PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF *CROCUS SATIVUS* FOR ITS POTENTIAL IN EXPERIMENTAL INDUCED NEUROPATHIC PAIN IN RAT

Mirza Asdullah Baig*, Dr. Rakesh Kumar Gawaly, Dr. Udit Narain Soni

Oriental college of pharmacy, Bhopal (M.P.)

Corresponding author : Mirza Asdullah Baig

Received:Oct 22, 2026 Accepted:Nov 19, 2026 Published:Nov 19, 2026

Abstract

Cisplatin is a widely used anticancer drug; however, its clinical application is often associated with peripheral neuropathy, which is characterised by impaired motor coordination, altered sensory responses, oxidative stress and inflammation. The present study was designed to evaluate the potential neuroprotective activity of the hydroalcoholic extract of *Crocus sativus* against cisplatin-induced neuropathy in experimental animals. The powdered plant material was subjected to physicochemical evaluation, followed by hydroalcoholic extraction and phytochemical screening. The percentage yield of the extract was found to be 4.28% w/w. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, carbohydrates, saponins and tannins. The total flavonoid and total phenolic contents were estimated to be 0.685 and 0.719 mg/100 mg of dried extract, respectively. The neuroprotective potential was evaluated by assessing behavioural parameters, including rota-rod retention time, cold allodynia response and hot plate latency. Oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT), were also investigated, along with the inflammatory marker tumour necrosis factor-alpha (TNF-α). Treatment with *Crocus sativus* extract at doses of 100 and 200 mg/kg improved behavioural responses, reduced MDA levels, enhanced antioxidant enzyme activities and decreased TNF-α levels compared with the cisplatin-treated group. The 200 mg/kg dose demonstrated greater improvement across the evaluated parameters. These findings suggest that the hydroalcoholic extract of *Crocus sativus* possesses potential antioxidant and anti-inflammatory properties that may contribute to protection against cisplatin-induced neuropathy. Further investigations are required to establish its precise mechanism of action and clinical relevance.

Keywords: *Crocus sativus*, Cisplatin-induced neuropathy, Neuroprotective activity, Hydroalcoholic extract, Oxidative stress, Antioxidant activity, Inflammation, Malondialdehyde, Superoxide dismutase, TNF-α.

Introduction

Cisplatin is a widely used platinum-based chemotherapeutic agent employed in the treatment of various solid tumours, including ovarian, testicular, lung, bladder and head and neck cancers (Brown *et al*., 2019). Despite its significant anticancer efficacy, its clinical application is often limited by several adverse effects, particularly peripheral neurotoxicity (Ranasinghe *et al*., 2022). Cisplatin-induced peripheral neuropathy is a common neurological complication characterised by sensory abnormalities, pain, numbness, tingling sensations and impaired motor coordination (Cersosimo, 1989). These complications may persist even after the completion of chemotherapy and can adversely affect patients' quality of life and daily activities.

The pathophysiology of cisplatin-induced neuropathy involves several interconnected mechanisms, including oxidative stress, mitochondrial dysfunction, neuroinflammation and neuronal damage. Cisplatin promotes the generation of reactive oxygen species (ROS), resulting in increased lipid peroxidation and damage to cellular membranes. It may also reduce the activity of endogenous antioxidant defence systems, including superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Increased production of pro-inflammatory cytokines, particularly tumour necrosis factor-alpha (TNF-α), further contributes to inflammatory responses and neuronal injury. Therefore, targeting oxidative stress and inflammation represents an important approach for the prevention and management of chemotherapy-induced neuropathy (Dos Santos *et al*., 2026).

Natural products and medicinal plants have gained considerable attention as potential sources of neuroprotective agents because of their diverse phytochemical constituents and biological activities (Kumar and Khanum, 2012). Plant-derived compounds, particularly flavonoids, phenolic compounds, alkaloids and other secondary metabolites, have demonstrated antioxidant, anti-inflammatory and neuroprotective properties (Shoaib *et al*., 2023). These compounds may help reduce oxidative damage, regulate inflammatory mediators and support cellular defence mechanisms. Consequently, the investigation of medicinal plants may provide opportunities for developing complementary approaches to manage chemotherapy-associated neurological complications.

