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Original Article

Ameliorative effect of *Jasminum grandiflorum* (L.) leaves against cyclophosphamide (CTX) induced renal and liver toxicity

Deepa[a,*], Arun Lodhi [a], Rajnish Srivastava [b], Amit K. Srivastava [c], Hemant Nagar [d]

[a] *Department of Pharmacology, NRI Institute of Pharmaceutical Sciences, Bhopal-462030, Madhya Pradesh, India.*
[b] *Moradabad Educational Trust, Group of Institutions–Faculty of Pharmacy, Moradabad-244001, (U.P) India.*
[c] *Department of Pharmacology, Sapience Bio-analytical Research Lab, Bhopal-462021, Madhya Pradesh,, India.*
[d] *Department of Pharmacology, Truba Institute of Pharmacy, Bhopal-462030, Madhya Pradesh, India.*

***Corresponding Author.** Tel .: +91 9027422094, *E-mail address:* chaudharydeepa22141@gmail.com

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ABSTRACT

In the present study, first we screened the ethanolic extract of *Jasminum grandiflorum* leaf (EEJG) through the in-vitro DPPH free radical scavenging method for its possible antioxidant activity and was found to posses good antioxidant potential. After that, ameliorative effect of the EEJG was evaluated against Cyclophosphamide (CTX) induced renal and hepatic toxicity. Wistar albino rats were divided into five groups, Group-I, control (0.5% w/v, Carboxy Methyl Cellulose); Group-II, negative control (CTX, 30 mg/kg, i.p, for 15 days); Group-III, (EEJG, 100 mg/kg, b.w., p.o., + CTX 30 mg/kg, b.w., i.p, for 15 days); Group-IV, (EEJG, 200 mg/kg, b.w., p.o. + CTX, 30 mg/kg, b.w., i.p, for 15 days) and Group-V, (received only EEJG, 200 mg/kg, b.w., p.o, for 15 days). Extract and CTX were given once daily for 15 days. Administration of CTX (30 mg/kg, b.w., i.p.) for 15 days induced renal and liver damage as indicated by the serum marker enzymes of liver alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), alkaline phosphatase (ALP), bilirubin and biochemical parameters for kidney function such as serum creatinine, urea and uric acid. Equivalent to these changes CTX also alters the hematological parameters like RBC, total WBC count and hemoglobin level. Concurrent treatment with EEJG (100 and 200 mg/kg, b.w., p.o.) effectively ameliorate the toxicological changes induced by the CTX through scavenging of free radicals. The *Jasminum grandiflorum* has potential as adjuvant treatment option to CTX for circumventing the side-effects associated with its antineoplastic applications.

1. INTRODUCTION

Cyclophosphamide (CTX) belongs to the nitrogen mustard class as a bifunctional cytotoxic alkylating agent. CTX is extensively used as a wide spectrum chemotherapeutic agent for treating various cancers as well as immunosuppressant in organ transplantation, systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis [1]. In recent years, use of CTX as a therapeutic antineoplastic agent is often restricted due to its devastating adverse effects both in the short and long term use [2-6]. Generally the antineoplastic action of prodrug CTX is an effect of its active metabolite phosphoramide, while the aldophosphamide metabolism generates the acrolein which interferes with the tissue antioxidant defense system producing highly reactive oxygen species (ROS) and free radicals. These free radicals and ROS could induce several toxic adverse effects to vital organs of the human body and may be mutagenic to normal mammalian cells [7, 8].

At present, herbal medications have been shown to be an outstanding and trustworthy source for the development of new treatment strategies. Flavonoids and phenolic compounds are found to demonstrate various biological properties, including hepatoprotective, and anti-cancer activities. The therapeutic

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efficacy of phenolic compounds is commonly thought to be due to their antioxidant and free radical scavenging properties [9].

Antioxidants are the compounds, able to slowing or preventing the oxidation of other molecules. They play a key role in the prevention of body from diseases by reducing the oxidative damage to cellular component caused by ROS [10]. Different synthetic antioxidants are available, but their use is limited due to their toxic and carcinogenic effect [11]. Alternatively, use of herbal plant extracts as anti-oxidant, due to their proved activity and lesser side effect is a promising approach. Recent studies also suggested that the plant derived antioxidants holds enormous therapeutic importance in free radical mediated diseases [12, 13].

Present study was designed to assess the ameliorative effect of *Jasminum grandiflorum* leaves against cyclophosphamide induced renal, and liver toxicity. Previous studies concluded that *Jasminum grandiflorum* (Family: Oleaceae) leaves enrich in flavonoids and also contain polyphenolic compounds [14], which may be beneficial for the present study. However, there are no established scientific reports for such type of activity. Therefore, the present study will be aimed to define more rationalize use of *Jasminum grandiflorum* leaves in cyclophosphamide induced toxicity.

