 0.88*	4.68 ± 1.54*
PAMC HD (400 mg/kg)	1.26±0.12**	3.20±0.09**	49.58±1.18**	10.90 ± 0.65**	4.90 ± 0.51**
Standard Treatment (Cystone syrup)	1.09±0.08**	3.24±0.18**	39.88±1.49**	12.38 ± 0.63**	5.10 ± 1.18**

Data are presented as mean ± SEM (n=6). *p<0.05, **p<0.01 compared to the cisplatin-only group.

3.4. Antioxidant Activity

3.4.1. Impact on SOD, CAT, Gpx, and GSH Levels

CIN is characterised by excessive oxidative stress, leading to the depletion of critical antioxidant enzymes. This research evaluated the antioxidant activity in kidney tissues by quantifying the amounts of SOD, CAT, Gpx, and decreased GSH. The injection of cisplatin led to a significant decrease in the activity of these antioxidant enzymes, indicating heightened oxidative stress.

In the cisplatin-only group, SOD levels were markedly decreased (4.86 ± 0.14 U/ml) compared to the control group (9.10 ± 0.42 U/ml), suggesting oxidative damage. However, in the LD PAMC group, SOD levels increased moderately (6.78 ± 0.25 U/ml), while the HD group showed a substantial restoration of SOD activity (7.31 ± 0.35 U/ml), approaching the levels observed in the control group.

Similar trends were observed with CAT activity. In the cisplatin-only group, CAT levels were significantly reduced (42.18 ± 2.60 U/ml), while the control group maintained higher levels (94.65 ± 1.02 U/ml). PAMC treatment, especially at the HD, restored CAT activity (66.93 ± 3.42 U/ml), suggesting a protective effect against oxidative damage.

Gpx, a critical enzyme in mitigating oxidative stress, was also significantly reduced in the cisplatin-only group (5.72 ± 0.21 U/ml). The HD PAMC group demonstrated a marked improvement in Gpx levels (8.71 ± 0.48 U/ml), similar to the control group.

Reduced GSH levels were significantly depleted in the cisplatin-only group (24.13 ± 1.29 mg/l), indicating oxidative damage to the renal tissues. The administration of PAMC, particularly at the HD, resulted in a notable restoration of GSH levels (46.11 ± 1.50 mg/l), indicating enhanced antioxidant capacity.

Table 5 summarises the antioxidant enzyme activity across the experimental groups.

3.4.2. Reduction of MDA Levels (Marker of Lipid Peroxidation) in Treated Groups

MDA is a byproduct of lipid peroxidation and a principal indicator of oxidative stress. Elevated MDA levels indicate significant damage to cell membranes due to ROS. In the cisplatin-only group, MDA levels were significantly increased (12.50 ± 0.28 nmol/mg), confirming the high degree of oxidative stress induced by cisplatin.

MDA levels were significantly lowered in the PAMC-treated groups in a dose-dependent manner. In the LD group, they were reduced to 6.69 ± 0.50 nmol/mg. In the HD group, they were further reduced to 5.66 ± 0.35 nmol/mg, approaching levels similar to those in the control group (6.85 ± 0.63 nmol/mg).

The standard treatment group, i.e., the cystone-treated group, also showed a substantial reduction in MDA levels (2.31 ± 0.37 nmol/mg), which was even lower than that of the control group, indicating strong protection against lipid peroxidation.

Table 6 presents the MDA levels across the experimental groups.

3.4.3. Dose-Dependent Antioxidant Effects of PAMC

The results from the antioxidant activity assessments demonstrate the dose-dependent effects of PAMC on enhancing the kidney's antioxidant defence mechanisms. The HD group (400 mg/kg) consistently showed better restoration of antioxidant enzymes (SOD, CAT, Gpx, and GSH) and a more significant reduction in MDA levels compared to the LD group (200 mg/kg). This indicates that a higher dose of PAMC offers more robust protection against cisplatin-induced oxidative stress.