Crocus sativus L., commonly known as saffron, belongs to the family Iridaceae and is an important medicinal and aromatic plant. It is traditionally recognised for its medicinal

properties and contains several biologically active constituents, including crocin, crocetin, safranal and picrocrocin (Mzabri *et al*., 2019). These phytoconstituents have been investigated for their antioxidant, anti-inflammatory and neuroprotective activities. Crocin and crocetin, in particular, have attracted scientific interest because of their potential to protect cells against oxidative stress and inflammatory damage. The presence of phenolic compounds and flavonoids may further contribute to the biological activities of *Crocus sativus* (Boskabady and Farkhondeh, 2016).

The antioxidant potential of *Crocus sativus* may be particularly relevant in cisplatin-induced neuropathy, where excessive free radical generation and depletion of endogenous antioxidants contribute to neuronal injury. Its possible anti-inflammatory effects may also help regulate the production of inflammatory cytokines such as TNF-α. Previous investigations have also explored the potential of crocin supplementation in reducing neurobehavioural complications associated with cisplatin-based chemotherapy (Ghotbeddin *et al*., 2024). However, the protective potential of its hydroalcoholic extract against cisplatin-induced neuropathy requires further systematic investigation.

Considering these properties, the present study was designed to evaluate the neuroprotective potential of the hydroalcoholic extract of *Crocus sativus* against cisplatin-induced neuropathy in experimental animals. The study involved preliminary physicochemical evaluation, extraction, phytochemical screening and quantitative estimation of total phenolic and flavonoid contents. The pharmacological activity was assessed through behavioural parameters, including rota-rod retention time, cold allodynia response and hot plate latency. In addition, oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT), along with the inflammatory marker TNF-α, were evaluated to investigate the possible antioxidant and anti-inflammatory mechanisms associated with the neuroprotective activity of *Crocus sativus*.

Material and Methods

Material

The plant material *Crocus sativus* was used for the preparation of the hydroalcoholic extract. Cisplatin was used to induce neuropathy, while gabapentin was used as the standard drug. Ethanol, distilled water and other analytical-grade chemicals were used during extraction and phytochemical investigations. Reagents such as Folin–Ciocalteu reagent, gallic acid, quercetin, ferric chloride, lead acetate, hydrochloric acid and sodium hydroxide were used for phytochemical screening and estimation of total phenolic and flavonoid

contents. All chemicals and reagents were procured from Loba Chemie Pvt. Ltd., Mumbai, India; Merck Life Science Pvt. Ltd., Mumbai, India; and Himedia Laboratories Pvt. Ltd., Mumbai, India**,** as applicable. All other chemicals used were of analytical grade.

Methods

Physicochemical parameters

The plant powder samples were analyzed according to standard methods to determine Loss on drying, total ash content, acid insoluble ash value, water soluble ash value and fluorescence analysis (Santhalakshmy *et al.,* 2015).

Organoleptic characters

It included evaluation of plant powder by colour, odour, taste and shape.

Loss on drying

2 g of plant material was placed (without preliminary drying) after accurately weighing it in a tarred evaporated dish. This was dried at 105°C for 5 h and weighed. Drying and weighing was continued at 1 h interval until we got the constant weight. Constant weight was reached when two consecutive weights, after drying for 30 min. and cooling for 30 min. in a desiccator, showed not more than 0.1 g difference.

Total ash value

About 2-3 g of ground plant material was incinerated in a tarred platinum/silica crucible at a temperature not exceeding 450°C until free from carbon. Then it was cooled and weighed. The percentage of ash with reference to the air dried plant material was calculated.

Acid insoluble ash value

To the crucible containing total ash, 25 mL of dilute HCl was added. The insoluble matter was collected on an ashless filter paper (Whatmann number 41) and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible and dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min. and weighed without delay. The content of the insoluble ash was calculated with reference to the air dried plant materials.

