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Research Article

PHYTO-PHARMACOLOGICAL EVALUATIONS OF *NELUMBO NUCIFERA* (GAERTN) IN CYCLOPHOSPHAMIDE-INDUCED NEPHROTOXITY IN RATS

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ABSTRACT

Objectives: *Nelumbo nucifera* (Gaertn) (Sacred Lotus) is a medicinally important plant conventionally used for the treatment of inflammatory and oxidative stress-related disorders. The present study aimed to evaluate the phytochemical profile, antioxidant potential, and protective effects of methanolic and aqueous extracts of *N. nucifera* against cyclophosphamide (CP)-induced toxicity.

Methods: Extractive values of methanolic and aqueous extracts were determined, followed by preliminary phytochemical screening and quantification of total phenolic and flavonoid contents. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Cytotoxicity and growth curve studies were performed using wild-type and Δtrx2 yeast strains to assess biocompatibility and stress response. *In vivo* nephroprotective effect was evaluated in CP-induced nephrotoxicity models by measuring serum creatinine, urea, uric acid, protein, globulin, alanine aminotransferase, aspartate aminotransferase, glutathione, catalase, and malondialdehyde levels, along with pro-inflammatory cytokines tumor necrosis factor-alpha and interleukin-1 beta.

Results: The aqueous extract yielded higher extractive value (13.0% w/w), whereas the methanolic extract exhibited higher phenolic (1.50 mg/100 mg) and flavonoid (2.33 mg/100 mg) content. DPPH assay revealed potent free-radical scavenging activity for methanolic extract (IC_{50}=42.32 µg/mL), closer to ascorbic acid. Yeast cytotoxicity studies confirmed the safety of the extracts and demonstrated their membrane-protective effects. *In vivo* studies showed that both extracts significantly ($p<0.05$–$p<0.001$) attenuated CP-induced alterations in renal and oxidative stress biomarkers, with methanolic extract (200 mg/kg) showing maximal protective effects.

Conclusion: *N. nucifera* extracts, particularly the methanolic fraction, possess strong antioxidant, nephroprotective, hepatoprotective, and anti-inflammatory activities. These findings support its potential therapeutic application in managing CP-induced nephrotoxity and oxidative stress-related disorders.

Keywords: *Nelumbo nucifera*, Methanolic extract, Phytochemical screening, 2,2-diphenyl-1-picrylhydrazyl assay, Antioxidant activity, Histopathology, Wild and knockout yeast strains, Cyclophosphamide, Nephroprotective, Oxidative stress, Cytokines, Glutathione, Catalase, Malondialdehyde.

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INTRODUCTION

Oxidative stress is a major contributor to the pathogenesis of several chronic diseases, including cancer, cardiovascular disorders, renal dysfunction, and liver injury [1]. It occurs due to an imbalance between the production of reactive oxygen species (ROS) and the endogenous antioxidant defense system, resulting in damage to proteins, lipids, and DNA [2].

Chemotherapeutic agents such as cyclophosphamide (CP) are widely used in cancer therapy but are associated with severe adverse effects, including nephrotoxicity and immunosuppression. CP undergoes metabolic activation to form phosphoramide mustard and acrolein, which induce oxidative stress, lipid peroxidation, and tissue injury [3].

Therefore, the use of natural antioxidants to mitigate CP-induced organ toxicity has gained significant interest. Medicinal plants serve as a rich source of bioactive phytochemicals with proven antioxidant, anti-inflammatory, and organ-protective properties [4,5].

Nelumbo nucifera Gaertn. (Family: *Nelumbonaceae*), commonly known as Sacred Lotus or Kamal, has been traditionally used in Ayurveda [6] and other systems of medicine for treating diarrhea, inflammation, bleeding disorders [7], liver dysfunction, and hyperlipidemia [8-10]. Various parts of the plant, including leaves, seeds, flowers, and rhizomes, have demonstrated pharmacological activities such as antimicrobial [11] anti-diabetic, anti-inflammatory, hepatoprotective, and antioxidant effects [12].

Phytochemical investigations have revealed that *N. nucifera* contains alkaloids (nuciferine, roemerine), flavonoids, phenolic acids, saponins, and tannins [13], which contribute to its therapeutic potential [14,15]. The presence of polyphenolic compounds makes it a promising candidate for scavenging free radicals and protecting against oxidative stress–mediated tissue injury [16,17]. The present study was designed to evaluate and compare the phytochemical profile, antioxidant potential, and protective effects of methanolic and aqueous extracts of *N. nucifera* against CP-induced nephrotoxicity and systemic oxidative stress. Phytochemical screening, total phenol and flavonoid content determination, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, yeast cytotoxicity tests, and nephroprotection supported by *in vivo* biochemical estimations were conducted to establish a scientific basis for the therapeutic use of *N. nucifera*.

