Pharmacological Role of *Capparis decidua* (Forssk.) Edgew in Preventing Cyclophosphamide-induced Myelosuppression and Modulating Innate and Adaptive Immune Response

Hafiz Muhammad Farhan Rasheed\(^1,2\) and Qaiser Jabeen\(^1\)

**Abstract**

**Background:** *Capparis decidua* (Forssk.) Edgew is reported to be practiced in the traditional system of medicine for the management of various immunological pathologies.

**Purpose:** The current study was designed to evaluate the modulatory effects of *C. decidua* on different immune responses.

**Research Design:** *C. decidua* was extracted in 70% methanol and the crude extract (Cd.Cr) was analyzed by FTIR and GCMS. In vivo models were employed to assess the actions of Cd.Cr on cyclophosphamide-induced myelosuppression, innate and adaptive immune responses.

**Results:** GCMS and FTIR analysis indicated the presence of flavonoids, phenols, terpenoids and lipids. Cd.Cr evoked a significant and dose-dependent increase in percent neutrophil adhesion (15.97 ± .81, 27.47 ± .79 and 38.35 ± 1.08) and the phagocytic index (3.1 ± .04, 3.96 ± .06 and 5.28 ± .13) at the doses of 30, 100 and 300 mg/kg. Cd.Cr also potentiated haemagglutinating antibody titre, immunoglobulins and cytokines (interferon-γ and interleukin-2) production for 4 weeks, after exposure to sheep erythrocytes, and delayed type hypersensitivity reaction significantly (\(P < .05\)). The restoration of hematological profile and antioxidant enzyme activities, by Cd.Cr, indicated the prevention of cyclophosphamide-induced myelosuppression and oxidative stress.

**Conclusions:** The findings of this study suggest that *C. decidua* holds immunomodulatory activity by thus possesses therapeutic potential for the management of immunological diseases.

**Keywords**
adaptive, *Capparis decidua*, immunomodulation, innate, cytokines, immunoglobulins

**Introduction**

The immune system is a comprehensive network of specialized tissues that protects the multicellular host organisms from development of various anomalies. The orchestrated defensive response, generated by effective immune cells, classically includes non-specific pathogen killing and elimination, antigen presentation, activation of lymphocytes, and ultimately producing different cytokines and antibodies\(^1\). These processes are critical and even slight alteration in one or

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Received 30 May 2022; accepted 13 August 2022

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more pathways may lead to many pathological conditions like organ transplant rejection, infectious diseases, autoimmune and/or malignant diseases. Immunomodulation is thought to be an effective therapeutic strategy to limit such pathologies during the past few years. Modulation of the immune response using all the therapeutic interventions is generally related to the enhanced functional efficiency of different cellular components like macrophages, granulocytes, complement, natural killer cells and lymphocytes, and the resultant secretion of various effector molecules generated by activated cells. Current therapeutic options for immunostimulation mainly include cytokines like interleukins and interferons, but the cost and adverse effects are the limitations. The indigenous system of medicines emphasizes the use of natural products as potential therapeutic alternatives, for immune system-related pathologies, due to their ability to target numerous immunological pathways of different pathologies.

Natural medicines originated from plant sources have recently gained more attention on a global scale due to their improved tolerance and lesser side effects. WHO estimates that over 75% of people worldwide today utilize herbs and other traditional remedies to treat a variety of ailments. Cholistan desert of Pakistan is a rich treasure of medicinal plants and local inhabitants use many plant species as folk remedies. The medicinal flora of Cholistan desert possesses variety of constituents mainly due to adaptation to several ecological stresses, including salinity, temperature and aridity. Many medicinally important phytochemicals including flavonoids, quinones, anthocyanidines, saponins, sterols and steroids, phenolics, fatty acids, terpenes and triterpenoids have been extracted and identified from the plants of Cholistan desert. Although the extreme climatic variation, unhygienic environment and lack of proper medical facilities of the under-developed area of the desert may cause different infectious disorders in the local population, the natural immunity in most of the local inhabitants against such infectious conditions indicates the presence of some immunomodulator especially immunostimulant type of activity in the natural products of the area. Undoubtedly, the plants, being the most typical natural products of the Cholistan desert, may be considered the potential candidates for evaluation, leading to characterization and isolation of newer and safer lead compound(s) having immunomodulator activities.