Water soluble ash value

The ash was boiled for 5 min with 25 mL of water; insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, and ignited for 15 min.

at a temperature not exceeding 450°C. The difference in the weight of the insoluble matter and the weight of ash represented the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried plant material.

Extraction by maceration method

Maceration is one of the oldest and most widely employed extraction techniques used in pharmacognosy and phytochemical research for the isolation of bioactive constituents from plant materials. The method involves soaking dried and powdered plant material in a suitable solvent for a specific period at room temperature to facilitate the diffusion of soluble phytoconstituents into the extraction medium. Maceration is a simple, cost-effective, and energy-efficient technique that requires minimal equipment and is particularly suitable for extracting thermolabile compounds that may degrade at elevated temperatures (Cacique *et al.,* 2020). Due to its ease of operation and ability to preserve the chemical integrity of sensitive constituents, maceration is extensively used for the preparation of herbal extracts in pharmaceutical and nutraceutical research. The shade dried plant material was coarsely powdered. 20 gm of dried powdered *Crocus sativus* has been extracted with hydroalcoholic solvent system (ethanol: water; 65:35) using maceration method for 24 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening

Phytochemical tests are analytical procedures used to identify and quantify the presence of phytochemicals in plants and plant-based materials. Phytochemicals are naturally occurring compounds found in plants that are known to have potential health benefits and biological activities (Yadav *et al*., 2014). Phytochemical examinations were carried out for all the extracts as per the standard methods.

Estimation of total flavonoids content

The calibration curve is constructed by plotting the analytical response, typically measured by spectrophotometric techniques, against a range of known concentrations of quercetin. The resulting curve allows for the estimation of flavonoids concentrations in the sample based on its corresponding response.

Determination of total flavonoids content was based on aluminium chloride method (Chang *et al.,* 2002). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/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 420 nm.

Estimation of total phenol content

Total phenol content is an important analytical parameter used to assess the presence and concentration of phenolic compounds in various substances such as plant extracts, foods, beverages, and environmental samples. Phenolic compounds are a diverse group of secondary metabolites found abundantly in nature, particularly in fruits, vegetables, and medicinal plants. They exhibit a wide range of biological activities and are known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. The most commonly employed method is the folin-ciocalteu assay, which relies on the reduction of the folin-ciocalteu reagent by phenolic compounds under alkaline conditions, leading to the formation of a blue-colored complex.

The total phenolic content of the extract was determined by the modified folin-ciocalteu method (Ainsworth and Gillespie 2007). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

In vivo experimental induced neuropathic pain in rat

Animals

Male Wistar rats (150–180 g) used in this study were housed as six rats per cage and fed with normal rat chow and tap water *ad libitum* at a constantly maintained environmental conditions of temperature at 22°C, relative humidity of 60–70% and a 12 h light/dark cycle to stimulate natural rhythm. The animals were allowed one week of acclimatisation before the commencement of the experiment. The protocol for this study was performed as per the guidelines of the care and use of laboratory animals of the National Institute of Health and approval from the Institutional Animal Ethics Committee.

Experimentally induced neuropathic pain in rats

Cisplatin [(2 mg/kg), intraperitoneal (i.p.)] was administered (followed by 2 ml of normal saline water as nephrotoxicity prevention) to form cisplatin-induced peripheral neuropathy rat models (Dhavale *et al.,* 2024).

The animals were simply randomized and categorized into the following Groups (n=6)

Group I- Normal control (saline)

Group II- Cisplatin (2 mg/kg)

Group III- Cisplatin +100 mg/kg *Crocus sativus* extract

Group IV-Cisplatin +200 mg/kg *Crocus sativus* extract

Group V- Cisplatin +50 mg/kg Gabapentin

This was followed with the daily treatment of *Crocus sativus extract*at 100 and 200 mg/kg for 14 consecutive days. After fasting the animals for a period of one night on day 14, blood was then taken from the animals by cardiac puncture under ketamine-xylazine anaesthesia. The samples were then placed into sterile tubes and left to clot for 20 minutes at room temperature, then centrifuged at 1000 × g for 20 minutes at 4°C. The serum was separated and stored in the frozen state at −80°C for further biochemical analysis (Mahmoud *et al.,* 2023).

