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Research Article

BIOCHEMICAL AND OXIDATIVE STRESS MODULATION BY *SWERTIA CHIRAYITA* EXTRACTS IN CYCLOPHOSPHAMIDE-INDUCED NEPHROTOXITY IN RATS

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ABSTRACT

Objective: The present study investigates the phytochemical profile, antioxidant potential, and protective effects of *Swertia chirayita* extracts against cyclophosphamide (CP)-induced biochemical and oxidative stress alterations.

Methods: Methanolic and aqueous extracts were prepared and screened for phytoconstituents, extractive values, and total phenolic and flavonoid content. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, where the methanolic extract exhibited superior free radical inhibition with a lower IC_{50} value compared to the aqueous extract. Cytotoxicity assessment in yeast models revealed no toxic effects, confirming extract safety. *In vivo* studies in CP-treated rats demonstrated significant improvement in biochemical parameters, including serum creatinine (Cr), urea, uric acid, protein, globulin, alanine aminotransferase, aspartate aminotransferase, glutathione, catalase, malondialdehyde, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β levels.

Results: Both extracts, particularly the methanolic extract at higher doses, showed significant ($p<0.05$–$p<0.001$) marked amelioration of biochemical parameters, suggesting a strong protective role. Moreover, *S. chirayita* demonstrated anti-inflammatory potential by markedly decreasing TNF-α and IL-1β levels compared to the CP group. Growth curve analysis demonstrated a protective effect of *S. chirayita* extract on oxidative stress–sensitive Δtrx2 yeast strains, indicating its antioxidant and stress-protective potential.

Conclusion: These findings highlight *S. chirayita* as a promising source of bioactive compounds with antioxidant and cytoprotective potential against CP-induced toxicity.

Keywords: *Swertia chirayita*, Phytochemical screening, Antioxidant activity, Cyclophosphamide, Oxidative stress, Biochemical parameters, Nephroprotection.

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INTRODUCTION

Cyclophosphamide (CP) is a widely used alkylating chemotherapeutic agent employed in the treatment of various malignancies and autoimmune disorders [1]. Despite its clinical efficacy, its therapeutic applications are often limited due to severe adverse effects, particularly nephrotoxicity and hematological toxicity, largely mediated through oxidative stress and free radical generation [2,3].

CP undergoes hepatic metabolism to form active metabolites, including acrolein, which induce lipid peroxidation, DNA damage, and depletion of endogenous antioxidants such as glutathione (GSH), catalase (CAT), and superoxide dismutase [4,5]. Therefore, there is growing interest in identifying natural bioactive compounds with antioxidant and protective effects that may mitigate CP-induced toxicity.

Medicinal plants rich in polyphenols, flavonoids, and alkaloids are being extensively studied for their ability to counter oxidative stress-related damage [6]. *Swertia chirayita* (Gentianaceae), commonly known as "chirata," is a traditional medicinal herb widely used in Ayurveda, Siddha, and Unani systems of medicine [7,8]. It is reported to possess diverse pharmacological properties, including antioxidant, anti-inflammatory, antidiabetic, and anticancer activities [9-12].

Phytochemical analyses have revealed that *S. chirayita* is a rich source of secoiridoid glycosides (swertiamarin, amarogentin, and mangiferin), xanthones, flavonoids, and alkaloids, which contribute to its therapeutic efficacy [13].

Recent studies have highlighted the antioxidant capacity of *S. chirayita* extracts in both *in vitro* and *in vivo* systems, demonstrating significant free radical scavenging activity and the ability to modulate oxidative stress markers [14]. However, limited research has focused on its role in attenuating drug-induced toxicity, particularly CP-mediated organ damage. Given its phytoconstituent profile, *S. chirayita* has the potential to reduce CP-induced biochemical alterations and oxidative stress by restoring antioxidant enzyme levels and reducing lipid peroxidation.

The present investigation aims to evaluate the biochemical and oxidative stress modulation by methanolic and aqueous extracts of *S. chirayita* in a CP-induced toxicity model. The study includes phytochemical screening, antioxidant potential assessment, and evaluation of serum biochemical, hepatic, renal, and oxidative stress parameters to explore the protective role of *S. chirayita* against CP toxicity.