*Capparis decidua* (Forsk.) Edgew, belonging to the plant family Capparidaceae, is a versatile woody shrub of desert and barren regions of Pakistan, India, Africa and Saudi Arabia. It dwells dry foothills and wastelands and matures in shallow soils. The phychemical studies of *C decidua* have reported high contents of alkaloids, terpenoids and glycosides, and specific constituents like isothiocyanate glycosides, glucocapparin, stachydrine, n-triacontane, n-triacontanol, n-pentacosane, β-carotene and β-sitosterol.

The plant is reported to be used in Unani system of medicine as carminative, tonic, emmenagogue and aphrodisiac. It is also used in the treatment of different ailments including rheumatism, cough, asthma and heart diseases. The shoots and leaves are employed for the treatment of diabetes, stomach problems, hypertension and intermittent fever. Decoction of young shoots is also used as herbal remedy to cure migraine. Wood coal is used for jaundice, muscular injuries, swellings and infection of joints.

*C. decidua* is one of the scientifically explored plants of the Cholistan desert. Pharmacological studies have reported CNS depressant, anti-hyperglycemic, antihyperlipidemic, antiatherosclerotic, anehamimetic, antioxidant, antimicrobial and hepatoprotective activities of the plant. The flowers have been reported to contain sulfur rich compounds having antimicrobial activity against several microorganisms. The crude extract of *C. decidua* has also showed inhibitory effects against prostaglandin synthesis by COX-2 inhibition and weak or no action against COX-1. Methanolic extract of *C. decidua* was also reported to decrease the force and rate of guinea-pig atrial contractions as well as dose-dependent fall in blood pressure and heart rate in experimental animals.

However, there is no scientific evidence found in the literature for the evaluation of immunopharmacological activities of *C. decidua*. Based on the facts regarding the folkloric uses of plant in various diseases with immunological pathologies and the scientific data, the study was planned to explore its effects on the immune system. The present study was aimed to evaluate the modulatory properties of aqueous methanolic extract of *C. decidua* for innate and adaptive immune responses. Cyclophosphamide-induced myelosuppression model was also utilized to assess the protective effects of plant against secondary immunodeficiencies related to chronic use of chemotherapies.

### Material and Methods

#### Plant Material

The aerial parts of *C. decidua* (2.6 kg) were collected from the desert area near Bagdad-ul-Jadeed campus, the Islamia University of Bahawalpur (IUB), Pakistan. The plant was identified through the standard routine procedure by the botanist of the department of Life Sciences, IUB, and a small portion was submitted in the herbarium section of the Pharmacology research lab, department of Pharmacology, faculty of Pharmacy, IUB, Pakistan, and the voucher number (CD-AP-04-14-64) was issued for the specimen. The plant material was processed by washing, shade-drying and screening to remove any adulteration.

#### Preparation of the Crude Extract of Capparis decidua

The plant material was ground to coarse powder and extracted in 70% methanol for 72 h. The macerate was filtered through Whatman filter paper and the residue was resoaked and filtered twice to obtain collective filtrate that was further processed for evaporation at 40°C and low pressure using rotary evaporator (Heidolph Laborota 4000 efficient, Germany). The process
was continued till a slurry like paste of crude extract (Cd.Cr) was obtained that was labeled, weighed to determine the yield and stored at −20°C.

**Phytochemical Analysis**

The phytochemical analysis of the crude extract of *C. decidua* (Cd.Cr) was performed to identify different classes of primary and secondary phytochemical constituents like glycosides, saponins, tannins, alkaloids, flavonoids and terpenes.\(^\text{12,13}\)

**Antioxidant Activity**

The antioxidant potential of Cd.Cr was determined by free radical scavenging ability of stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical,\(^\text{14}\) the amount of total phenolics by Folin-Ciocalteau method and total flavonoid contents by aluminum chloride method.\(^\text{15}\) About .1 mM solution of DPPH was prepared in methanol and mixed (1 mL) with 1 mL of standard/extract solution of varying concentration (25-300 μg/mL). L-ascorbic acid solution was used as the reference standard and all the solutions were incubated (in the dark) for 30 min at 37°C and optical density was measured at 517 nm.

For total phenolic contents, 5 mL of Folin-Ciocalteu reagent was added to 1 mL methanolic solution of Cd.Cr (1 mg/mL) followed by the addition of 4 mL of 7.5% sodium bicarbonate solution. The tubes were kept at room temperature for 30 min and the optical density was measured at 765 nm. The amount of total phenolic contents of Cd.Cr was derived using gallic acid calibration curve (prepared for gallic acid 10-μg/mL) and expressed as mg of gallic acid equivalent to 1 g (mg of GAE/g) of the plant extract.