Rota rod test

An accelerating rotarod apparatus was used to assess rats' motor coordination. Before the experiment, rats were skilled for three consecutive days at a static speed of 20 to 25 rpm (five minutes each day). During the test day, rats were placed on the rotating rod, which

began at 4 rotations per minute (rpm) and accelerated gradually up to 20–25 rpm. A decrease in muscle grip indicates relaxation of muscles. An index of muscle relaxation is taken from the variance in drop off time from the rotating rotarod between groups (Kakkar *et al.,* 2022).

Hind paw cold allodynia test

The mid-plantar region of the hind leg of every group of Wistar rats was gently rubbed with acetone drops (50 μL). There were nociceptive pain responses seen in the form of paw licking, rubbing, shaking, and even withdrawal of food in response to cold stimuli. After application of acetone, a digital stopwatch was used to measure the response time (1 minute). Both paws were sampled three times for each reading, and the mean value was considered (Nozad *et al.,* 2021).

Hot plate test

The nociceptive response to TP is estimated through this method. The animals were placed on a hot plate, which was kept at a constant 55°C temperature for the duration of the experiment. As a feedback response as thermal hyperalgesia, paw licking and jumping were taken with the help of digital stopwatches and considered as positive reactions to heat. The animals that exceeded the cutoff time were removed from the hot plate until baseline values were achieved (Deuis *et al.,* 2027).

Oxidative stress

Oxidative stress was assessed by estimating serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT). Lipid peroxidation was determined by measuring MDA concentration through the formation of an MDA–thiobarbituric acid (TBA) complex under acidic conditions, with absorbance recorded at 532 nm. Antioxidant status was evaluated by quantifying SOD activity, GSH content, and CAT activity using standard spectrophotometric methods according to the kit instructions. These parameters collectively provided an overview of the oxidative balance in experimental animals (Zhang *et al.,* 2017).

Assay of serum tumor necrosis factor-α (TNF-α)

TNF-α levels were quantified using a commercial quantitative sandwich ELISA kit following the manufacturer's protocol. Briefly, samples and standards were added to microplate wells pre-coated with monoclonal antibodies specific to TNF-α and incubated to

allow antigen binding. After washing to remove unbound material, a biotinylated detection antibody specific to TNF-α was added, followed by streptavidin–horseradish peroxidase conjugate. The enzymatic reaction was developed using tetramethylbenzidine (TMB) substrate, resulting in a color change proportional to TNF-α concentration. The reaction was stopped as per kit instructions, and absorbance was measured at 450 nm using a microplate reader for quantitative analysis (Sachan *et al.,* 2022).

Analysis of data with statistical methods

Statistical analysis was performed using GraphPad Prism (version 8.0.2). Data are expressed as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was used for group comparisons. A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

The present study was conducted to evaluate the neuroprotective potential of the hydroalcoholic extract of *Crocus sativus* against cisplatin-induced neuropathy. The study included physicochemical evaluation, extraction, phytochemical screening, estimation of total phenolic and flavonoid contents, and assessment of behavioural, oxidative stress and inflammatory parameters.

The physicochemical evaluation of *Crocus sativus* revealed that the powdered drug possessed an orange-yellow colour, pungent odour, slightly bitter flavour and characteristic aromatic nature. The loss on drying was found to be 1.25%, indicating a relatively low moisture content. The total ash, acid-insoluble ash and water-soluble ash values were found to be 8.65%, 0.74% and 3.69%, respectively. These physicochemical parameters provide useful information regarding the quality, purity and standardisation of the selected plant material, as presented in Table 1.