Table 5. Antioxidant enzyme activity across the experimental groups

Group	SOD (U/ml)	CAT (U/ml)	Gpx (U/ml)	GSH (mg/l)
Control	9.10 ± 0.42	94.65 ± 1.02	9.60 ± 0.42	57.84 ± 1.40
Cisplatin-only	4.86 ± 0.14	42.18 ± 2.60	5.72 ± 0.21	24.13 ± 1.29
PAMC LD (200 mg/kg)	6.78 ± 0.25*	55.75 ± 1.29*	8.13 ± 0.25*	40.07 ± 0.92*
PAMC HD (400 mg/kg)	7.31 ± 0.35**	66.93 ± 3.42**	8.71 ± 0.48**	46.11 ± 1.50**
Standard Treatment (Cystone syrup)	8.57 ± 0.46**	83.38 ± 1.81**	9.92 ± 0.59**	53.04 ± 1.82**

Data are presented as mean ± SEM (n=6). *p<0.05, **p<0.01 compared to the cisplatin-only group.

Table 6. MDA levels across the experimental groups

Group	MDA (nmol/mg)
Control	6.85 ± 0.63
Cisplatin-only	12.50 ± 0.28
PAMC LD (200 mg/kg)	6.69 ± 0.50*
PAMC HD (400 mg/kg)	5.66 ± 0.35**
Standard Treatment (Cystone syrup)	2.31 ± 0.37**

Data are presented as mean ± SEM (n=6). *p<0.05, **p<0.01 compared to the cisplatin-only group.

3.5. Histopathological Findings

3.5.1. Renal Histology in Control, Cisplatin, and Treated Groups

Histopathological examination of kidney tissues directly proved the amount of renal damage caused by cisplatin and preserved by PAMC. The control group's results were normal histological architecture with the complete presence of glomeruli, renal tubules, and interstitial spaces. However, the effects of cisplatin only revealed severe damage to the kidneys, including extensive tubular necrosis, inflammation, and loss of tubular architecture, characteristic features of nephrotoxicity caused by cisplatin.

In the treatment groups, LD of 200 mg/kg and HD of 400 mg/kg PAMC exhibited mixed modes of renal protection:

The tubular injury was mild to moderate in the LD Group (200 mg/kg), with less necrosis and inflammation than in the cisplatin group. The effect of LD treatment proved that the kidney's architecture was preserved.

The HD Group (400 mg/kg) significantly protected the kidney tissues; histopathological findings were mild. The tubular structures mainly appeared well maintained with no features of necrosis or inflammation, apparently with a shape similar to the histology of the control group.

The Standard Treatment (Cystone syrup) showed similar results to the HD group; the Cysteine-Treated group displayed substantial protection against cisplatin-induced damage, with minimal necrosis and inflammation.

Table 7 summarises the histopathological grading of renal damage across the experimental groups, evaluating the extent of necrosis, inflammation, and tubular damage on a scale of 0 to 4 (0 = no damage, 4 = severe damage).

3.5.2. Protection of Renal Tubules from Necrosis and Inflammation by PAMC

Microscopic examination of kidney tissues confirmed that cisplatin caused severe necrosis and inflammatory infiltration in the renal tubules, particularly in the proximal tubules most vulnerable to cisplatin toxicity. In the cisplatin-only group, widespread tubular necrosis, degeneration of epithelial cells, and extensive inflammatory infiltration in the interstitial regions were evident.

In the HD PAMC Group (400 mg/kg), minimal necrosis was observed, and the renal tubules appeared structurally intact, with reduced signs of inflammation. This group's preservation of tubular architecture suggests strong nephroprotective effects of HD PAMC.

The LD PAMC Group (200 mg/kg) showed some degree of necrosis and inflammation, but the damage was significantly lower than in the cisplatin-only group. This indicates a moderate protective effect at this dose.

The Standard Treatment (Cysteine syrup) was similar to the HD group. The Cysteine-Treated group showed significant protection against necrosis and inflammation, with minimal histopathological changes.