For the determination of total flavonoid content, rutin was used as standard. In brief, 1 mL of extract (1 mg/mL), 30 mL of methanol (30%), .5 mL of NaNO₂ (.5 M) and .5 mL of AlCl₃,6H₂O (.3 M) were mixed. After 5 min, 3 mL of NaOH (1 M) was added to alkalinate the solution. The solution was mixed well and the optical density was measured at 506 nm. The standard curve for total flavonoids was plotted using rutin standard solution (0-100 μg/mL). The total flavonoids were expressed as milligrams of rutin equivalents per g (mg of RE/g) of the extract.

**Fourier Transform Infrared Spectroscopy**

The dried sample was pulverized with potassium bromide to prepare the pellet. This KBr disc (pellet) was scanned and the transmittance spectrum recorded in the range of 4000 →650 cm⁻¹ using Bruker FTIR (Tensor 27 series, Germany).

**GC-MS Analysis**

The methanolic solution (1 mg/mL) of the crude extract of *C. decidua* (Cd.Cr) was subjected to GC-MS analysis using Agilent Technologies GC systems consisting of Agilent 7890B equipped with column HP-5 ms ultra inert (length 30 m, i.d. 250 μm, film thickness 25 μm) and mass spectrometer Agilent Technologies (5977A). The spectrometric detection was performed in the EI (electron impact) mode with 70 eV; mass range 50-650 amu and scan time 1.5 s. The detector temperature was maintained at 250°C, with split injection mode at 10:1. The oven temperature was programmed initially at 110°C (6 min), then increased to 280°C at a ramp rate of 5°C/min (34 min), then stabilized at 280°C (6 min), while the detector temperature was kept at 280°C. Helium gas (99.999%) was used as mobile phase at a constant flow rate of 1.5 mL/min and the volume of injected sample was 2.0 μL. Identification of the constituents was carried out by comparing the spectral patterns with NIST 05 and Willey spectral library.\(^\text{16,17}\)

**Chemicals**

All the chemicals utilized during research work were of analytical grade and obtained from different sources; ie dexamethasone, nigrosine and xylon pellets from Sigma Aldrich (USA), cyclophosphamide from Pharmedic, Lahore (Pakistan), levarasole from ICI (Pakistan), ketamine from Global Pharmaceuticals (Pakistan) and xylazine from mylab, Bahawalpur (Pakistan).

**Kits**

ELISA kits for Interferon-γ (Catalog No: E-EL-R0009), Interleukin-2 (Catalog No: E-EL-R0013), Immunoglobulin type G (Catalog No: E-EL-R0518) and type M (Catalog No: E-EL-R3016) while colorimetric assay kits for malondialdehyde (MDA; E-BC-K025-M), total superoxide dismutase (T-SOD; E-BC-K020-M), glutathione peroxidase (GSH-Px; E-BC-K096-M) and catalase (CAT; E-BC-K031-M) were purchased from Elabscience Biotechnology, USA.

**Experimental Animals**

Wistar Albino rats weighing 150-250 g of either sex and Swiss albino mice (either sex) weighing 20-30 g were used for the studies. The animals were kept in the animal housing facility of Pharmacology research laboratory, department of Pharmacology, faculty of Pharmacy, IUB, Pakistan. The animals were housed in polycarbonate cages with a maximum of six animals per cage and provided with standard temperature conditions (25 ± 2°C) and humidity (50-55%), along with standard animal diet and tap water *ad libitum*, throughout the study. The animals were acclimatized, for minimum 1 week, to the experimental conditions. The study protocols and procedures were approved by the Pharmacy Animal Ethics Committee (PAEC) of the faculty of Pharmacy, IUB with Ref. No. PAEC/2021/43.
Acute Toxicity Assay

Acute toxicity studies were performed according to OECD guidelines 425 and the limit dose was 10 000 mg/kg. For this purpose, twenty-five mice of either sex were selected and divided into 5 groups each consisting of 5 mice and kept fasted overnight. The normal control group was given normal saline (10 mL/kg p.o.) while other groups were given different doses of Cd.Cr; ie 1000, 3000, 5000 and 10 000 mg/kg p.o. as single dose. The animals were observed, using a check list proforma, for changes in acute behavioral and physiological parameters including grooming, hyperactivity, convulsions, alertness, lacrimation, urination, corneal reflex, writhing reflex, righting reflex and gripping strength for 12 h, on hourly basis, and for mortality for 48 h and then for 14 days, on daily basis.18