MATERIAL AND METHODS

Material

The whole plant of *S. chirayita* was obtained from a certified herbal supplier and authenticated by a botanist. Methanol (analytical grade) and other solvents were procured from Merck, India, whereas phytochemical screening reagents such as Wagner's, Hager's, Salkowski's, ferric chloride, and Folin-Ciocalteu reagent were purchased from HiMedia Laboratories. CP was obtained as a gift sample and used to induce toxicity. Standard ascorbic acid served as the reference antioxidant. Male Wistar albino rats (150–200 g) were used for the study and maintained under standard laboratory conditions with free access to food and water. All experimental procedures were approved

by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines.

Methods

Collection of S. chirayita

Aerial parts of *S. chirayita* 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. 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 soxhlation method (hot continuous extraction)

Soxhlet has a similar assembly, and it also works on the same principle of condensation as reflux extraction. Extracts were obtained by the continuous hot filtrate extraction method using soxhlet apparatus [15]. Aerial parts of *S. chirayita* were dried in shade and moderately coarsely powdered. 100 g of powder was passed through sieve no. 18, 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 was determined.

Quantitative estimation of bioactive compound

Estimation of total phenol content

The total phenolic content of the extract was determined by the modified folin-ciocalteumethod [16]. 10 mg gallic acid was dissolved in 10 mL methanol, and 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.

Estimation of total flavonoid content

Determination of total flavonoid content was based on the aluminum chloride method [17]. 10 mg quercetin was dissolved in 10 mL methanol, and various aliquots of 5–25 µg/mL were prepared in methanol. A 10 mg of dried extract was dissolved in 10 mL of methanol and filtered. 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 [17].

In vitro* antioxidant activity of extracts of *S. chirayita

2,2-diphenyl-1-picrylhydrazyl (DPPH) method

The total free radical scavenging capacity of the extract from *S. chirayita* was estimated according to the previously reported method with slight modification. Solution of DPPH (6 mg in 100 mL methanol) was prepared and stored in a dark place. Different concentrations of standard and test (10–100 µg/mL) were prepared. 1.5 mL of DPPH and 1.5 mL of each standard and test were taken in separate test tubes; the absorbance of this solution was taken immediately at 517 nm. 1.5 mL of DPPH and 1.5 mL of methanol were taken as a control absorbance at λmax 517 nm [18].

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 [19].

Choice of yeast strains and optimization of stressing agent

Based on the literature survey and the facts from the 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) was tested, and it was found that 4 mM gave the optimum stressing effect on selected strains [20].

Growth curve assay

Different cultures were prepared when both the wild strain BY4743 and the deletion strain Δ*trx2* 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, 10 µl of either extract in strengths (0.4, 0.8, 1.6 mg/mL) and ascorbic acid (10 mM) were added 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 [20].

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. *S. chirayita* aqueous (SCA) (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 (12 000 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 the 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 the dark for 5 min post PI-treatment, the cells were harvested, and PBS was added to it. The slides were observed under an apotome axio imager (Carl Zeiss, Gottingen, Germany) at 100 X.

In-vivo* nephroprotective activity of extracts of *S. chirayita

Animals

The animal studies were approved by the 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 (150–200 g) were used. During 1 week of acclimatization (22±1°C temperature and 50–80% humidity), with a 12 h cycle variation between the light and dark, freely, animals consumed a standard diet for rodents and water filtered beforehand [21].

Acute toxicity study

The extracts of *S. chirayita* were assessed for acute oral toxicity using OECD ANNEX-423 standards. According to prior toxicity studies, the extract of *S. chirayita* was delivered orally to rats (2000 mg/kg body weight) [22].

Experimental design

Experimental-CP

- Group I (Normal group): Acquired daily vehicle (saline, p.o.).
- Group II (CP group): Administered 4-CP injections (150 mg/kg/day, i.p.) every 7 days.
- Group III (Standard group): Administered4-N-acetylcysteine, 150 mg/kg, p.o./day+CP injections (150 mg/kg/day, i.p.) every 7 days.
- Group IV (Test group): CP-induced nephrotoxicity rats treated with bioactive methanolic extract of *S. chirayita* – 100 mg/kg/p.o./day.

- Group V (Test group): CP-induced nephrotoxicity rats treated with bioactive methanolic extract of *S. chirayita* – 200 mg/kg/p.o./day.
- Group VI (Test group): CP-induced nephrotoxicity rats treated with bioactive Aqueous extract of *S. chirayita* – 100 mg/kg/p.o./day.
- Group VII (Test group): CP-induced nephrotoxicity rats treated with bioactive Aqueous extract of *S. chirayita*-200 mg/kg/p.o./day.
- A 10-day experimental study was conducted. CP (150 mg/kg, i.p.) was administered on Day 4. *S. chirayita* extracts were administered orally once daily from Day 1 to Day 10.