MATERIALS AND METHODS

Materials

The present investigation employed a range of analytical-grade chemicals and reagents; all procured from reputed suppliers. Methanol and ethanol (Merck, India) were used as solvents for extraction due to their excellent polarity profiles. The standard phytochemical reagents,

such as Mayer's, Dragendorff's, and Wagner's for alkaloid detection, and ferric chloride and lead acetate for phenol and flavonoid identification, were sourced from Loba Chemie Pvt. Ltd., Mumbai. Ascorbic acid, used as a standard for antioxidant assays, was obtained from S.D. Fine Chemicals Ltd., Mumbai. Distilled water used throughout the study was freshly prepared in the laboratory. All reagents and chemicals used were of analytical or AR grade, ensuring the reliability and accuracy of phytochemical screening and antioxidant assessments.

Methods

Collection of flowers with stamen of N. nucifera

Flowers with stamen of *N. nucifera* were collected from Minor Forest Produce Processing and Research Centre, Vindhya Herbals, Bhopal. The plant materials were washed thoroughly with normal tap water followed by sterile distilled water, and then dried under shaded conditions at room temperature. Dried plant materials were crushed into powder using a grinding machine. Powder was stored at 4 °C in a tight air container bottle.

Extraction by Soxhlet method (hot continuous extraction)

Soxhlet is having similar assembly as well as it also works on the same principle of condensation as reflux extraction. Extracts were obtained by the continuous hot filtrate extraction method using a Soxhlet apparatus [18]. Freshly collected flowers of *N. nucifera* were dried in shade and moderately coarsely powdered. 40 g of powder was passed through sieve no. 18 and which was placed in the middle chamber of the Soxhlet apparatus, and extracted using solvents such as methanol and distilled water as solvents. The filtrate was concentrated in a rotary vacuum evaporator, dried in a desiccator, and the percentage yield.

Phytochemical screening

The qualitative chemical experiments were carried out with some modifications for different extracts according to the methods mentioned in Mukherjee, in 2019 [10].

Quantitative estimation of bioactive compound

Estimation of total phenol content

The total phenolic content of the extract was determined by the modified Folin–Ciocalteu method [19]. 10 mg Gallic acid was dissolved in 10 mL methanol; various aliquots of 10–50 µg/mL were prepared in methanol. 10 mg of dried extracts were dissolved in 10 mL of methanol and filtered. 2 mL (1 mg/mL) of this solution was used for the estimation of phenol. 2 mL of each extract or standard was mixed with 1 mL of Folin–Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 mL (7.5 g/L) of sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 15 min for color development. The absorbance was measured at 765 nm using a spectrophotometer [20].

Estimation of total flavonoid content

Determination of total flavonoid content was based on aluminum chloride method 10 mg quercetin was dissolved in 10 mL methanol, and various aliquots of 5–25 µg/mL were prepared in methanol. 10 mg of dried extract of were dissolved in 10 mL methanol and filter. 3 mL (1 mg/mL) of this solution was used for the estimation of flavonoid. 1 mL of 2% $AlCl_3$ solution was added to 3 mL of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at λmax 420 nm [21].

In vitro* antioxidant activity of extracts of *N. nucifera

DPPH method

Total free radical scavenging capacity of the methanolic extract from *N. nucifera* was estimated according to the previously reported method with slight modification [22]. Solution of DPPH (6 mg in 100 mL methanol) was prepared and stored in a dark place. Different concentration of standard and test (10–100 µg/mL) was prepared. 1.5 mL of DPPH and 1.5 mL of each standard and test were taken in separate test tube; the absorbance of this solution was taken immediately at 517 nm. 1.5 mL of DPPH and 1.5 mL of methanol was taken as a control absorbance at λmax 517 nm [23].

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Antioxidant assay in wild and mutant yeast strains

Yeast strains and growth conditions

The strain wild-type (WT) BY4743 (MATa/MATa*his3Δ1/his3Δ1leu2Δ0/leu2Δ0met15Δ0*/MET 15LYS2/*lys2Δ0g/ura3Δ0*) was used as WT yeast strain, and *TRX2* (Δ*trx2*) was used in the study. The yeast culture was prepared by allowing a single colony of WT/knockout yeast strain in yeast extract peptone dextrose (YPD) medium (1% [w/v] yeast extract, 2% [w/v] peptone, and 2% [w/v] glucose) overnight to exponential phase (OD_{600}=0.6), in an orbital shaker, at 30°C, and 180 rpm, with a ratio of flask volume/medium volume of 5:1.

Choice of yeast strains and optimization of the stressing agent

Based on the literature survey and the facts from *Saccharomyces* genome database, one H_2O_2 sensitive deletion yeast strain was chosen and tested (trx2) for H_2O_2 sensitivity. The stressing agent, H_2O_2, at various strengths (1 mM, 2 mM, 4 mM, and 6 mM) were tested, and it was found that 4 mM gave the optimum stressing effect on selected strains [24].