Macrophage Phagocytic Response

Wistar albino rats of either sex were divided into six groups each comprising of six animals; the control group (normal saline, 4 mL/kg), the two standard control groups (levamisole 100 mg/kg and cyclophosphamide; 25 mg/kg p.o.), treatment groups (Cd.Cr; 30, 100 and 300 mg/kg). All the animals of different groups received respective treatments for 7 days and after 24 h of the last dose, the animals were injected, through tail vein, .3 mL/30 g of colloidal carbon suspension (1.6% of nigrosine stabilized in 1% gelatin). 50 μL of blood samples were collected from each animal by retro-orbital puncture at an interval of 2 and 10 min and mixed with 4 mL of .1% sodium carbonate solution to lyse the erythrocytes. The optical density of the diluted blood samples was measured at 675 nm using pre-injection blood samples as blank. After final blood collection, the animals were dissected out for liver and spleen. Rate of carbon clearance (κ) and phagocytic index (α) were calculated using the formula; κ = (log₂ - log₁₀)/(t₂ - t₁), α = κ¹/³ × body weight/(liver weight + spleen weight).19

Neutrophil Adhesion Test

The control group was given normal saline (4 mL/kg), whereas levamisole and cyclophosphamide were administered to the two standard control groups; and, the extract-treated groups received the treatment of Cd.Cr at the doses of 30, 100 and 300 mg/kg. All the groups were treated for two weeks and subsequently, blood samples were collected from retro-orbital plexus in heparin containing vials to avoid blood clotting. The blood samples from all the animals were subjected to total leukocyte count (TLC) and differential leukocyte count (DLC) followed by incubation with nylon pellets (80 mg/mL) for 15 min at 37°C. Afterward, the blood samples were re-analyzed to determine TLC and DLC.20 The extent of neutrophil adhesion (NA) and neutrophil index (NI) was evaluated: NI = TLC x percentage of neutrophils and percent NA = (NIu → NI/C0 x 100); where NIu: NI of untreated blood samples and NI: NI of treated blood samples.

Adaptive Immune Response Against Sheep Erythrocytes

Antigen. The sheep blood was taken from the jugular vein aseptically in the local government slaughterhouse of Bahawalpur, Pakistan, and preserved in Alsever’s solution at 4°C. Sheep erythrocytes were washed thrice with pyrogen-free phosphate buffer saline (PBS) and adjusted at .5 × 10⁹ cells/ml.21

Induction of Immunogenicity. Wistar albino rats of either sex were randomly distributed in different groups, with six animals in each group. On day 0, all the animals were injected with .1 mL of SRBCs (.5 × 10⁹ cells/mL) followed by different treatments for 7 days. Normal saline (4 mL/kg) was administered to the normal control animals, while levamisole (100 mg/kg) was given as standard immunostimulant and dexamethasone, at the dose of .5 mg/kg, was given as standard immunosuppressant; the treatment groups received Cd.Cr, at the doses of 100, 300 and 500 mg/kg. After 7 days of first exposure to SRBCs (antigen), the animals were subjected to humoral immune response and cell-mediated immunity.

Humoral Immune Response. Blood samples were collected from animals after 7, 14, 21 and 28 days of first exposure to the antigen by puncturing the retro-orbital plexuses and sera were separated by centrifugation at 5000 rpm for 15 min and divided into two portions for further analysis. One part of the sera was subjected to the determination of immunoglobulins (IgG and IgM) through ELISA kits on microplate spectrophotometer (Synergy™ HTX Multi-Mode Microplate Reader, USA) using manufacturer protocols.22 The second portion of sera was utilized for the determination of haemagglutinating antibody titre. 25 μl of test serum was pooled in V-shaped 96-well microtiteration plates and phosphate-buffered saline (PBS) was used to prepare 2-fold serial dilutions of sera. 10% v/v (25 μL) of SRBCs in PBS was poured in the wells and mixed gently followed by incubation at 37°C for 2 h. The agglutination of erythrocytes was determined through serological titrimetry and expressed as percentage of agglutination.23

Cell-Mediated Immune Response by Delayed-type Hypersensitivity (DTH) Reaction. After 7 days of sensitization with SRBCs, the animals were injected, in the right paw, with .5 × 10⁹/mL of SRBCs (50 μL) for second exposure to the same
antigen and development of delayed-type hypersensitivity response. 50 μL of PBS was injected into the left paw to serve as a control for DTH reaction. The paw thickness was determined through digital vernier caliper after 24, 48 and 72 h of paw injection and the difference among right and left paw thickness (mm) was observed to calculate percentage swelling (DTH response to a specific antigen). Furthermore, blood samples were collected after 10, 14, 18 and 21 days of initial immunization with SRBCs and sera were separated and subjected to the determination of INF-γ and IL-2 using ELISA kits.