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 in direction to disperse the serum. On the 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 [23].

Estimation of biochemical parameters

After 24 h of the last day, blood samples were collected by retro-orbital puncture. The serum was rapidly separated and processed for the determination of serum Cr, serum urea, serum uric acid, etc., as an indicator of kidney damage, using commercially available kits from Span Diagnostics Private Ltd. [23].

Statistical analysis

Graph Pad Prism (version 8.0.2) is used to represent all statistical analyses as mean±standard error of the mean. Data were compared with the control using Tukey's *post hoc* test after one-way analysis of variance, where $p<0.05$ was deemed statistically significant. *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ indicated a significance.

RESULTS AND DISCUSSION

The preliminary phytochemical evaluation of *S. chirayita* extracts revealed the presence of several bioactive compounds that may contribute to its pharmacological activity. Extractive values indicated a higher yield with the aqueous extract (3.0%) compared to the methanolic extract (1.7%), both showing characteristic green coloration (Table 1). Phytochemical screening demonstrated that alkaloids, flavonoids, diterpenes, phenols, proteins, saponins, and sterols were variably present in both extracts, with the methanolic extract showing a broader phytoconstituent profile (Table 2). The quantitative estimation further supported this observation, where the methanolic extract contained higher levels of total phenols (1.22 mg/100 mg) and flavonoids (1.84 mg/100 mg) than the aqueous extract (Table 3).

The antioxidant activity determined by DPPH assay revealed concentration-dependent inhibition of free radicals, with the methanolic extract exhibiting stronger activity (IC50=54.65 µg/mL) compared to the aqueous extract (IC50=81.17 µg/mL), although both were less potent than ascorbic acid (IC50=21.87 µg/mL) (Table 4). Importantly, cytotoxicity studies in yeast cells showed no toxicity of the extract at tested concentrations, confirming its safety profile (Fig. 1). Growth curve analysis demonstrated a protective effect of *S. chirayita* extract on oxidative stress–sensitive Δtrx2 yeast strains, indicating its antioxidant and stress-protective potential (Fig. 2).

In vivo studies using a CP-induced toxicity model highlighted the protective efficacy of *S. chirayita* extracts. Administration of bioactive methanolic and aqueous extracts significantly ameliorated altered biochemical parameters. The methanolic extract, particularly at 200 mg/kg, was more effective in reducing serum Cr and urea levels (Table 5), as well as uric acid (UA) and protein imbalance (Table 6). Nephroprotective activity was evident through the restoration of globulin and a marked reduction in AST and ALT levels (Tables 7 and 8). Antioxidant defense markers such as CAT and GSH were significantly restored, while malondialdehyde (MDA), a lipid peroxidation marker, was reduced in treated groups (Tables 8 and 9).

Moreover, *S. chirayita* demonstrated anti-inflammatory potential by markedly decreasing tumor necrosis factor (TNF)-α and interleukin (IL)-1β levels compared to the CP group (Table 10). The methanolic extract at 200 mg/kg showed the most pronounced effect, nearly restoring values close to the normal control.

Table 1: Extractive values of extracts of *Swertia chirayita*

S. No.	Extracts	Percentage of yield (w/w)	Color of extractive
1	Methanolic	1.7	Sticky green
2	Aqueous	3.0	Solid green

Table 2: Result of phytochemical screening of extracts of *Swertia chirayita*

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 *Swertia chirayita*

S. No.	Extracts	Total phenol content	Total flavonoids content
		mg/100 mg	
1	Methanolic	1.22	1.84
2	Aqueous	0.91	1.56

Table 4: Percentage of Inhibition of ascorbic acid and the extract of *Swertia chirayita*

S. No.	Concentration (µg/mL)	Percentage of inhibition		
		Ascorbic acid	Methanolic extract	Aqueous extract
1	10	37.627	19.548	9.831
2	20	50.734	27.458	19.548
3	40	64.407	41.808	24.520
4	60	74.237	54.124	37.062
5	80	88.136	67.797	45.198
6	100	92.655	77.627	64.746
IC_{50} value		21.87	54.65	81.17

These findings establish that the methanolic extract of *S. chirayita* possesses superior phytochemical richness, antioxidant activity, nephroprotective, and anti-inflammatory effects compared to the aqueous extract. The observed protective role is likely attributed to its higher phenolic and flavonoid content, supporting its traditional use as a medicinal herb for oxidative stress and inflammation-associated disorders.