Growth curve assay

Different cultures were prepared when both wild strain BY4743 and deletion strain Δtrx2 were seeded into YPD media, at 180 rpm/30°C for the whole night. Adjusted the culture obtained to get an initial seeding density of yeast in a 96-well plate at an OD_{600} of 0.1. Then added 10 µL of *N. nucifera* extract in strengths (0.4, 0.8, 1.6 mg/mL) and ascorbic acid (10 mM) to the yeast culture (100 µL) and incubated at 30°C/24 h in a multi-well plate reader with continuous shaking at medium intensity. OD_{600} was measured every hour [25].

Membrane integrity assay by propidium iodide (PI) staining

The cultures of wild and deletion yeast strains (*WT* and *trx2*) were adjusted to OD_{600}~0.5. All the groups were stressed with H_2O_2 (4 mM), leaving the control group. *N. nucifera* aqueous (NNA) extract (1.6 mg/mL) or ascorbic acid (10 mM) was added to the treatment groups. The tubes were kept in the dark at 30°C for 3 h. Post-incubation, the cells were pelleted (12000 rpm, 30 s), washed, and added in 200 µL of phosphate-buffered saline (PBS) buffer and mixed with 0.2 µL of PI, from a stock (1 mg/mL), to get a strength of 1 µg/mL. After keeping for 5 min in dark, the cells were harvested and washed with PBS. An appropriate amount of PBS (≈100 µL) was used to get a final cell suspension. After keeping in dark for 5 min post-PI-treatment, the cells were harvested, and PBS was added to it. The slides were observed under Apotome Axio Imager (Carl Zeiss, Gottingen, Germany) at ×100 [24].

In vivo nephroprotective activity ofextract of N. nucifera

The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), (Approval letter no.- BU/Pharma/IAEC/A/23/02) constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India. In the present study, Wistar rats 3–4 old weeks (60–80 g) were used. The animals were divided into 6 groups. During 1 week of acclimatization (22±1°C temperature and 50–80% humidity), with 12 h cycle variation between the light and dark, freely, animals consumed a standard diet for rodents and water filtered beforehand. The animals were divided into 6 groups.

Acute toxicity study

The extract of *N. nucifera* (Flower with stamen) was assessed for acute oral toxicity using OECD ANNEX-423 standards. According to prior

toxicity studies, the extract of *N. nucifera* (flower with stamen) was delivered orally to rats (2000 mg/kg body weight).

Experimental design- CP [11]

- Group I (Normal group): Acquired daily vehicle (saline, p.o.)
- Group II (CP group): Provided 4-CP injections (150 mg/kg/day, i.p.) in every 7 days
- Group III (Treated): CP-induced nephrotoxicity rats were treated with the bioactive methanolic extract of *N. nucifera*-100 mg/kg/p.o. per day
- Group IV (Treated group): CP-induced nephrotoxicity rats were treated with bioactive methanolic extract of *N. nucifera*-200 mg/kg/p.o. per day
- Group V (Treated group): CP-induced nephrotoxicity rats were treated with bioactive aqueous extract of *N. nucifera*-100 mg/kg/p.o. per day
- Group VI (Treated group): CP-induced nephrotoxicity rats were treated with the bioactive aqueous extract of *N. Nucifera* - 200 mg/kg/p.o. per day.

Biochemical assessment was carried out by sacrificing the animals on the last day of study.

Biochemical assessment

Kidney tissue homogenate and biological sample preparations

A blood sample using the retro-orbital plexus was collected and centrifuged for 20 min at 1000 rpm to disperse the serum. Last day of the experiment, urine samples were collected from 24-h urine samples. Biochemical analysis was then conducted on both samples. The rodent was sacrificed, and the kidneys were extracted for biochemical assessment using 10% w/v tissue homogenates in 0.1 M Tris-HCl buffer (pH 7.5), after centrifugation at 3000 rpm for 15 min [25].

Estimation of biochemical parameters

After 24 h of the last day, blood samples were collected by retroorbital puncture. The serum was rapidly separated and processed for the determination of serum creatinine, serum urea, serum uric, etc., as an indicator of kidney damage, using commercially available kits from Span Diagnostics Private Ltd. The animals were sacrificed, and the kidney was isolated for histopathological examination.

Histological examination

For histological examination, the longitudinal part of the left kidney will be excised from each animal. Renal tissue specimens will be set for 7 days in 10% neutral formalin buffered. Fixed tissue samples will be embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological examination. Using light microscopy, three sections of each group of animals will be subjected to microscopic study. Changes in renal structure have been classified as mild, moderate, or severe compared to the control group. Kidney sections were observed under a microscope (BX40; Olympus, Tokyo, Japan) for any histological changes [26].

Statistical analysis

GraphPad Prism (version 8.0.2) is used to represent all statistical analyses as mean±standard error the mean. Data were compared with the control and CP control using Tukey's *post hoc* test after one-way analysis of variance and shown statistically significant in between "#p<0.001 versus normal control (Group I)" and "*p<0.05, **p<0.01, ***p<0.001 versus CP Control (Group II)."