Figure 1 displays microscopic images of renal tissue from the control, cisplatin-only, and PAMC-treated groups. These images would illustrate the extent of renal damage and recovery in each group.

Table 7. Histopathological grading of renal damage across the experimental groups

Group	Necrosis (0-4)	Inflammation (0-4)	Tubular Damage (0-4)
Control	0	0	0
Cisplatin-only	4	4	4
PAMC LD (200 mg/kg)	2	2	2
PAMC HD (400 mg/kg)	1	1	1
Standard Treatment (Cystone syrup)	1	1	1

Grading was performed based on the severity of observed histopathological changes.

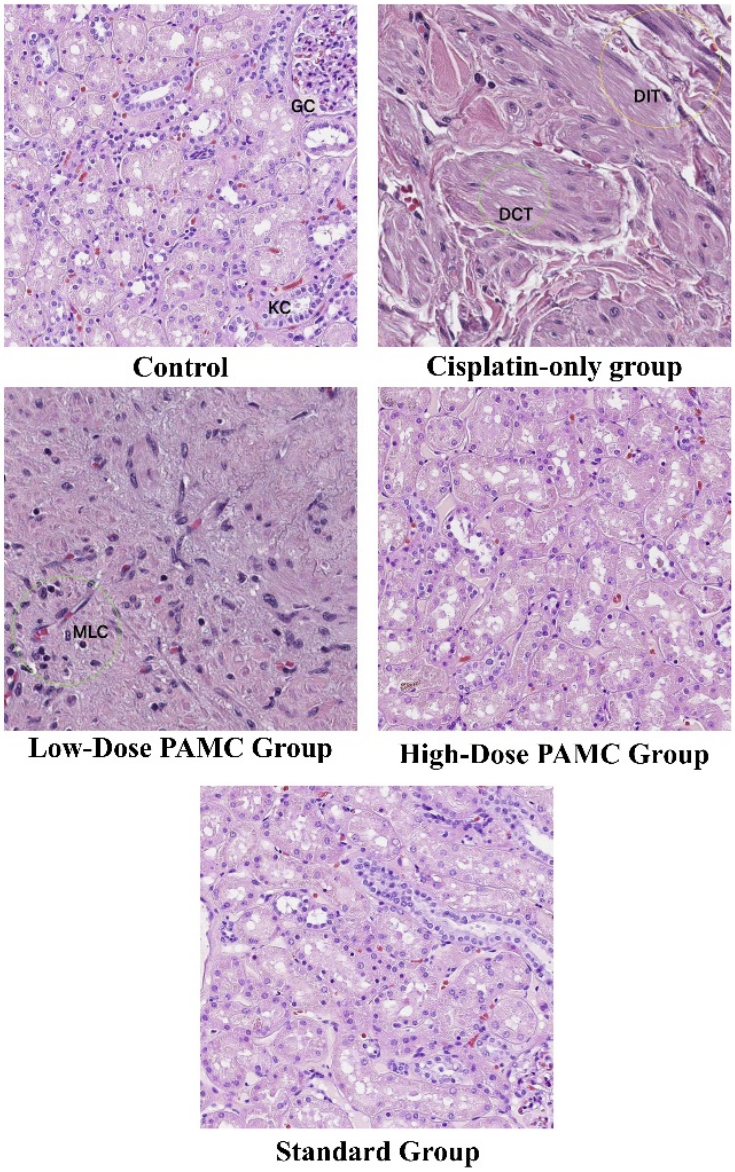

Figure 1. Histopathological images of renal tissue

3.5.3. Comparison of Tissue Recovery in LD, HD, and Standard Treatment Groups

The histopathological evaluation revealed a clear dose-dependent recovery in the PAMC-treated groups. The HD group (400 mg/kg) showed near-complete preservation of renal tissue, with minimal necrosis, inflammation, and tubular damage. This group exhibited histological features comparable to the control group, indicating robust protection against CIN.