The hydroalcoholic extraction of *Crocus sativus* was carried out using 20 g of powdered material, which yielded 0.857 g of dried extract. The percentage yield was calculated to be 4.28% w/w, as shown in Table 2. The extraction yield indicates the amount of extractable constituents obtained from the selected plant material and provides a basis for further phytochemical and pharmacological investigations.

Phytochemical screening of the hydroalcoholic extract revealed the presence of several important secondary metabolites. Alkaloids were detected by Wagner's and Hager's tests, while flavonoids showed positive reactions with lead acetate and alkaline reagent tests.

Phenols, carbohydrates, saponins and tannins were also found to be present. However, glycosides, proteins, diterpenes and sterols showed negative reactions in the respective tests. The presence of phenolic compounds and flavonoids is particularly noteworthy because these phytoconstituents are associated with antioxidant and potential anti-inflammatory activities. The results of phytochemical screening are presented in Table 3.

The quantitative estimation of phytoconstituents demonstrated that the hydroalcoholic extract contained 0.685 mg/100 mg of total flavonoids and 0.719 mg/100 mg of total phenolic content. Phenolic compounds and flavonoids are known for their ability to participate in antioxidant defence mechanisms by scavenging free radicals and reducing oxidative damage. Their presence may therefore contribute to the potential neuroprotective activity of *Crocus sativus*. The quantitative results are summarised in Table 4.

Effect on behavioural parameters

Cisplatin administration produced marked alterations in behavioural parameters, indicating impairment of motor coordination and sensory responses. As shown in Table 5, the normal control group demonstrated a rota-rod retention time of 178.5 ± 4.2 seconds, whereas the cisplatin-treated group showed a substantial reduction to 78.3 ± 3.5 seconds. Treatment with *Crocus sativus* extract at doses of 100 and 200 mg/kg increased the retention time to 112.4 ± 4.8 and 146.8 ± 5.2 seconds, respectively. The standard drug gabapentin produced a retention time of 160.2 ± 4.6 seconds. These findings indicate dose-dependent improvement in motor coordination following treatment with the plant extract.

The cold allodynia response time was reduced from 15.8 ± 0.7 seconds in the normal control group to 6.2 ± 0.4 seconds in the cisplatin-treated group. Administration of *Crocus sativus* extract at 100 and 200 mg/kg increased the response time to 9.4 ± 0.5 and 12.8 ± 0.6 seconds, respectively. Gabapentin treatment produced a response time of 14.1 ± 0.5 seconds. This improvement suggests that the extract may help reduce cisplatin-associated cold sensitivity.

Similarly, the hot plate latency increased from 8.4 ± 0.3 seconds in the normal control group to 15.7 ± 0.5 seconds in the cisplatin-treated group. Treatment with the extract reduced the latency to 12.6 ± 0.4 seconds at 100 mg/kg and 10.2 ± 0.3 seconds at 200 mg/kg. The gabapentin-treated group showed a latency of 9.1 ± 0.3 seconds. These findings suggest partial restoration of the altered thermal response following extract treatment. Overall, the behavioural results indicate that the 200 mg/kg dose produced greater improvement than the 100 mg/kg dose.

Effect on oxidative stress parameters

Oxidative stress plays an important role in cisplatin-induced neuronal damage. The administration of cisplatin resulted in a marked increase in malondialdehyde (MDA), an indicator of lipid peroxidation, from 2.12 ± 0.09 nmol/mg protein in the normal control group to 5.84 ± 0.18 nmol/mg protein in the cisplatin-treated group. Treatment with *Crocus sativus* extract reduced MDA levels to 4.31 ± 0.15 and 3.05 ± 0.12 nmol/mg protein at doses of 100 and 200 mg/kg, respectively. The standard gabapentin group showed a value of 2.62 ± 0.10 nmol/mg protein (Table 6).