RESULTS AND DISCUSSION

It may be assumed that the fundamental role of CP-induced nephrotoxicity is the generation of oxidative stress and inflammation; a loop of damage amplification and a connection between mechanisms of tubular and glomerular changes [25]. Marked elevations of serum creatinine and urea concentration were suggested as an important purposeful destruction of the kidney in CP-induced nephrotoxicity. Serum creatinine concentration is a stronger marker than the urea in the initial pathogenesis of kidney disease. In addition, urea concentrations commence to augment just after parenchymal injury [26]. Numerous studies have reported that oxygen-free radicals are considered to be significant peacekeepers of CP-induced acute renal failure. Consequently, among the major approaches used to improve CP-induced nephrotoxicity is the use of formulations with antioxidant properties [27]. The extractive values of *N. nucifera* were determined, and results showed that the aqueous extract provided a significantly higher yield (13.0% w/w) compared to the methanolic extract (3.6% w/w), indicating a higher content of water-soluble constituents (Table 1).

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, diterpenes, phenols, saponins, and sterols in both extracts, with glycosides, proteins, and carbohydrates being prominent only in the aqueous extract. The methanolic extract showed higher flavonoid content, which may explain its stronger antioxidant activity (Table 2).

Quantitative estimation confirmed that the methanolic extract possessed higher total phenol and flavonoid content (1.50 mg/100 mg and 2.33 mg/100 mg, respectively) compared to the aqueous extract (Table 3).

Table 1: Extractive values of extracts of *Nelumbo nucifera*

S. No.	Extracts	Percentage yield (w/w)	Color of extractive
1	Methanolic	3.6	Solid brown
2	Aqueous	13.0	Solid brown

Table 2: Result of phytochemical screening of extracts of *Nelumbo nucifera*

S. No.	Constituents	Methanolic extract	Aqueous extract
1.	Alkaloids		
	Wagner's test	+ve	+ve
	Hager's test	−ve	+ve
2.	Glycosides		
	Conc. H_2SO_4 Test	−ve	+ve
3.	Flavonoids		
	Lead acetate test	+ve	+ve
	Alkaline reagent test	+ve	−ve
4.	Diterpenes		
	Copper acetate test	+ve	+ve
5.	Phenol		
	Ferric chloride test	−ve	+ve
	Folin–Ciocalteu test	+ve	+ve
6.	Proteins		
	Xanthoproteic test	−ve	+ve
7.	Carbohydrate		
	Fehling's test	−ve	−ve
	Benedict's test	−ve	+ve
8.	Saponins		
	Froth test	−ve	+ve
9.	Tannins		
	Gelatin test	−ve	−ve
10.	Sterols		
	Salkowski's test	+ve	+ve

+Ve: Positive, −Ve: Negative

Table 3: Results of total phenol and flavonoid content of the extract of *Nelumbo nucifera*

S. No.	Extracts	Total phenol content	Total flavonoids content
		mg/100 mg	
1	Methanolic	1.50	2.33
2	Aqueous	1.15	2.12

The antioxidant potential of the extracts was evaluated by the DPPH radical scavenging method. The methanolic extract showed a concentration-dependent increase in free radical scavenging activity with an IC_{50} value of 42.32 µg/mL, which was significantly lower than the aqueous extract (60.50 µg/mL) and closer to that of ascorbic acid (21.87 µg/mL) (Table 4).

Safety evaluation demonstrated no cytotoxicity in WT yeast cells, even at higher concentrations of *N. nucifera* extract, suggesting good biocompatibility (Fig. 1). A decrease in the number of yeast cells was observed (WT) in H_2O_2-treated group relative to the normal, after incubation-24 h in a significant ($p<0.01$) manner. The H_2O_2-induced growth halt was repaired by NNA, which was concentration-dependent in both the wild and *Δtrx-2* strains. Initially, the extract showed a lag phase; after 6 h, it helped the cells to overcome the stress significantly ($p<0.01$), and the recovered cells showed a normal exponential phase as that of the control.

Table 4: % Inhibition of ascorbic acid and extract of *Nelumbo nucifera* by DDPH method

S. No.	Concentration (µg/mL)	Percentage inhibition		
		Ascorbic acid	Methanolic extract	Aqueous extract
1	10	37.627	30.508	15.819
2	20	50.734	37.627	25.311
3	40	64.407	45.198	36.949
4	60	74.237	62.034	51.186
5	80	88.136	75.028	62.034
6	100	92.655	83.616	74.689
IC_{50} value		21.87	42.32	60.50