In contrast, the LD group (200 mg/kg) displayed moderate protection, with some residual necrosis and inflammation still present. While the renal architecture was not fully restored, the damage was significantly reduced compared to the cisplatin-only group.

The standard treatment group (Cystone syrup) performed similarly to the HD PAMC group, suggesting that PAMC at higher doses has comparable nephroprotective effects to an established nephroprotective agent.

3.6. Comparison of PAMC with N-Acetylcysteine and Alpha-Lipoic Acid)

PAMC showed significant nephroprotective effects similar to those of pharmaceutical agents like N-acetylcysteine (NAC) and alpha-lipoic acid (ALA), which are known for their antioxidant properties.

N-acetylcysteine (NAC): NAC has been widely studied for its ability to counteract oxidative stress by replenishing intracellular glutathione (GSH) levels. Literature has shown that NAC significantly reduced serum creatinine, BUN, and oxidative stress markers (such as MDA), similar to the effects observed with PAMC treatment. However, NAC generally requires higher doses (typically 600–1200 mg/kg) for effective nephroprotection in animal models [19]. Our findings with PAMC (both low and high doses) indicate comparable reductions in serum creatinine and BUN levels, with the HD PAMC group showing results near those of the standard pharmaceutical treatment, Cystone syrup.

Alpha-lipoic acid (ALA): ALA is a potent antioxidant that reduces oxidative damage and improves renal function by restoring antioxidant defences. Literature has demonstrated ALA's efficacy in protecting against nephrotoxicity, especially in cases of drug-induced kidney injury. ALA improved serum creatinine and antioxidant enzyme activities, but PAMC demonstrated a broader range of protective mechanisms [20]. PAMC’s effects on both oxidative stress and inflammation were more pronounced, as evidenced by the enhancement of SOD, CAT, and Gpx activity, alongside a reduction in MDA levels. These improvements were observed at both low and high doses of PAMC, with the HD group showing maximal renal protection.

4. Discussion

4.1. Nephroprotective Effects of PAMC

4.1.1. Dose-Dependent Protection against CIN

The current study's results exhibit a dose-dependent nephroprotective effect with PAMC in attenuating CIN. Maximum improvement was found in parameters for renal function markers, antioxidant activity, and histopathological findings in the HD group treated with 400 mg/kg. The LD group treated with 200 mg/kg also showed significant protection, although significantly less than the HD group.

This dose-dependent protection suggests that the higher dosages would be more effective due to the improved availability of bioactive compounds exerting antioxidant, anti-inflammatory, and nephroprotective actions in vivo. Those herbal key ingredients used in the preparation of PAMC, such as *Boerhavia diffusa* [21], *Tribulus terrestris*, and *Crataeva nurvala* [22], have been discussed and studied for their diuretic, anti-inflammatory, and antioxidant properties, thus protecting the kidneys against CIN.

4.1.2. Reduction in Creatinine, Urea, and Uric Acid Levels as Evidence of Improved Renal Function

One of the most compelling indicators of PAMC's nephroprotective effects is the significant reduction in serum creatinine, urea, and uric acid levels observed in the treatment groups, particularly in the HD group. Elevated levels of these markers in the cisplatin-only group indicated severe renal dysfunction, as CIN impairs glomerular filtration, accumulating nitrogenous wastes in the bloodstream.

PAMC, especially at 400 mg/kg, effectively restored these markers to near-normal levels, comparable to the control group. This improvement in renal function suggests that the herbal formulation enhances the kidneys' ability to filter and excrete metabolic waste products, preventing the accumulation of toxic substances in the bloodstream. The reduction in creatinine, urea, and uric acid levels can be attributed to the anti-inflammatory and antioxidant effects [23] of the churna, which likely reduced oxidative stress and preserved the structural integrity of the renal tissues.

4.1.3. Prevention of Cisplatin-Induced Weight Loss and Renal Failure

Cisplatin administration is known to cause significant systemic toxicity, including marked weight loss due to reduced food intake, dehydration, and general malaise [24]. In this study, the cisplatin-only group exhibited substantial weight loss over the 8 days, reflecting the severity of renal impairment and overall systemic decline.