2. EXPERIMENTAL

Plant Collection and Authentication

Fresh leaves of *Jasminum grandiflorum* were collected from the Truba Institue of Pharmacy, Bhopal, Madhya Pradesh, India. It was identified and authenticated by Dr. Zia Ul Hasan, Head of Department, Department of Botany, Safia Science College, Bhopal, Madhya Pradesh, India, and a specimen voucher assigned was 493/Bot/Safia/14.

Preparation of Extract

Shade dried leaves of *Jasminum grandiflorum* were pulverized to a moderately coarse powder, and passed through sieve no. 16 to maintain uniformity. Coarsely dried powder was first defatted with petroleum ether (60-80ºC) for 72 hours and then extracted with ethanol using a soxhlet apparatus for 36 hours. The dark green extract was collected and concentrated in vacuum under reduced pressure using a rotary flash evaporator and the dried crude extract was stored in airtight container at 4ºC for further study.

Preliminary Phytochemical Screening

Ethanolic Extract of *Jasminum Grandiflorum* leaves (EEJG) was subjected to various phytochemical tests for the identification of phytoconstituents present therein, following the standard procedures. [15, 16]

Animal Care and Handling

Healthy Wistar albino rats (140-200 g) were obtained from Sapience Bio-analytical Research Lab, Bhopal, Madhya Pradesh, India. One week before the initiation of the experiment, animals were acclimatized to the standard laboratory conditions of temperature 25±2°C, relative humidity 44-56%, and 12:12 hours light and dark cycles, fed with standard pellet diet and water *ad libitum* during the study period. Study protocol was approved by the Institutional Animal Ethics Committee (IAEC) as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Approval No. SBRL/IAEC/June 2014/13).

Acute Oral Toxicity Study

The acute oral toxicity study was performed as per the Organization for Economic Cooperation and Development (OECD) guidelines no. 425 on Wistar albino rats (140-200 g). Three overnight fasted rats received 2000 mg/kg, b.w., of EEJG. Extract was given in the form of suspension with 0.5% carboxy methyl celluloe in distilled water by oral gavage. Each animal was observed individually for any toxicity sign of gross changes like convulsion, tremor, circling movement, depression, and mortality after dosing, for 24 hours, with special attention given during the first 4 hours. All observations were systematically recorded with individual records being maintained for each animal.

In-vitro Antioxidant Activity by DPPH Method

The free radical scavenging activity of the EEJG was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) method [17]. 0.1 mM solution of DPPH was prepared in methanol, and 1 ml of it was added to different concentrations of EEJG (100, 200, 300, 400 and 500 **µg**/ml) in the test tube and final volume of 3 ml was made with methanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Absorbance of the resulting mixture was measured at 517 nm against methanol as blank, by using a UV-visible spectrophotometer (Systronics, 2203, Japan). Each sample was then measured in triplicate and results were represented as mean. The ascorbic acid was used as a standard antioxidant in this method. Percentage of DPPH free radical scavenging activity (FRSA) was determined as follows:

$$\% \text{ (FRSA)} = \frac{\text{(Absorbance of control-Absorbance of Text Sample)}}{\text{Absorbance of Control}} \times 100$$

In-vivo Cyclophosphamide (CTX) induced Toxicity [18]

Randomization, grouping and dosing of animals

Wistar albino rats were divided randomly into five groups consisting of six animals in each group. Group-I, control (0.5% w/v, Carboxy Methyl Cellulose); Group-II, negative control (CTX, 30 mg/kg, i.p, for 15 days); Group-III, (EEJG, 100 mg/kg, b.w., p.o., + CTX, 30 mg/kg, b.w., i.p, for 15 days); Group-IV, (EEJG, 200 mg/kg, b.w., p.o. + CTX, 30 mg/kg, b.w., i.p, for 15 days) and Group-V, (received only EEJG, 200 mg/kg, b.w., p.o, for 15 days). Extract and CTX were given once daily for 15 days. Extract and CTX were given once daily for 15 days.

Evaluation of effect of EEJG on Cyclophosphamide (CTX) induced toxicity

Change in Body Weight

Body weight of each animal of all the groups was measured every 7th day to assess the percentage change in body weight during the overall study period.

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Evaluation of hematological parameters

At the end of study, on the 15th day, blood samples were collected from all groups of animals by retro-orbital puncture technique under mild anesthesia. The blood samples were collected into two fractions; one fraction was used for the study of hematological parameters like RBC, total WBC count and hemoglobin level, while remaining second fraction of blood was utilized for serum analysis.