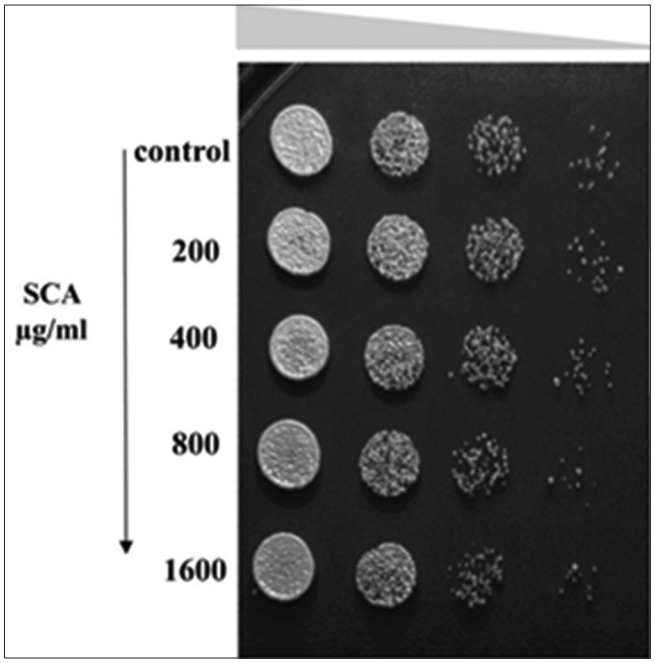

Fig. 1: Cytotoxicity study in wild (BY4743) type of yeast cell treated with different concentrations of *Swertia chirayita* aqueous (0.2, 0.4, 0.8, and 1.6 mg) on a normal yeast extract-peptone-dextrose plate, and the extract showed no toxicity

Toxicological study of SCA extract

Toxicological test of the extracts at tested concentrations proved to be safe to yeast in all the experimental groups.

Growth curve assay

A decrease in the number of yeast cells was observed (WT) in the H_2O_2-treated group relative to the normal, after incubation for 24 h in a significant ($p<0.01$) manner. The H_2O_2-induced growth halt was repaired by SCA, 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. 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), SCA (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 of incubation in WT yeast cells, whereas SCA failed to revert the H_2O_2-induced growth arrest in *Δtrx-2* strains.

PI staining method

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 of about 39.25±1.00% in the case of WT cells and 68.4±2.9% in the 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 SCA 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 SCA and ascorbic acid bring about adaptive changes in cells, which

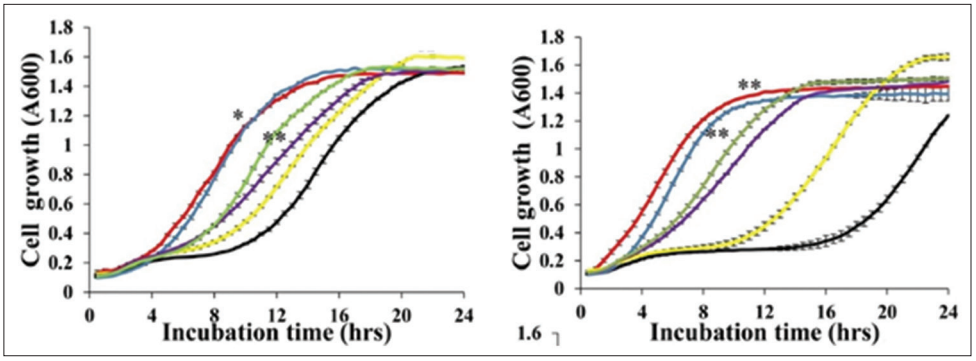

Fig. 2: (a) Growth curve for wild-type yeast strains treated with *Swertia chirayita* aqueous (0.4 mg/mL, 0.8 mg/mL, 1.6 mg/mL). (b) Growth curve for Δtrx2 yeast strains treated by SCA (0.4 mg/mL, 0.8 mg/mL, 1.6 mg/mL)

Table 5: Effect of extract of *Swertia chirayita* on serum creatinine and serum urea induced by CP

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	Standard group	0.80	0.04**	30.1	1.4**
Group IV	CP+bioactive Me-OH extract of *S. chirayita* (100)	1.15	0.06**	42.6	1.8**
Group V	CP+bioactive Me-OH extract of *S. chirayita* (200)	0.89	0.05***	36.9	1.6***
Group VI	CP+bioactive aqueous extract of *S. chirayita* (100)	1.37	0.07*	49.2	2*
Group VII	CP+bioactive aqueous extract of *S. chirayita* (200)	0.98	0.04**	33.8	1.5**