The treated groups, especially the HD group, lost minimal weight compared with cisplatin controls. The well-documented maintenance of BWs in the treated groups indicates that PAMC served to protect not only against nephrotoxicity but also against the drug's systemic activity. This formulation accounted for the advancement of BW by its diuretic property, which maintained fluid balance and evaded dehydration, and its antioxidant property, which reduced oxidative stress and improved general health status.

Furthermore, the prevention of CIN was evident from improved renal function markers with better antioxidant enzyme activity and reduced histopathological damage,

which is the therapeutic potential of PAMC as a nephroprotective agent. Significant protection against renal failure underlines the value of this herbal formulation in preserving kidney function during chemotherapy.

4.2. Antioxidant Properties and Mechanism of Action

4.2.1. Role of Punarnava, Gokshura, and Other Ingredients in Reducing Oxidative Stress

PAMC is a polyherbal formulation that consists of multiple constituents. This formulation consists of many ingredients rich in potent antioxidant and nephroprotective properties. In the preparation process, some of the essential constituents such as *Boerhavia diffusa*, *Tribulus terrestris*, *Crataeva nurvala*, and *Albizia lebbeck* (Shirish) that form this formulation with the influential role in reducing oxidative stress along with inhibiting renal damage, have been added.

Boerhavia diffusa has diuretic, anti-inflammatory, and antioxidant activity. It protects the kidneys from oxidative injury, reduces ROS, and enhances endogenous antioxidants in the body. Furthermore, it has a definite anti-inflammatory effect, reducing tissue inflammation and fibrosis [25].

Tribulus terrestris is an Ayurvedic drug for treating disorders of the urinary and kidney; *Tribulus terrestris* acts through the mediation of oxidative stress by scavenging free radicals and reducing lipid peroxidation. It also contributes to renal functionality through the elimination of toxins and reduction in the damage caused by renal stress from oxidative stress [26].

Crataeva nurvala has historically been used to treat urinary calculi and irritation. *Crataeva nurvala* possesses antioxidant activity, which protects the renal tissue from oxidative damage due to free radicals, thus leading to the regeneration of damaged cells [27].

Albizia Lebbeck has detoxifying properties. It would help lessen oxidative stress and inflammation while protecting against the toxic substance cisplatin. It would strengthen the antioxidant defence, making the formulation more effective in preserving kidney tissues [28].

Each ingredient synergises in reducing oxidative stress, terminating free radicals, and protecting renal cells from damage [25-28]. For this reason, it is fundamental to shut down or weaken an important process by which cisplatin induces nephrotoxicity-oxidative damage.

4.2.2. Enhancement of Antioxidant Enzyme Activity (SOD, CAT, Gpx) and Reduction of Lipid Peroxidation (MDA)

In the present study, it appears that the activity of some SOD, CAT, and Gpx in enzymic antioxidant defence was augmented at the same time, while the concentration of the lipid peroxidation marker MDA decreased.

SOD, CAT, and Gpx enzymes play an essential role in scavenging ROS and protecting the cells from oxidative damage [29]. SOD catalyses the conversion of superoxide radicals into hydrogen peroxide and oxygen [30]. CAT decomposes hydrogen peroxide into water and oxygen, safeguarding the cell from oxidative harm [31]. Gpx reduces hydrogen peroxide and lipid hydroperoxides and protects cellular membranes from damage [16]. In both, the dosages of PAMC, SOD, CAT, and Gpx activities were significantly increased in all three enzymes when compared to the cisplatin-alone group. This means an efficient antioxidant defence system must be present in the formulation to save renal tissues from oxidation.

MDA is the product of lipid peroxidation and is also viewed as a marker of oxidative damage to cell membranes [32]. Increased MDA levels are reported with CIN through increased oxidative stress. The HD PAMC group revealed a significant decrease in the MDA level, thereby ensuring that the formulation inhibited lipid peroxidation. Thus, through decreasing the MDA, PAMC protects the structural integrity of renal cell membranes from oxidative damage.

