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Original Article

Protective Effects of *Tinospora cordifolia* on Cyclophosphamide-induced Nephrotoxicity in Wistar Rats

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ABSTRACT

Background: Nephrotoxicity induced by drugs is a common cause of renal failure, particularly with agents like cyclophosphamide used in cancer treatment. Natural antioxidants found in plants offer diverse therapeutic benefits. *Tinospora cordifolia*, a medicinal plant known for its hepatoprotective, diuretic, carminative, digestive, and anti-helminthic properties, was investigated for its potential to counter cyclophosphamide-induced kidney damage in rats. **Materials and Methods:** Extracts were prepared from dried *Tinospora* powder, rich in flavonoids, polyphenolics, and alkaloids. Rats were divided into control and treatment groups, with varying doses of the *Tinospora* extract alongside cyclophosphamide. The blood sample was evaluated for creatinine, urea and BUN. **Results:** Treatment with the extract, particularly at 400 mg/kg, significantly reduced creatinine levels, as well as serum urea and blood urea nitrogen ($p<0.01$). These results highlight *Tinospora cordifolia*'s nephroprotective potential, attributed to its flavonoid and polyphenol content. **Conclusion:** This study underscores the promise of plant-derived compounds in mitigating drug-induced kidney damage, suggesting *Tinospora cordifolia* as a potential adjunct therapy to preserve renal function during chemotherapy. Further research is needed to elucidate mechanisms and validate these findings for clinical application.

Keywords: *Tinospora cordifolia*, Nephroprotection, Cyclophosphamide, Renal function, Wistar rats.

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INTRODUCTION

The kidneys, bilaterally positioned in the lumbar region adjacent to the vertebral column, are reddish-brown vital organs tasked with multifaceted homeostatic functions.[1] Their primary role involves meticulous regulation of the intracellular environment's chemical composition. This intricate regulation encompasses the precise management of water, sodium chloride, potassium, phosphate, and various other solutes within the body.[2] Through finely tuned mechanisms, the kidneys meticulously control electrolyte balance, fluid volume, and pH levels, crucial for sustaining physiological equilibrium. Additionally, they play a pivotal role in eliminating metabolic waste products and toxins, ensuring systemic detoxification.[3] The kidney's intricate nephron structure, comprising diverse functional units, facilitates these intricate processes, including filtration, reabsorption, and secretion. Following ingestion, drugs undergo various biochemical transformations in the liver and other tissues, generating metabolites that are often water-soluble and readily excreted by the kidneys.[4] This renal elimination pathway ensures the removal of potentially harmful substances and maintains drug concentration within therapeutic ranges.[5] However, the excretion of drug metabolites can also have detrimental effects on kidney function. Certain drugs and their metabolites can accumulate within the kidney tissues, leading to nephrotoxicity, which manifests as acute or chronic kidney injury.[6] Nephrotoxic drugs may interfere with renal blood flow, disrupt tubular function, or induce inflammation and oxidative stress, ultimately impairing kidney function. Common examples include Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), certain antibiotics, chemotherapeutic agents, and contrast media used in medical imaging procedures.[7]

Cyclophosphamide is an alkylating agent commonly used in chemotherapy to treat various cancers and autoimmune diseases. While effective in its therapeutic action, cyclophosphamide can also induce nephrotoxicity, leading to kidney injury. The molecular mechanisms underlying cyclophosphamide-induced nephrotoxicity involve several interconnected pathways.[8] One prominent mechanism involves the activation of cyclophosphamide into its active enzymes. These metabolites are then distributed systemically, where they can accumulate in renal tissues. Phosphoramide mustard can directly damage renal tubular cells by inducing DNA cross-linking and oxidative stress, leading to cellular dysfunction and apoptosis. Acrolein, a reactive

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aldehyde, exacerbates nephrotoxicity by inducing oxidative stress, lipid peroxidation, and inflammation within the kidney. It can also impair mitochondrial function and disrupt cellular signaling pathways, further contributing to renal damage.[9] Furthermore, cyclophosphamide and its metabolites can elicit an immune response, triggering inflammatory cascades within the kidney. This immune-mediated injury involves the activation of pro-inflammatory cytokines, chemokines, and immune cells, leading to tissue inflammation and injury.[10]