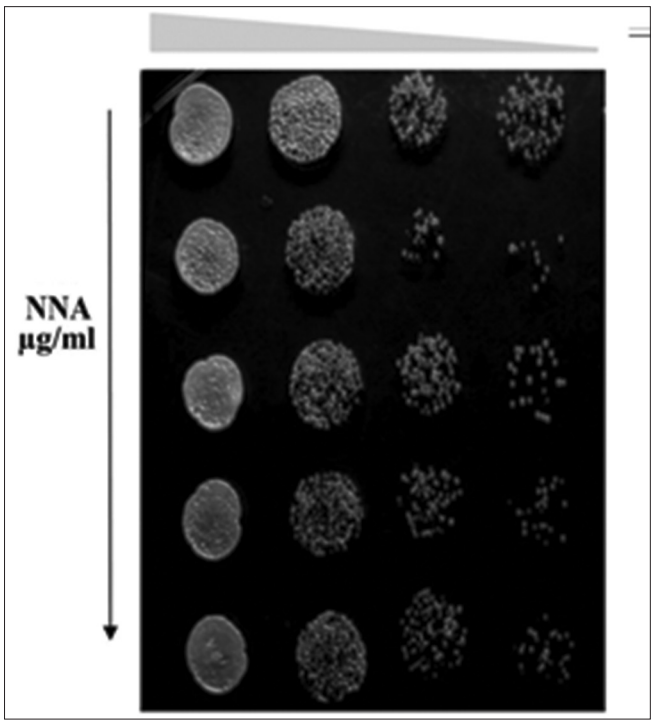


Fig. 1: Cytotoxicity study in wild (BY4743) type of yeast cell treated with different concentrations of *Nelumbo nucifera* aqueous (0.2, 0.4, 0.8, and 1.6 mg) on a normal yeast extract peptone dextrose plate, and the extract showed no toxicity

Ascorbic acid served as the standard, which totally arrested the challenge and encouraged a normal growth curve for the treatment group.

Comparable to the standard (ascorbic acid), NNA (1.6 mg/mL)-treated culture was significantly ($p<0.01$) able to overcome the growth inhibitory effect of the H_2O_2 after 24 h incubation in WT yeast cells, while NNA failed to revert the H_2O_2-induced growth arrest in *Δtrx-2* strains.

Growth curve studies revealed a dose-dependent but non-lethal effect on yeast growth kinetics in both WT and Δtrx2 strains (Fig. 2). From the previous investigations on yeast, we are not able to conclude about the viability of the cells; we confirmed it by another method, staining with PI, which is the nucleic acid-binding fluorescent probe. The moment membrane integrity is lost, the cell internalizes PI, and it reacts with double-stranded nucleic acid by intercalating between the bases, showing red fluorescence. Our findings show that incubating with H_2O_2 allowed PI infiltration about 39.25±1.00% in case of WT cells and 68.4±2.9% in case of Δ*trx2*, yeast cells. The ascorbic acid-treated group resulted in approximately 5 times reduction in PI-stained WT cells and *Δtrx2 cells* in comparison to the negative control. At the same time, the adaptive treatment with NNA resulted in a reduction in PI-stained WT cells and *Δtrx2* strain by approximately 2.5 and 2 times, respectively, as compared to the negative control. The results indicate that NNA and ascorbic acid bring about adaptive changes in cells that could counteract ROS, and can keep the cell membrane intact against H_2O_2 challenge. The cell membrane protective effect of NNA is comparable to that of the standard.

Plasma membrane integrity assays further confirmed the protective effect of the extract by showing reduced PI uptake in treated cells compared to CP control (Fig. 3). The study demonstrates that NNA has potential antioxidant activity by decreasing the growth inhibition of *WT* and *trx2* yeast strains against H_2O_2 challenge. The potent radical scavenging capacity of NNA (DPPH study) depicts effective antioxidative property *in vitro* which was identical to the results of nephroprotection against chemotherapeutic agent, i.e., CP. These results are in accordance to quantity of total phenols and flavonoids in the aqueous extract of *N. nucifera*. Moreover, *in vivo* research utilizing the budding yeast cells, both in WT and a Δ*trx2* strains established for cytoprotection against oxidative stress. Likewise, the usage of yeast in such types of study gives us a different alternative to lessen the load on animals in experimentation. The vital role played by the *Trx* gene as redox regulator makes the *TRX*2 gene knock out an invaluable model for screening cellular antioxidant activity of the extract.

Currently, a lot of importance is given to investigations elucidating mechanisms responsible for pathogenesis owing to ROS stress. To identify cell viability post-exposure to oxidants, yeast was propagated in YPD media.

The growth curve assay helped in calculating the cell viability (% of living cells in a population). NNA was able to decrease membrane damage, as suggested by the significant decrease in the number of PI-stained cells.

The NNA was able to reduce mitochondrial ROS, a biomarker of oxidative stress; these findings are proof of linking NNA with the conditional mutant Δ*trx2* linked to specific cellular pathways.

CP-induced nephrotoxicity was evident by elevated serum creatinine and urea levels in the CP-treated group, which were significantly reduced on treatment with both extracts, with the methanolic extract (MNN) (200 mg/kg) producing the most prominent effect (Table 5).