Enhanced activity of antioxidant enzymes and decreased MDA levels in cisplatin-induced groups highlight that the crude powder of PAMC possesses vigorous antioxidant activity, which is relatively necessary for countering oxidative stress induced by cisplatin.

4.2.3. Comparative Analysis with Standard Antioxidant Treatments

The findings of the current investigation indicate that the nephroprotective and antioxidant effects of PAMC, particularly at a dosage of 400 mg/kg, are equivalent to the usual antioxidant therapy, Cystone syrup, used for comparison in this experiment. Both treatments showed significantly improved activity of the antioxidant enzymes (SOD, CAT, Gpx) and reduced MDA values, which strongly points to protection against oxidative stress.

Comparatively, both treatments restored renal function drastically with a marked reduction in oxidative stress; this multi-herb formulation PAMC may also provide better benefits from the various individual herbs and ingredients that show potent nephroprotective activity. Indeed, the formulation of herbs for antioxidant activity and anti-inflammatory properties could impart broader systemic protection. Accordingly, Cystone mainly appears to act on the former path.

The experiment overall demonstrates a consistent trend of the groups having higher doses outperforming the LD group with a clear dose-response relationship. Such relationships establish that the higher doses of the herbal preparation convey stronger antioxidant protection and greater efficacy in preventing CIN.

Therefore, it can be concluded that PAMC possesses highly potent antioxidant properties and boosts the body's antioxidant defence, decreasing oxidative damage to renal tissues. Its effects on lipid peroxidation reduction and improvement in antioxidant enzyme activity reflect its role as a natural multi-targeted therapy for cisplatin-induced

nephrotoxic effects equal to conventional antioxidant treatments.

4.3. Histopathological Analysis and Renal Protection

4.3.1. Preservation of Tubular Architecture and Prevention of Necrosis

Histopathological analysis of the kidney tissues provided critical insights into the protective effects of PAMC against cisplatin-induced renal damage. In the cisplatin-only group, severe tubular necrosis, loss of tubular architecture, and extensive cellular degeneration were observed, particularly in the proximal tubules. CIN typically affects the renal tubules, leading to cell death, which ultimately impairs kidney function.

In contrast, treatment with PAMC, particularly at the HD (400 mg/kg), demonstrated significant preservation of tubular architecture. The kidneys in the HD group exhibited minimal necrosis, with well-preserved tubular structures and only minor histological alterations. This suggests that PAMC effectively protected renal tubular cells from the damaging effects of cisplatin, likely by reducing oxidative stress and inflammation.

Even in the LD group (200 mg/kg), there was a noticeable reduction in tubular damage compared to the cisplatin-only group, although some degree of necrosis was still present. Preserving tubular integrity in both PAMC-treated groups highlights its capacity to mitigate cisplatin-induced tubular necrosis and preserve kidney function.

4.3.2. Anti-Inflammatory Effects and Reduced Cellular Damage in the Renal Interstitium

The nephrotoxicity accompanying cisplatin exposure always involves an inflammatory response, as shown by the infiltration of immune cells into the renal interstitium, subsequently leading to inflammation and fibrosis. Gross infiltration of inflammatory cells, particularly neutrophils and macrophages, was observed in the renal interstitial space of the cisplatin-only-treated animals of this study.

The treatment with PAMC significantly reduced interstitial inflammation. This was most significant in the HD group, which pointed to minimal infiltration of inflammatory cells and a marked reduction in fibrosis. Thus, this churna had very significant anti-inflammatory effects associated with it. The LD of the churn showed less inflammation but was less extreme. These anti-inflammatory effects are probably due to bioactive compounds in herbal formulations like Punarnava and *Albizia lebbeck*, which are well-known for their anti-inflammatory effects.