Analysis of serum for liver and kidney function test

Blood samples were collected, allowed to clot and the serum was separated by the centrifugation at 3000 rpm at for 15 minutes and stored at 4ºC for further biochemical analysis. Different biochemical enzymes for liver function test like serum alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), alkaline phosphatase (ALP), bilirubin and biochemical parameters for kidney function test such as serum creatinine, urea and uric acid were measured spectrophotometrically by the standard enzymatic methods using the commercial kits (Span Diagnostics Ltd., India).

3. RESULT AND DISCUSSION

Preliminary phytochemical screening suggests the presence of flavonoids, glycosides, alkaloids, tannins, phenolic compounds, and carbohydrates, in the EEJG [Table 1].

Table 1. Phytochemical screening of EEJG

S.No.	Chemical Test	Inference
1.	Carbohydrates	(+)
2.	Tannins	(+)
3.	Alkaloids	(+)
4.	Glycosides	(+)
5.	Flavonoids	(+)
6.	Steroids and sterols	(+)
7.	Proteins and amino acids	(+)
8.	Saponins	(–)

(+) Sign indicates presence and (–) sign indicates absence of phytoconstituent

EEJG was found to be non-toxic up to 2000 mg/kg, b. w., as no change in the behavioral pattern and not any sign of toxicity and mortality was reported during overall acute oral toxicity study period [Table 2]. Finally, the dose of 100 mg/kg and 200 mg/kg were chosen for further studies.

Table 2. Mortality data of acute oral toxicity study of EEJG

Group	No. of Animal	No. of Animals Dead	Mortality Ratio
EEJG (2000 mg/kg, b.w., p.o.)	3	Nil	Nil

The results of the free radical scavenging activity of EEJG tested by the DPPH method are depicted in Table–3. EEJG showed gradual increasing percentage inhibition with increasing concentration at 517 nm as anti oxidative agent by DPPH assay (maximum % inhibition: 66.67% at 500 µg/ml). Ascorbic acid as a standard antioxidant showed a gradual increase in percentage inhibition with increasing concentration at 517 nm by DPPH assay [Table 3].

Table 3. Antioxidant activity by DPPH method

S.No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	EEJG
1.	100	41.65 ± 1.01	22.12 ± 1.15
2.	200	58.65 ± 1.29	34.52 ± 2.21
3.	300	70.69 ± 0.92	47.70 ± 2.32
4.	400	82.50 ± 1.16	58.13 ± 1.43
5.	500	89.39 ± 0.62	66.67 ± 1.29

The body weight of the negative control animals (group II) was consistently reduced till day 15. Administration of EEJG at dose levels of 100 mg/kg and 200 mg/kg, b.w., respectively inhibit the change in body weight of the test animals (group III and IV) dose dependently at all week. The effect of EEJG at 200 mg/kg, b.w., was more prominent as there was less % change in the body weight of animals (group IV) in comparison to EEJG at a dose level of 100 mg/kg, body weight (group III) [Table 4]. However, alone treatment of EEJG at the dose level of 200 mg/kg, b.w., (Group V) did not affect the body weight of animals.

Table 4. Percentage change in body weight

Group	Body weight (g) ± SEM			% change in body weight
	Initial	Day 7	Day 15	
I	145.54±2.43	147.9±3.04	148.5±1.04	-
II	162.48±3.43	156.5±3.49	150.4±3.65	7.43
III	155.5±3.75	150.6±2.25	146.2±2.98	5.98
IV	182.13±4.05	177.34±3.55	174.9±2.5	3.96
V	160.4±3.29	158.3±1.63	157.85±2.0	1.58

Table 5. Haematological parameters

Group	RBC (Millions/ mm^3)	WBC (Thousands/ mm^3)	Hb (g/dl)
I	8.02 ± 1.17	7.74 ± 0.23	14.72 ± 1.25
II	5.74 ± 1.26a***	5.6 ± 0.28a***	11.23 ± 0.14a*
III	6.59 ± 0.58a**	6.26 ± 0.28a**	12.03 ± 1.34
IV	7.77 ± 0.84b**	7.12 ± 0.11b**	13.35 ± 1.67
V	8.58 ± 1.54b***	7.66 ± 0.19b***	13.25 ± 0.76

All values are represented as mean ± SEM, n = 6 animals in each group, Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test, a–Significant difference as compared to normal control group (group-I), b–Significant difference as compared to the negative control group (group-II) and *P<0.05, **P<0.01, ***P<0.001.