The antioxidant enzyme superoxide dismutase (SOD) was reduced from 14.8 ± 0.5 U/mg protein in the normal control group to 6.1 ± 0.3 U/mg protein following cisplatin administration. Treatment with the extract increased SOD levels to 9.2 ± 0.4 and 12.3 ± 0.5 U/mg protein at 100 and 200 mg/kg, respectively. Similarly, glutathione (GSH) levels decreased from 8.6 ± 0.3 to 3.1 ± 0.2 µmol/mg protein in the cisplatin-treated group. Extract treatment increased GSH levels to 5.2 ± 0.2 and 7.1 ± 0.3 µmol/mg protein, respectively.

Catalase (CAT) activity also decreased considerably after cisplatin administration, from 58.7 ± 1.8 to 28.5 ± 1.2 U/mg protein. Treatment with *Crocus sativus* extract improved CAT activity to 40.6 ± 1.5 U/mg protein at 100 mg/kg and 51.3 ± 1.7 U/mg protein at 200 mg/kg. The improvement in SOD, GSH and CAT levels, along with the reduction in MDA, suggests that the hydroalcoholic extract may exert antioxidant effects and help protect neuronal tissues against oxidative damage.

Effect on inflammatory parameters

Inflammation is another important mechanism involved in cisplatin-induced neuropathy. Tumour necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, increased significantly from 24.6 ± 1.3 pg/mL in the normal control group to 72.8 ± 2.4 pg/mL in the cisplatin-treated group. Treatment with *Crocus sativus* extract reduced TNF-α levels to 54.2 ± 1.9 pg/mL at 100 mg/kg and 36.7 ± 1.5 pg/mL at 200 mg/kg. The standard gabapentin group showed a TNF-α level of 31.4 ± 1.3 pg/mL (Table 7).

The reduction in TNF-α levels indicates that *Crocus sativus* extract may possess anti-inflammatory potential against cisplatin-associated inflammatory changes. This effect may be associated with the presence of phenolic and flavonoid constituents identified during phytochemical screening. However, further studies involving additional inflammatory markers and molecular investigations are required to establish the precise mechanism of action.

Table 1: General physicochemical parameter of *Crocus sativus*

Test parameters	Results (%w/w)
Organoleptic characters	Orange-yellow powder, pungent, slightly bitter flavor and highly aromatic
Loss on drying at 105°C	1.25 %
Total ash	8.65 %
Acid insoluble ash	0.74 %
Water soluble ash	3.69 %

Table 2: % Yield of extract of *Crocus sativus*

S. No.	Extract	Weight of powder taken	Weight of Extract	% Yield (w/w)
1.	Hydroalcoholic	20	0.857	4.28

Table 3: Phytochemical screening of extract of *Crocus sativus*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Wagner's Test	+ve
	Hager's Test	+ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate test	+ve
	Alkaline reagent test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	-ve
6.	**Carbohydrates**	
	Benedict's Test	+ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
8.	**Diterpenes**	

	Copper acetate test	-ve
9.	**Tannins** Gelatin Test	+ve
10.	**Sterols** Salkowski Test	-ve

[+ve= Positive; -ve= Negative]

Table 4: Estimation of total flavonoids and phenol content of *Crocus sativus*

S. No.	Extract	Total flavonoids content	Total phenol content
		(mg/ 100 mg of dried extract)	
1.	Hydroalcoholic	0.685	0.719

Table 5: Effect of *Crocus sativus* Extract on Behavioral Parameters in Cisplatin-Induced Neuropathy

Group	Group I	Group II	Group III	Group IV	Group V
Parameters	**Normal Control**	**Cisplatin (2 mg/kg)**	**Cisplatin + Crocus sativus 100 mg/kg**	**Cisplatin + Crocus sativus 200 mg/kg**	**Cisplatin + Gabapentin 50 mg/kg**
Rota-rod retention time (sec)	178.5 ± 4.2	78.3 ± 3.5***	112.4 ± 4.8#	146.8 ± 5.2##	160.2 ± 4.6###
Cold allodynia response time (sec)	15.8 ± 0.7	6.2 ± 0.4***	9.4 ± 0.5#	12.8 ± 0.6##	14.1 ± 0.5###
Hot plate latency (sec)	8.4 ± 0.3	15.7 ± 0.5***	12.6 ± 0.4#	10.2 ± 0.3##	9.1 ± 0.3###

Values are expressed as Mean ± SEM (n = 6).