The drug protects renal cells from immune-mediated damage by reducing the infiltration of immune cells and limiting interstitial inflammation. Most importantly, it inhibits the development of fibrosis, a critical factor in chronic kidney disease. This indicates that the formulation's action is not merely a simple antioxidant but includes modulation of the inflammatory response.

4.3.3. Comparison with Histopathological Recovery in Standard Treatment Groups

The histopathological recovery observed in the HD PAMC group was comparable to that in the standard treatment group (Cystone syrup). Both treatments significantly protected against cisplatin-induced tubular necrosis, inflammation, and overall renal damage. The standard treatment group, which received Cystone, showed near-complete recovery of tubular architecture and minimal signs of necrosis or inflammation, similar to the HD PAMC group.

However, the advantage of PAMC lies in its multi-herb composition, which provides a broad range of bioactive compounds that target both oxidative stress and inflammation. The combination of herbs such as *Tribulus terrestris* and *Crateva nurvala* contributes to the prevention of tubular damage and the reduction of interstitial inflammation and cellular damage [33]. This multi-targeted approach gives PAMC a slight edge regarding comprehensive renal protection, as it addresses the oxidative and inflammatory pathways involved in CIN.

The histopathological findings demonstrate that PAMC offers strong nephroprotective effects comparable to established nephroprotective treatments like Cystone. Its ability to preserve tubular architecture, prevent necrosis, and reduce inflammation suggests that this polyherbal formulation has significant potential for clinical use in managing chemotherapy-induced nephrotoxicity.

4.4. Overall Therapeutic Potential of PAMC

4.4.1. Summary of Nephroprotective and Antioxidant Effects

The results of this study highlight the significant nephroprotective and antioxidant effects of polyherbal formulation, clearly demonstrating a dose-dependent protective effect. At the same time, the HD group, 400 mg/kg, showed near-complete preservation of renal function and structure. Main Findings:

In PAMC, serum creatinine, urea, and uric acid levels were significantly decreased, indicating regeneration and enhancement of the kidneys' filtration and excretory functions. Results in the HD group were comparable to those in the control group, signifying strong nephroprotective efficacy.

It increased the activity of key antioxidant enzymes such as SOD, CAT, and Gpx, with a resultant reduction in MDA contents as the marker of lipid peroxidation. Thus, these results suggest that this treatment with PAMC effectively lessens oxidative stress in renal tissues.

Histological study shows that PAMC maintained the tubular structure, suppressed necrosis, and caused less inflammation in the renal interstitium. This, therefore, implies that the formulation confers the full protective action of the kidneys, not only in the aspect of oxidative stress but also in the inflammatory factor.

This will lead to a multi-targeted approach by the PAMC through the synergistic action of its key ingredients, such as Punarnava, *Tribulus terrestris*, *Crateva nurvala*, and Shirish. It is, therefore, a valuable candidate for managing chemotherapy-induced renal damage.

4.4.2. Implications for Clinical Use in Managing Cisplatin-Induced Renal Damage

This work carries important clinical applicability for the use of PAMC in the treatment of CIN, which is often associated with dose-limiting toxicity following cisplatin chemotherapy. Cisplatin is a chemotherapeutic agent employed for the treatment of cancer patients; however, it causes nephrotoxicity that limits its therapeutic value. It may be an adjunct therapy for patients treated with cisplatin because it protects the renal tissues.

Since PAMC improves renal function, reduces oxidative stress, and maintains kidney architecture, it seems helpful in preventing or reducing CIN. Its multi-herb composition provides wide-ranging protection against both oxidative damage and inflammation.

It is an herbal formulation that, being natural, could provide a safety profile as an alternative to synthetic nephroprotective agents. This is useful for patients preferring natural formulations or those with adverse reactions to conventional nephroprotective drugs.

More clinical research is urgently needed to establish whether PAMC would be effective and safe in cancer patients. However, the preclinical data indicate a high therapeutic potential for its application in chemotherapy regimens to prevent renal damage.