For millennia, plants have served as medicinal remedies worldwide. According to a bulletin from the World Health Organization, around 80% of people in developing nations continue to rely predominantly on plant-based medicines for their primary healthcare needs. This longstanding tradition underscores the enduring significance of botanical remedies in diverse cultural and geographic contexts.[11] Despite advancements in modern medicine, the accessibility, affordability, and cultural relevance of plant-based therapies remain vital for many communities. Recognizing the therapeutic potential of plants contributes not only to addressing healthcare disparities but also to preserving traditional knowledge and biodiversity.[12] *Tinospora cordifolia*, commonly known as Guduchi or *Tinospora*, is a medicinal plant revered in traditional systems of medicine like Ayurveda for its diverse therapeutic potential. Numerous studies have reported its beneficial effects in various animal models, validating its traditional use.[13] Research suggests that *Tinospora cordifolia* exhibits potent immunomodulatory properties, enhancing the activity of immune cells such as macrophages and lymphocytes, which play pivotal roles in defending against infections and diseases.[14] Additionally, it demonstrates anti-inflammatory activity by inhibiting pro-inflammatory mediators and cytokines, thereby alleviating inflammatory conditions.[15] Furthermore, *Tinospora cordifolia* exhibits antioxidant properties, scavenging free radicals and reducing oxidative stress, which is implicated in various chronic diseases.[15] Moreover, it shows hepatoprotective effects by safeguarding liver cells against damage and promoting liver regeneration.[16] *Tinospora cordifolia* is renowned for its rich content of bioactive compounds such as alkaloids, diterpenoids, glycosides, steroids, and polysaccharides.[17] Additionally, it contains phytochemicals like berberine, palmatine, and magnoflorine, which contribute to its therapeutic potential.[18] The present study aims to assess the efficacy of *Tinospora cordifolia* in mitigating cyclophosphamide-induced renal injury in male Wistar rats.

EXPERIMENTAL

Chemicals

Cyclophosphamide, obtained from Central Drug House in India, was used in this study. Other necessary chemicals were also procured from the same source, CDH, India. This included all substances required for experimental procedures and analyses.

Extraction and Phytochemical Screening

Tinospora cordifolia stems were sourced locally from Jabalpur, Madhya Pradesh, India, during the winter season (November 2023). After shade drying, the stems were pulverized using a grinder, and the resulting powder was stored in an airtight container. Subsequently, 600 g of the dried powder was soaked in 1500 mL of 90% ethanol for ten days with intermittent shaking. The mixture was then filtered using clean cotton and Whatman filter paper. The filtrate was dried using a vacuum rotary evaporator at 40°C to retain essential plant constituents, yielding 20 g of crude ethanolic extract. This extract was further partitioned using the ethyl acetate fractionation method.[19]

Selection of dose

As per studies performed by Zou *et al.*,[20] extract in the dose of 200 and 400 mg/kg of body weight was used in present study.

Induction of Nephrotoxicity

Cyclophosphamide in the dose of 3000 mg/ kg was used to induce hepatotoxicity in rats.[21]

Animal grouping

Animals were divided into five groups with six animals in each

Group I: Normal Control

Group II: Cyclophosphamide (3000 mg/kg)

Group III: Cyclophosphamide (3000 mg/kg) + Extract (200 mg/ Kg)

Group IV: Cyclophosphamide (3000 mg/kg) + Extract (400 mg/ kg)

On the 21st day that is, after 48 hr of pharmacological treatments, blood was withdrawn by a retro-orbital puncture for the estimation of biochemical parameters.

Biochemical Estimations

Blood samples were collected via retro-orbital puncture at the end of 24 hr; the serum was rapidly separated and processed for determination of serum creatinine, serum urea, and Blood Urea Nitrogen (BUN) using of Span Diagnostic kits. Body weight of animal was also recorded.

Statistical Analysis

The results were expressed as mean ± Standard Error of the Mean (SEM). Statistical analysis was carried out by using one-way Analysis of Variance (ANOVA) followed by *post hoc* Dunnett's test and $p < 0.001$ was considered significant.