CTX at the dose of 30 b.w., i.p, (group II) caused a significant decrease in RBC, total WBC count, and Hb% as compared to the normal control group [Table 5]. EEJG (Group III and IV) dose dependently showed significant ($P < 0.01$) elevation in total WBC count and significant ($P < 0.01$) increase in RBC and Hb% when compared with the negative control group (Group II) which

was dose dependent. However, the changes in the hematological parameters were more significant (P < 0.001) with e alone EEPG 200 mg/kg, b.w., (Group V) as compared to the normal control animals at the mentioned doses [Table 5].

Levels of the serum marker enzymes associated with liver function, SGPT, SGOT, ALP and bilirubin were found to be elevated in the CTX treated rats, whereas in the EEJG treated animals, marker enzymes were significantly reduced in comparison to the control group [Table 6].

Table 6. Serum analysis for liver function test

Group	SGOT (IU/l)	SGPT (IU/l)	ALP (KA Unit)	Bilirubin (mg/dl)
I	11.72 ± 1.58	21.02 ± 1.72	6.56 ± 1.24	0.68±0.06
II	24.5 ± 3.54 a***	33.75 ± 2.78 a***	15.40 ± 0.57 a**	2.74±0.04
III	20.83 ± 2.97 a***	30.48 ± 2.37 a***	11.18 ± 0.58 a*	2.06±0.02
IV	16.46 ± 1.06 a**, b***	27.76 ± 0.86 a**, b***	8.08 ± 0.27 b**	1.57±0.09
V	12.09±3.0 b***	20.91 ± 3.09 b***	6.03 ± 0.76 b**	0.62±0.04

All values are represented as mean ± SEM, n = 6 animals in each group, Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test, a–Significant difference as compared to the normal control group (group-I), b–Significant difference as compared to the negative control group (group-II) and *P<0.05, **P<0.01, ***P<0.001.

Biochemical parameters associated with kidney function test, creatinine and urea were found to be elevated along with uric acid in the CTX treated rats, in the EEJG treated animals, these markers were significantly therapeutically ameliorated in comparison to the control group [Table 7].

Table 7. Serum analysis for kidney function test

Group	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
I	0.54 ± 0.051	15.65 ± 1.76	5.58 ± 0.63
II	0.86 ± 0.035 a**	22.04 ± 2.06 a***	13.45 ± 0.89 a***
III	0.72 ± 0.018 a*	20.43 ± 1.23 a**	11.29 ± 0.78 a**
IV	0.68 ± 0.025 b*	19.64 ± 1.52 a**, b*	9.49 ± 0.56 b*
V	0.59 ± 0.013 b**	16.53 ± 2.45 b**	5.78 ± 0.93 b**

All values are represented as mean ± SEM, n = 6 animals in each group, Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test, a–Significant difference as compared to the normal control group (group-I), b–Significant difference as compared to the negative control group (group-II) and *P<0.05, **P<0.01, ***P<0.001.

The present studies revealed that the ethanolic extract of *Jasminum grandiflorum* leaves shows the ameliorative effect against Cyclophosphamide (CTX) induced oxidative stress, renal and liver toxicity.

Recent studies postulated that elevated free radicals associated with CTX metabolites Acrolein, increases the lipid peroxidation, which can alter the hepatocytes cell membrane structure and function [19, 20]. It is also well known that increased levels of SGPT, SGOT and ALP enzymes in the serum are diagnostic biomarkers of hepatotoxicity. In the present study, treatment of animals with CTX leads to oxidative stress as apparent from significant increase in the level of serum marker enzymes associated with liver function, SGPT, SGOT, ALP and bilirubin. EEJG significantly decreased the serum SGPT, SGOT, ALP and bilirubin levels, indicating the shielding activity against the liver damage.

Phytochemical study showed the presence of polyphenolic compounds and flavonoids. Flavonoids are well known for their diverse biological activities. In the present study, the presence of flavonoids and phenolic compounds in extract directly contribute to the antioxidant activity by neutralizing the generated free radicals [21]. Inhibition of the CTX induced oxidative stress probably may be due to the antioxidant properties of the extract. The precise molecular mechanism of ameliorative effect of ethanolic extract of *Jasminum grandiflorum* leaves is not clear. Probably it may be attributed due to its antioxidant property.

4. CONCLUSION

In conclusion, the present studies indicate that ethanolic extract of *Jasminum grandiflorum* leaves attenuates the CTX induced renal and liver damage. Thus, *Jasminum grandiflorum* has potential as adjuvant treatment option to CTX for circumventing the side-effects associated with its antineoplastic applications. Further studies are needed to understand the more insight towards the mechanism of action of isolated phytoconstituents responsible for the activity.

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