4.4.3. Nephroprotective Potential of PAMC vs NAC and ALA

PAMC's nephroprotective effects are comparable to those of NAC and ALA, but it offers broader protection through both antioxidant and anti-inflammatory mechanisms. While NAC and ALA effectively reduce oxidative stress, PAMC's multi-targeted action leads to better preservation of renal architecture and reduced inflammation. These results highlight PAMC as a promising adjunct therapy for preventing chemotherapy-induced renal damage, providing a natural alternative to synthetic drugs. Further clinical studies are necessary to validate these findings.

4.4.4. Limitations of the Current Study and Suggestions for Future Research

While valuable information was derived on the nephroprotective and antioxidant properties of the PAMC, several limitations exist that need more investigation:

This study focused on acute CIN over a relatively short experimental period of 8 days. Further studies must address the chronic toxicity models for the formulation's long-term efficacy and safety assessment. Chronic exposure to cisplatin or other nephrotoxic agents may reveal further protective mechanisms of the formulation.

Although the study demonstrated significant protection against oxidative stress and inflammation, more detailed mechanistic studies are required to fully elucidate the molecular pathways through which PAMC exerts its effects. Investigating specific signalling pathways involved in oxidative stress, apoptosis, and inflammation would provide deeper insights into its therapeutic action.

Preclinical findings, while promising, must be validated in human clinical trials. Controlled clinical studies should assess the efficacy of PAMC in preventing or reducing CIN in cancer patients. Dosage optimisation, safety profiles, and potential interactions with chemotherapy drugs also need to be explored.

As an herbal product, the consistency of PAMC's active compounds can vary depending on the source and preparation method. Future research should focus on standardising the formulation to ensure consistent therapeutic outcomes.

Although our findings suggest promising nephroprotective effects, future studies should explore the molecular mechanisms, including inflammatory pathways (e.g., NF-κB, TNF-α) and apoptosis-related genes. Longer studies are needed to assess PAMC's sustained efficacy and its ability to prevent chronic nephrotoxicity. Pharmacokinetic data, including bioavailability and ADME profiling, will also be investigated for human application. Additionally, future research will evaluate PAMC's safety profile, side effects, toxicity, and interactions with chemotherapy to determine its clinical applicability.

5. Conclusions

PAMC demonstrated significant efficacy in preventing CIN by improving renal function, reducing oxidative stress, and preserving renal tissue architecture, particularly at 400 mg/kg HD. It restored key biomarkers of renal health, such as serum creatinine, urea, and uric acid, and enhanced antioxidant defences by boosting SOD, CAT, and Gpx activity while lowering lipid peroxidation (MDA levels). With its potent nephroprotective and antioxidant properties, it holds promise as an adjunct therapy during chemotherapy, enabling effective cisplatin doses with reduced nephrotoxic risks. Future research should focus on chronic toxicity models, optimal dosing, safety, formulation standardisation, molecular mechanisms, Pharmacokinetics, longer experimental duration, and safety profile assessment to advance its clinical applicability in oncology and nephrology.

Abbreviations

PAMC- Planet Ayurveda Mutrakrichantak churna, ROS- Reactive Oxygen Species, CIN- Cisplatin Induced

Nephrotoxicity, BW- Body Weight, LD- Low Dose, HD- High Dose, i.p.– Intraperitoneal, IAEC- Institutional Animal Ethics Committee, GFR- Glomerular Filtration Rate, BUN- Blood Urea Nitrogen, SOD- Superoxide Dismutase, CAT- Catalase, Gpx- Glutathione Peroxide, GSH- Glutathione, MDA- Malondialdehyde, TBARS- Thiobarbituric acid reactive substances, PBS- Phosphate Buffered Saline, NAC- N-acetylcysteine, ALA- Alpha-lipoic acid.

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Declaration of Competing Interest

The author declares no conflict of interest.

Statement

During the preparation of this work, the author(s) used **quillbot.com** and **grammarly.com** to remove plagiarism and make grammar corrections, respectively. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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