RESULTS

Creatinine

The study evaluates the protective effects of *Tinospora cordifolia* extract on cyclophosphamide-induced nephrotoxicity in rats. Group II, subjected to high-dose cyclophosphamide, exhibited significant renal damage, as indicated by elevated creatinine during nephrotoxicity. This underscores the severe nephrotoxic potential of cyclophosphamide. In contrast, Groups III and IV, which received cyclophosphamide in conjunction with different doses of *Tinospora cordifolia* extract, showed a considerable reduction in creatinine. The results were dose-dependent, with the higher extract dose in Group IV providing greater protection. This suggests that *Tinospora cordifolia* extract mitigates renal damage likely through its antioxidant properties, which counteract the oxidative stress induced by cyclophosphamide. Group I, serving as the normal control, maintained baseline renal function, affirming the model's validity.

Serum urea

The study explores the impact of *Tinospora cordifolia* extract on serum urea levels in rats with cyclophosphamide-induced nephrotoxicity. Group II, receiving cyclophosphamide alone, demonstrated a significant elevation in serum urea levels, indicating substantial kidney damage and impaired renal function due to the drug's nephrotoxic effects. In contrast, Group

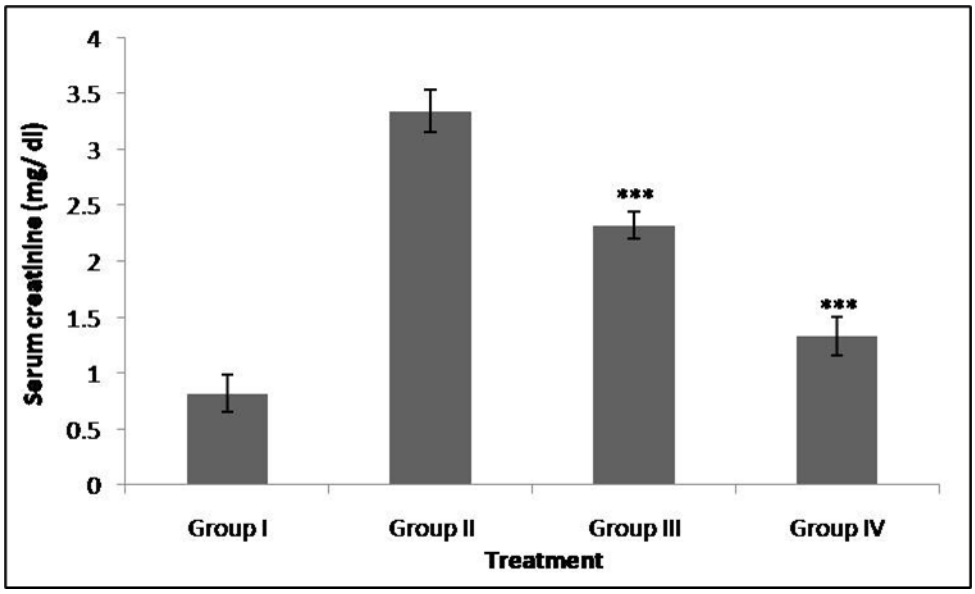

Figure 1: Effect of ethanol extract of *Tinospora cordifolia* on serum creatinine in Cyclophosphamide treated rats. Results are given as mean ± SEM of sixanimals in each group. Significance at *p<0.01.