Similarly, treatment with the extracts significantly reduced serum uric acid and restored total protein levels (Table 6), improved globulin concentration and reduced aspartate aminotransferase levels (Table 7), normalized alanine aminotransferase levels and enhanced glutathione concentration (Table 8), increased catalase activity and reduced malondialdehyde levels (Table 9), and significantly lowered pro-inflammatory cytokines tumor necrosis factor-alpha and interleukin-1 beta (Table 10).

Histopathological results

Histopathological results demonstrating structural changes in renal tissue. Histopathological view of renal sections in CP treated group showed the degeneration, desquamation and necrosis in tubules and swelling in glomerulus, as compared to control group. Glomerular and tubula repithelial changes were considerably mild in the groups treated with CP+ aqueous extract of NN at dose of 100 mg/kg and CP+ aqueous extract of NN 200 mg/kg showed karyopicnosis and mild tubular

Table 5: Effect of extract of *Nelumbo nucifera* on serum creatinine and serum urea induced by cyclophosphamide

Group	Drug and dose	Serum creatinine (mg/dL)		Serum urea (mg/dL)	
		Mean	SEM	Mean	SEM
Group I	Normal Control (saline)	0.76	0.04	28.4	1.3
Group II	CP	2.88	0.12#	71.8	2.7#
Group III	CP+bioactive Me-OH extract of *Nelumbo nucifera* (100)	1.24	0.05**	46.1	1.6**
Group IV	CP+bioactive Me-OH extract of *Nelumbo nucifera* (200)	0.91	0.06***	35.7	1.9***
Group V	CP+bioactive Aqueous extract of *Nelumbo nucifera* (100)	1.2	0.07*	48.6	2.1*
Group VI	CP+bioactive Aqueous extract of *Nelumbo nucifera* (200)	0.95	0.06**	36.3	1.8**

SEM: Standard error of the mean, ANOVA: Analysis of variance, CP: cyclophosphamide. Values are expressed in mean±SEM (n=6). A one-way ANOVA followed by Tukey's *post hoc* test, #p<0.001 versus normal, *p<0.05, **p<0.01, ***p<0.001 vs. CP

Table 6: Effect of extract of *Nelumbo nucifera* on uric acid and protein induced by cyclophosphamide

Group	Drug and dose	Uric acid (mg/dL)		Protein (g/dL)	
		Mean	SEM	Mean	SEM
Group I	Normal control (saline)	2.8	0.2	6.9	0.2
Group II	CP	6.5	0.3#	4.2	0.2#
Group III	CP+bioactive Me-OH extract of *Nelumbo nucifera* (100)	4.5	0.2**	5.7	0.2**
Group IV	CP+bioactive Me-OH extract of *Nelumbo nucifera* (200)	3.6	0.3***	6.1	0.2***
Group V	CP+bioactive Aqueous extract of *Nelumbo nucifera* (100)	4.7	0.3*	5.9	0.2*
Group VI	CP+bioactive Aqueous extract of *Nelumbo nucifera* (200)	3.9	0.2**	5.5	0.2**

SEM: Standard error of the mean, ANOVA: Analysis of variance, CP: cyclophosphamide. Values are expressed in mean±SEM (n=6). A one-way ANOVA followed by Tukey's *post hoc* test, #p<0.001 verasu normal, *p<0.05, **p<0.01, ***p<0.001 versus CP

Table 7: Effect of extract of *Nelumbo nucifera* on globulin and AST levels induced by cyclophosphamide

Group	Drug and dose	Globulin (g/dL)		AST levels (IU/L)	
		Mean	SEM	Mean	SEM
Group I	Normal control (saline)	2.9	0.1	74.3	3.1
Group II	CP	1.6	0.1#	182.5	5.4#
Group III	CP+bioactive Me-OH extract of *Nelumbo nucifera* (100)	2.5	0.1**	108.4	4.1**
Group IV	CP+bioactive Me-OH extract of *Nelumbo nucifera* (200)	2.6	0.1***	90.5	3.5***
Group V	CP+bioactive Aqueous extract of *Nelumbo nucifera* (100)	2.3	0.1*	109.6	4.3*
Group VI	CP+bioactive Aqueous extract of *Nelumbo nucifera* (200)	2.5	0.1**	97.8	3.8**

SEM: Standard error of the mean, ANOVA: Analysis of variance, CP: cyclophosphamide, AST: Aspartate aminotransferase. Values are expressed in mean±SEM (n=6). A one-way ANOVA followed by Tukey's *post hoc* test, #p<0.001 versus Normal, *p<0.05, **p<0.01, ***p<0.001 versus CP