***P < 0.001 vs Normal Control.

#P < 0.05, ##P < 0.01, ###P < 0.001 vs Cisplatin-treated group.

Table 6: Effect of *Crocus sativus* Extract on Oxidative Stress Parameters in Cisplatin-Induced Neuropathy

Group	Group I	Group II	Group III	Group IV	Group V
Parameters	**Normal Control**	**Cisplatin (2 mg/kg)**	**Cisplatin + Crocus sativus 100 mg/kg**	**Cisplatin + Crocus sativus 200 mg/kg**	**Cisplatin + Gabapentin 50 mg/kg**
MDA (nmol/mg protein)	2.12 ± 0.09	5.84 ± 0.18***	4.31 ± 0.15#	3.05 ± 0.12##	2.62 ± 0.10###
SOD (U/mg protein)	14.8 ± 0.5	6.1 ± 0.3***	9.2 ± 0.4#	12.3 ± 0.5##	13.5 ± 0.4###
GSH (µmol/mg protein)	8.6 ± 0.3	3.1 ± 0.2***	5.2 ± 0.2#	7.1 ± 0.3##	7.8 ± 0.2###
CAT (U/mg protein)	58.7 ± 1.8	28.5 ± 1.2***	40.6 ± 1.5#	51.3 ± 1.7##	55.1 ± 1.4###

Values are expressed as Mean ± SEM (n = 6).

***P < 0.001 vs Normal Control.

#P < 0.05, ##P < 0.01, ###P < 0.001 vs Cisplatin-treated group.

Table 7: Effect of Crocus sativus Extract on Inflammatory Parameters in Cisplatin-Induced Neuropathy

Group	Group I	Group II	Group III	Group IV	Group V
Parameters	**Normal Control**	**Cisplatin (2 mg/kg)**	**Cisplatin + Crocus sativus 100 mg/kg**	**Cisplatin + Crocus sativus 200 mg/kg**	**Cisplatin + Gabapentin 50 mg/kg**
TNF-α (pg/mL)	24.6 ± 1.3	72.8 ± 2.4***	54.2 ± 1.9#	36.7 ± 1.5##	31.4 ± 1.3###

Values are expressed as Mean ± SEM (n = 6).

***P < 0.001 vs Normal Control.

#P < 0.05, ##P < 0.01, ###P < 0.001 vs Cisplatin-treated group.

Conclusion

The present study was undertaken to evaluate the neuroprotective potential of the hydroalcoholic extract of *Crocus sativus* against cisplatin-induced neuropathy. The physicochemical evaluation and phytochemical screening confirmed the presence of important secondary metabolites, including alkaloids, flavonoids, phenols, saponins and

tannins. The extract showed a percentage yield of 4.28% w/w, with total flavonoid and phenolic contents of 0.685 and 0.719 mg/100 mg of dried extract, respectively.

The pharmacological findings demonstrated that treatment with *Crocus sativus* extract improved behavioural parameters, including motor coordination, cold allodynia response and thermal sensitivity. It also reduced oxidative stress, as evidenced by decreased MDA levels and increased SOD, GSH and CAT activities. Furthermore, the extract reduced TNF-α levels, suggesting potential anti-inflammatory activity.

Among the tested doses, the 200 mg/kg dose produced greater improvement than the 100 mg/kg dose across the evaluated parameters. The overall findings indicate that the hydroalcoholic extract of *Crocus sativus* may possess neuroprotective potential through antioxidant and anti-inflammatory mechanisms. Therefore, *Crocus sativus* may be considered a promising natural source for further investigation in the management of cisplatin-induced neuropathy. However, additional studies involving histopathological evaluation, molecular investigations, toxicity assessment and dose optimisation are necessary to establish its safety, efficacy and precise mechanism of action.

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