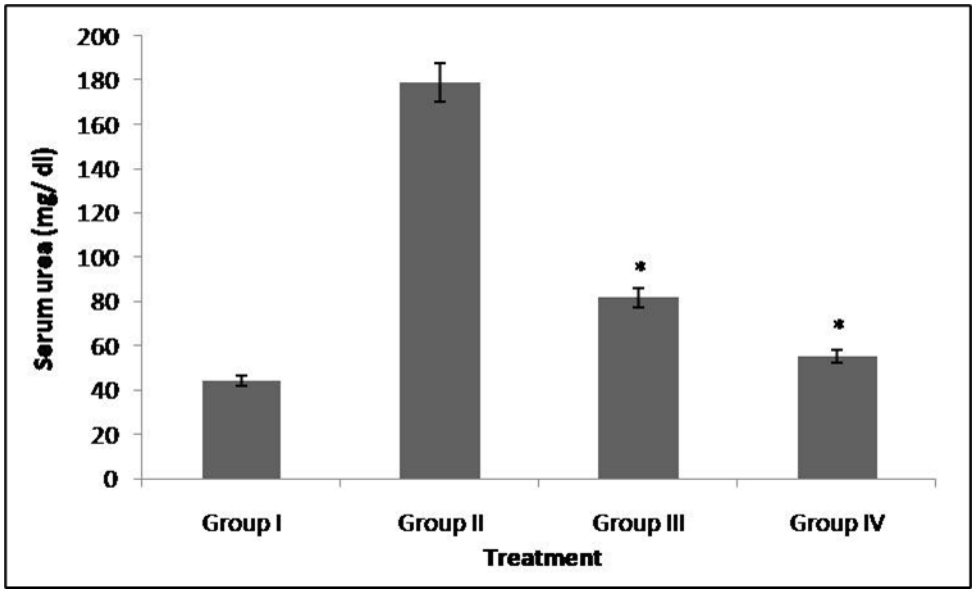

Figure 2: Effect of ethanol extract of *Tinospora cordifolia* on serum urea in Cyclophosphamide treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *p<0.01.

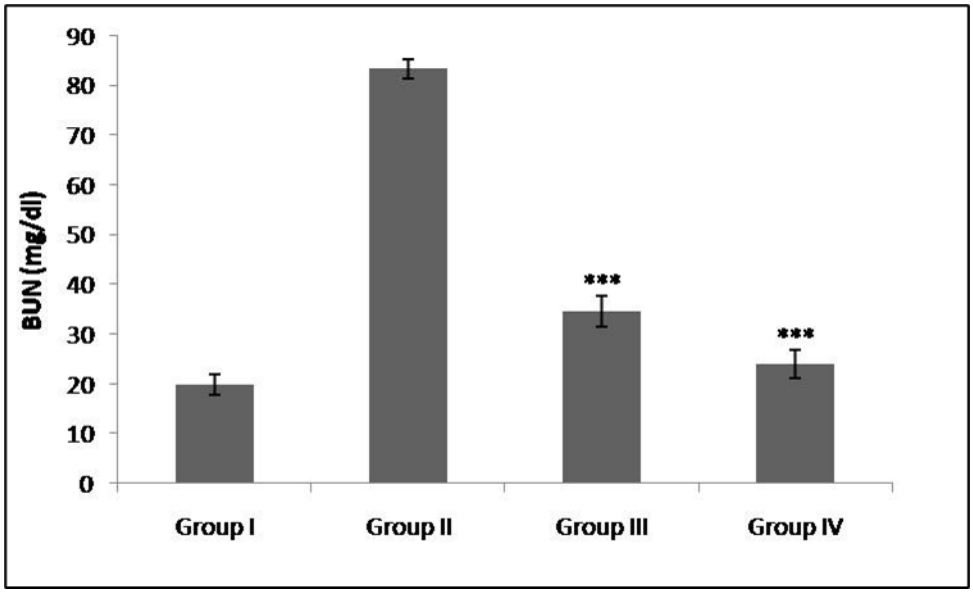

Figure 3: Effect of ethanol extract of *Tinospora cordifolia* on BUN in Cyclophosphamide treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *p<0.01.

III, treated with a combination of cyclophosphamide and a lower dose of *Tinospora cordifolia* extract, showed a marked reduction in serum urea levels compared to Group II. This suggests a protective effect of the extract, likely through its antioxidative properties, which help mitigate renal injury. Group IV, which received a higher dose of *Tinospora cordifolia* extract along with cyclophosphamide, exhibited an even greater reduction in serum urea levels, approaching those of the normal control group.

BUN

The study investigates the effect of *Tinospora cordifolia* extract on Blood Urea Nitrogen (BUN) levels in rats subjected to cyclophosphamide-induced nephrotoxicity. Cyclophosphamide administration in Group II led to a significant increase in BUN levels, reflecting severe kidney damage and impaired renal function due to its nephrotoxic effects. However, Group III, which received a combination of cyclophosphamide and a lower dose of *Tinospora cordifolia* extract, showed a notable reduction in BUN levels compared to Group II. This indicates the extract's potential in attenuating renal damage, likely through its antioxidative and nephroprotective properties. Group IV, treated with a higher dose of the extract alongside cyclophosphamide, exhibited BUN levels closer to those of the normal control group, demonstrating an enhanced protective effect.