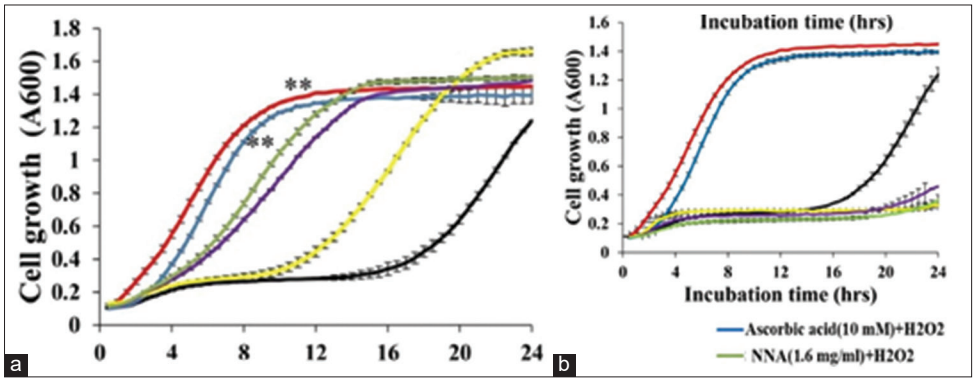


Fig. 2: (a) Growth curve for wild-type yeast strains treated with *Nelumbo nucifera* aqueous (NNA) (0.4 mg/mL, 0.8 mg/mL, 1.6 mg/mL) (b) Growth curve for Δtrx2 yeast strains treated with NNA

Table 8: Effect of extract of *Nelumbo nucifera* on ALT levels induced by cyclophosphamide

Group	Drug and dose	ALT levels (IU/L)		GSH levels (µmol/mg)	
		Mean	SEM	Mean	SEM
Group I	Normal control (saline)	48.6	2.1	7.45	0.22
Group II	CP	154.8	4.9#	2.38	0.15#
Group III	CP+bioactive Me-OH extract of *Nelumbo nucifera* (100)	92.4	3.7**	5.23	0.18**
Group IV	CP+bioactive Me-OH extract of *Nelumbo nucifera* (200)	72.1	3.1***	6.12	0.21***
Group V	CP+bioactive aqueous extract of *Nelumbo nucifera* (100)	101.3	3.9*	4.78	0.18*
Group VI	CP+bioactive Aqueous extract of *Nelumbo nucifera* (200)	78.6	3.3**	5.91	0.2**

SEM: Standard error of the mean, ANOVA: Analysis of variance, CP: cyclophosphamide, ALT: Alanine aminotransferase, GST: Glutathione S-transferase. Values are expressed in mean±SEM (n=6). A one-way ANOVA followed by Tukey's *post hoc* test, #p<0.001 versus Normal, *p<0.05, **p<0.01, ***p<0.001 versus CP

Table 9: Effect of extract of *Nelumbo nucifera* on CAT and MDA levels induced by cyclophosphamide

Group	Drug and dose	CAT levels (U/mg)		MDA levels (nmol/mg)	
		Mean	SEM	Mean	SEM
Group I	Normal control (saline)	45.6	1.8	1.8	0.12
Group II	CP	18.4	1.2#	5.6	0.25#
Group III	CP+bioactive Me-OH extract of *Nelumbo nucifera* (100)	31.7	1.3**	3.5	0.19**
Group IV	CP+bioactive Me-OH extract of *Nelumbo nucifera* (200)	39.2	1.6***	2.8	0.14***
Group V	CP+bioactive Aqueous extract of *Nelumbo nucifera* (100)	31.9	1.3*	3.9	0.2*
Group VI	CP+bioactive aqueous extract of *Nelumbo nucifera* (200)	37.8	1.5**	3	0.15**

SEM: Standard error of the mean, ANOVA: Analysis of variance, CP: cyclophosphamide, CAT: Catalase, MDA: Malondialdehyde. values are expressed in mean±SEM (n=6). A one-way ANOVA followed by Tukey's *post hoc* test, #p<0.001 versus Normal, *p<0.05, **p<0.01, ***p<0.001 versus CP

Table 10: Effect of extract of *Nelumbo nucifera* on TNF-α levels induced by cyclophosphamide

Group	Drug and dose	TNF-α levels (pg/mL)		IL-1β levels (pg/mL)	
		Mean	SEM	Mean	SEM
Group I	Normal control (saline)	15.2	1.1	12.8	1
Group II	CP	78.5	3.8#	65.7	3.5#
Group III	CP+bioactive Me-OH extract of *Nelumbo nucifera* (100)	46.1	2.3**	40.7	2**
Group IV	CP+bioactive Me-OH extract of *Nelumbo nucifera* (200)	33.8	1.8***	30.6	1.6***
Group V	CP+bioactive Aqueous extract of *Nelumbo nucifera* (100)	50.9	2.5*	43.9	2.2*
Group VI	CP+bioactive aqueous extract of *Nelumbo nucifera* (200)	36.7	1.9**	31.7	1.7**

SEM: Standard error of the mean, ANOVA: Analysis of variance, CP: cyclophosphamide, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1 beta. Values are expressed in mean±SEM (n=6). A one-way ANOVA followed by Tukey's *post hoc* test, #p<0.001 versus control, *p<0.05, **p<0.01, ***p<0.001 versus cyclophosphamide