CP: Cyclophosphamide, SEM: Standard error of the mean. Values are expressed in mean±S.E.M (n=6). A one-way analysis of variance followed by Tukey's ***post hoc*** test, #$p<0.001$ versus Normal, *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus CP

Table 6: Effect of extract of *Swertia chirayita* on uric acid and protein induced by CP

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	Standard group	3.1	0.2**	5.9	0.2**
Group IV	CP+bioactive Me-OH extract of *S. chirayita* (100)	4.2	0.2**	6.3	0.2**
Group V	CP+bioactive Me-OH extract of *S. chirayita* (200)	3.5	0.2***	5.8	0.2***
Group VI	CP+bioactive Aqueous extract of *S. chirayita* (100)	4.8	0.3*	6.4	0.2*
Group VII	CP+bioactive aqueous extract of *S. chirayita* (200)	3.3	0.2**	5.6	0.2**

CP: Cyclophosphamide, SEM: Standard error of the mean. Values are expressed in mean±S.E.M (n=6). A one-way analysis of variance followed by Tukey's *post hoc* test, #p<0.001 versus Normal, *p<0.05, **p<0.01, ***p<0.001 versus CP

Table 7: Effect of extract of Swertia *chirayita* on globulin and AST levels induced by CP

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	Standard Group	3	01**	88.2**	3.2**
Group IV	CP+bioactive Me-OH extract of *S. chirayita* (100)	2.4	0.1**	110.2	4.2*
Group V	CP+bioactive Me-OH extract of *S. chirayita* (200)	2.7	0.1***	91.7	3.6***
Group VI	CP+bioactive aqueous extract of *S. chirayita* (100)	2.3	0.1*	120.6	4.4*
Group VII	CP+bioactive aqueous extract of *S. chirayita* (200)	2.8	0.1**	95.3	3.7**

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

Table 8: Effect of extract of Swertia *chirayita* on ALT levels induced by CP

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	Standard group	59.7	2.2**	4	0.2**
Group IV	CP+bioactive Me-OH extract of *S. chirayita* (100)	95.2	3.8*	5.12	0.2**
Group V	CP+bioactive Me-OH extract of *S. chirayita* (200)	74.6	3.2**	6.03	0.21***
Group VI	CP+bioactive aqueous extract of *S. chirayita* (100)	106.9	4*	4.85	0.19*
Group VII	CP+bioactive Aqueous extract of *S. chirayita* (200)	80.7	3.4**	5.94	0.2*

CP: Cyclophosphamide, SEM: Standard error of the mean, ALT: Alanine aminotransferase, GSH: Glutathione. Values are expressed in mean±S.E.M (n=6). A one-way analysis of variance 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 *Swertia chirayita* on CAT and MDA levels induced by CP

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	Standard group	40.2	1.4**	2	0.14**
Group IV	CP+bioactive Me-OH extract of *S. chirayita* (100)	32.1	1.5**	3.4	0.18**
Group V	CP+bioactive Me-OH extract of *S. chirayita* (200)	38.9	1.6***	2.9	0.15***
Group VI	CP+bioactive aqueous extract of *S. chirayita* (100)	30.5	1.4*	3.7	0.17*
Group VII	CP+bioactive aqueous extract of *S. chirayita* (200)	36.5	1.5**	3.1	0.16**

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

could counteract against the reactive oxygen species, and can keep the cell membrane intact against H_2O_2 challenge. The cell membrane protective effect of SCA is comparable to that of the standard (Fig. 3).

CP can interfere with the antioxidant defense system and produce highly reactive oxygen-free radicals [24,25], which can be responsible for the hepatic oxidative damage (indicated by the high level of MDA) and AST disturbance in this study. Our finding indicates that treatment with CP decreases CAT and GSH (non-enzymatic) levels while increasing MDA levels in the tissues of the kidney in comparison to the negative control group levels. Both extracts at doses of 100 mg/kg and 200 mg/kg boost the antioxidant capacity in the damaged kidney, which could be explained by the nephroprotective mechanisms of naringenin on CP-induced renal damage. These findings are in agreement with a previous report [26]. Hence, a multifactorial approach exists for the management of central pontine myelinolysis-induced nephrotoxicity. In line with this, it was found that natural products have been explicitly investigated for their role in various disease conditions, including as an adjuvant with