DISCUSSION

Cyclophosphamide, a clinically effective anticancer agent, is widely used in the treatment of various carcinomas.[22] Scientific studies have shown that cyclophosphamide augments the production of harmful mediators such as Reactive Oxygen Species (ROS) and pro-inflammatory cytokines.[23] These mediators contribute to oxidative stress and inflammation, leading to cellular and tissue damage. Furthermore, cyclophosphamide-induced lipid peroxidation promotes significant damage to the structure and function of cellular membranes. Lipid peroxidation results in the formation of Malondialdehyde (MDA) and 4-Hydroxynonenal (4-HNE), which can disrupt membrane integrity, increase permeability, and impair normal cellular functions.[24]

Plant-based pharmaceuticals have garnered significant attention for their role in managing various diseases and disorders. These natural compounds offer a diverse range of bioactive substances, including antioxidants, anti-inflammatory agents, and immune modulators.[25] Their efficacy and lower side effect profiles make them attractive alternatives or complements to conventional therapies. Research has highlighted the potential of plant-derived extracts in treating conditions such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders.[26] As the demand for safer and more effective treatments grows, the exploration and utilization of plant-based pharmaceuticals continue to expand, promising new avenues for disease management and health improvement.

Natural antioxidants are renowned for their capacity to protect cells and organisms from oxidative stress, a fundamental cause of aging and degenerative diseases. These antioxidants, found abundantly in medicinal plants, include phenolic compounds such as phenolic acids and flavonoids, as well as carotenoids, tocopherol, and ascorbic acid.[23] Phenolic compounds help neutralize free radicals, reducing oxidative damage to cellular components. Carotenoids protect lipid membranes, tocopherol

acts as a potent lipid-soluble antioxidant, and ascorbic acid (Vitamin C) enhances overall antioxidant defense.[27]

In the present work, the chemo preventive role of *Tinospora cordifolia* against cyclophosphamide-induced renal damage was studied in a rat model. *Tinospora cordifolia*, an important medicinal plant, is known for its immunomodulatory, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic potential. These properties make it a promising candidate for mitigating drug-induced toxicities. *Tinospora cordifolia*'s ability to scavenge free radicals helps in reducing oxidative stress, a major factor in drug-induced nephrotoxicity. Additionally, it has been shown to protect cellular structures from damage and improve overall cellular health. By modulating inflammatory pathways, *Tinospora cordifolia* can reduce tissue inflammation and promote healing.

In the present study, administration of cyclophosphamide induced nephrotoxicity, evident from biochemical examinations. Elevated levels of urea, creatinine, and blood urea nitrogen in serum indicated renal damage.[28] However, treatment with *Tinospora cordifolia* extract prevented the increase in these parameters, suggesting its protective effect against cyclophosphamide-induced renal toxicity. The extract's ability to attenuate the rise in urea, creatinine, and blood urea nitrogen levels underscores its potential in preserving renal function and mitigating nephrotoxicity. This protective effect may be attributed to the extract's antioxidative and anti-inflammatory properties,[14] which counteract oxidative stress and inflammation induced by cyclophosphamide. The study highlights *Tinospora cordifolia* extract as a promising therapeutic agent for preventing chemotherapy-induced renal damage, offering a natural and effective approach to mitigate the adverse effects of cyclophosphamide treatment on renal function.

CONCLUSION

Tinospora cordifolia exhibits significant phytochemical nephroprotective potential, with flavonoids and polyphenols identified as crucial constituents. These compounds likely contribute to its observed protective effects against cyclophosphamide-induced nephrotoxicity in rats. Nonetheless, further systematic investigations are imperative to validate its nephroprotective properties in human subjects. Robust clinical studies are warranted to elucidate the safety, efficacy, and optimal dosage regimens of *Tinospora cordifolia* extract in preventing chemotherapy-induced renal damage. Such research endeavors will facilitate the translation of these promising preclinical findings into practical therapeutic strategies for safeguarding renal function in cancer patients undergoing chemotherapy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of Variance; **BUN:** Blood Urea Nitrogen; **CDH:** Central Drug House; **DNA:** Deoxyribonucleic Acid; **MDA:** Malondialdehyde; **NSAIDs:** Nonsteroidal Anti-Inflammatory Drugs; **ROS:** Reactive Oxygen Species; **SEM:** Standard Error of the Mean; **WHO:** World Health Organization; **4-HNE:** 4-Hydroxynonenal.

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