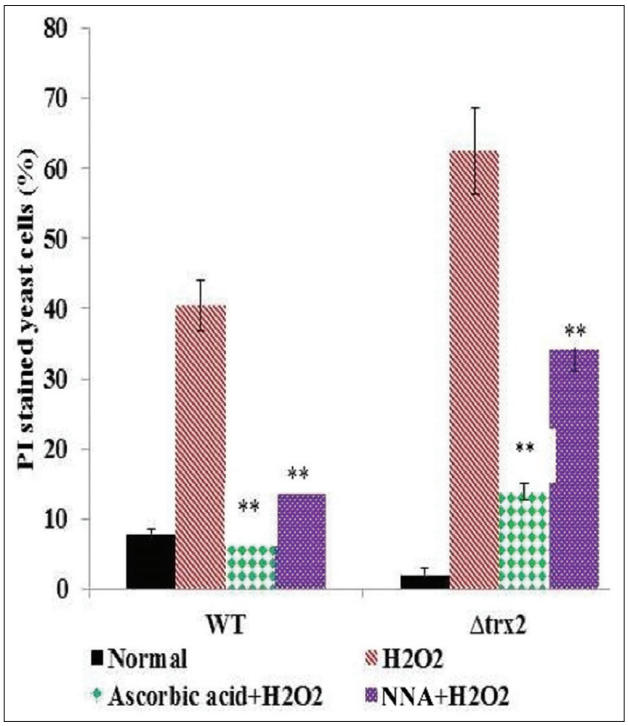
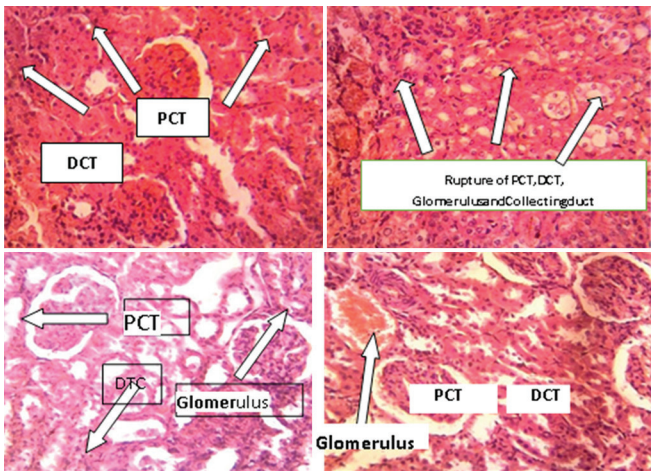


Fig. 3: (a) Plasma membrane integrity determination in yeast wild-type (A1-D1) and Δ*trx2* (A2-D2) cells stained with Propidium iodide

epithelial changes while, the same tubula repithelial changes were considerably mild in the group streated with CP+ methanolic extract of NN at dose of 100 mg/kg and CP+ methanolic extract of NN at dose of 200 mg/kg showed regeneration in tubula repithelial cells.



Group 1. Normal saline treated group showed clear structure of proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and glomerulus of nephron; Group 2. Cyclophosphamide (CP) treated group shown rupture of nephrons; Group 4. CP+ aqueous extract of NN 200 mg/kg showed recovered clear structure of PCT, DCT, collecting duct and glomerulus of nephron. Group 6: CP methanolic extract of NN 200

mg/kg showed recovered clear structure of PCT, DCT, collecting duct and glomerulus of nephron.

CONCLUSION

The study effectively highlights how natural antioxidants can bridge the gap between necessary medical treatments, like chemotherapy, and the preservation of organ health. The study identifies three primary pathways through which the extracts counteract the damage caused by CP, (1) Antioxidant Defense: The phenolic and flavonoid compounds act as "scavengers," neutralizing ROS before they can damage cellular structures. (2) Anti-inflammatory response: The extracts likely inhibit the signaling pathways that lead to tissue swelling and cellular distress in the kidneys. (3) Membrane stabilization: By protecting the lipid bilayer of cells, the extracts prevent the leakage of renal function markers and maintain cellular integrity against CP-induced "insults." The findings support the traditional use of *N. nucifera* and highlight its potential as a nephroprotective therapeutic candidate.

The present study successfully demonstrated the phytochemical richness, antioxidant capacity, and protective potential of methanolic and NNA against CP-induced systemic toxicity. The *N. nucifera* methanolic extract (MNN), having higher phenolic and flavonoid content, exhibited superior free-radical scavenging activity and significant protection of renal function markers and supported by histopathological findings as well as oxidative stress biomarkers.

The results suggest that both kinds of *N. nucifera extracts* exert its protective effects through antioxidant, anti-inflammatory and membrane-stabilizing mechanisms, thereby mitigating CP-induced nephrotoxicity. These findings support the traditional use of *N. nucifera* and highlight its potential as a natural therapeutic candidate for managing oxidative stress-related disorders and chemotherapy-induced organ damage.

AUTHOR'S CONTRIBUTION

Sulakshana Pawar is worked on her Ph.D research work topic. Raghuveer Irchhiaya is contribute to design the research concept and mentoring on research project.

CONFLICTS OF INTEREST

None.

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