Table 10: Effect of extract of *Swertia chirayita* on TNF-α levels induced by CP

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	Standard group	32.2	1**	28.6	2**
Group III	CP+bioactive Me-OH extract of *S. chirayita* (100)	48.7	2.4**	42.6	2.1**
Group IV	CP+bioactive Me-OH extract of *S. chirayita* (200)	35.9	1.9***	30.9	1.8***
Group V	CP+bioactive aqueous extract of *S. chirayita* (100)	52.3	2.7*	45.8	2.3*
Group VI	CP+bioactive aqueous extract of *S. chirayita* (200)	38.4	2**	33.5	1.9**

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

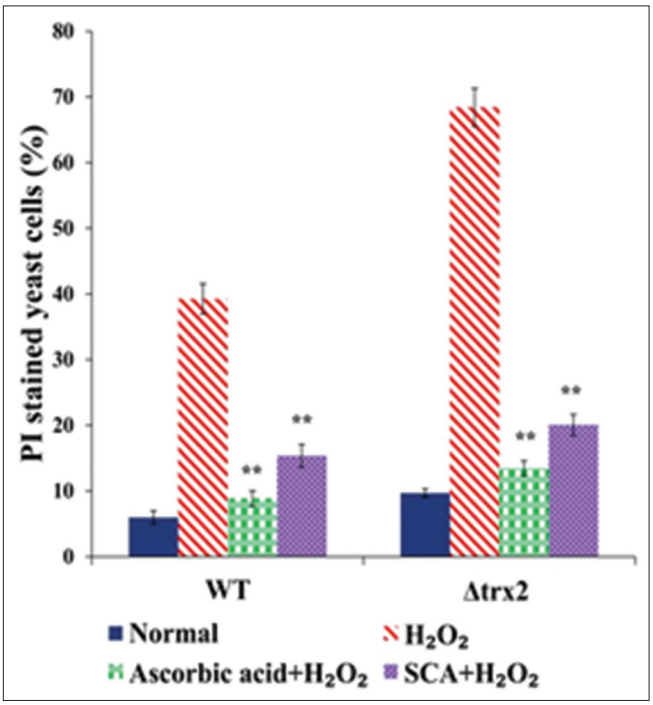

Fig. 3: Percentage of yeast strains (wild-type and Δ*trx2*), stained with propidium iodide**

chemotherapeutic agents [27]. The natural products possess significant antioxidant, anti-inflammatory, and nephroprotective properties. In addition, most of the natural products are considered safe to use, as these are used in traditional systems of medicine.

It also became necessary to highlight that being important mediators and mechanisms, such as oxidative stress and inflammation, these are not renal-specific markers. Henceforth, it became permissive to estimate and evaluate the level of renal-specific injury markers such as serum Cr, UA, blood urea nitrogen (BUN), and various other electrolytes [28,29]. UA, in normal physiology, undergoes renal excretion, and when its serum level increases beyond a certain level, it signifies compromised renal function and renal toxicity. Like UA, Cr, and BUN also indicate the extent of healthy renal activity, and their derailed level signifies damaged renal function [29]. Kidney injury molecule-1 (KIM-1) is another important and sensitive renal injury marker and has been extensively studied in various preclinical and clinical models [30,31]. Considering this fact, we will evaluate the serum levels of renal injury markers and KIM-1 in future studies along with the histopathological findings. It was found that CP administration effectively elevated the urea, UA, Cr, and BUN, validating the compromised renal function. *S. chirayita*, both extracts showed a promising renal protective effect, where the urea, UA, Cr, and BUN levels were reduced significantly.

CONCLUSION

The findings of the present investigation demonstrate that *Swertia chirayita* possesses significant antioxidant and cytoprotective activities, which effectively counteract CP-induced biochemical and oxidative stress alterations. The methanolic extract, rich in phenolic and flavonoid compounds, exhibited superior free radical scavenging activity and provided better restoration of liver and kidney function markers compared to the aqueous extract. Improvement in enzymatic antioxidants (GSH, CAT) and reduction in oxidative markers (MDA) as well as pro-inflammatory cytokines (TNF-α, IL-1β) further confirm the therapeutic potential of the plant. *S. chirayita* can be considered a promising natural candidate for the management of drug-induced toxicity and related oxidative stress disorders.

AUTHOR CONTRIBUTION

Sulakshana Pawar is working on her Ph.D research work topic. Raghuveer Irchhiaya is contributing to designing the research concept and mentoring on the research project.

CONFLICTS OF INTEREST

None.

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