**Cyclophosphamide-Induced Myelosuppression.** The animals of either sex were divided into six groups, each consisting of six animals. Normal control and negative control groups were administered normal saline (4 mL/kg). One group received levamisole (100 mg/kg) as a standard drug while treatment groups received different doses of Cd.Cr (30, 100 and 300 mg/kg). The treatments were continued orally for 14 days with concomitant administration of cyclophosphamide (30 mg/kg; p.o.) for the last three consecutive days (12th to 14th day). Afterward, blood samples were collected from all animals with subsequent determination of complete blood profile (RBC, WBC, Platelets and Hemoglobin) and sera separation. The sera were used for the estimation of malondialdehyde (MDA) levels, total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities. All oxidative stress biochemical markers were determined through colorimetric assay kits following manufacturer protocols (Elabsciences biotechnology, USA): 5, 5-dithiobis-p-nitrobenzoic acid method for GSH-Px, the xanthine oxidase method at 550 nm for SOD activity, the ammonium molybdate method for CAT activity and thiobarbituric acid (TBA) method for MDA levels in the serum.

**Statistical Analysis**

The statistical analysis of the data obtained from experiments was performed using Graphpad Prism version 8. The results were expressed as mean ± SEM. One-way and two-way ANOVA followed by Bonferroni and Tukey post hoc multi comparisons, respectively, to assess statistical significance in different models, and P value < .05 was considered as significant.

**Results**

**Percent Yield**

About 2.6 kg plant material yielded 364 g of crude extract (14%).

**Phytochemical Analysis**

Qualitative phytochemical studies signify the importance of plants in diverse pathologies as several important phytoconstituents with very high therapeutic values have been reported to be associated with the treatment of various diseases. The chemical analysis of Cd.Cr confirmed the presence of important constituents like alkaloids, glycosides, saponins, terpenes, proteins, tannins and coumarins.

**Antioxidant Activity**

The free radical scavenging assay of Cd.Cr and ascorbic acid (standard) at different concentrations (25, 50, 75, 100, 150, 200, 250, 300 μg/mL) was performed and the results were expressed as percent inhibition of replicates for three readings as mean ± SEM; ie 3.68 ± .7, 18.58 ± 2.08, 30.36 ± 1.13, 45.88 ± 2.51, 57.85 ± 1.27, 65.47 ± 1.62, 72.32 ± 1.31, 72.22 ± 1.94 for Cd.Cr and 16.14 ± .34, 26.2 ± .67, 67.24 ± .96, 75.91 ± .21, 83.91 ± .44, 86.73 ± .56, 87.36 ± .79, 87.6 ± .46 for ascorbic acid, respectively (Figure 1). Moreover, the aqueous methanolic extract of *C. decidua* (Cd.Cr) displayed high total phenolic and flavonoid contents (37.63 ± 3.85 mg GAE/g and 44.34 ± .76 mg RE/g, respectively, of Cd.Cr).

**FTIR Analysis**

FTIR results of Cd.Cr identified and categorized major functional groups based on the peak values of transmittance bands and the spectral features are indicated in Figure 2. The group frequency region (4000-1800 cm⁻¹) reflected that the characteristic broad peak at 3274 cm⁻¹ was due to –OH stretching (mainly of polyphenols) and N–H stretching of major alkaloids, while the minor spectral peak at 2916 cm⁻¹ was indicative of anti-symmetric stretching absorbance representing the C–H stretching frequency of –CH₃ of methyl groups mainly from terpenoids. The fingerprint region (1800-550 cm⁻¹) described the phytochemical composition, especially the moieties of alkaloids, phenolic substances,
terpenoids, carbohydrate and protein secondary structures in the plant extract. A distinctive peak confirmed secondary amide and phenyl ring at the wavenumber of 1610 cm\(^{-1}\) (corresponds to N–H bending and ring C–C stretch). The band at 1395 cm\(^{-1}\) was due to CH\(_3\) asymmetric deformation and the spectral peak region between 1350 and 1150 cm\(^{-1}\) was due to the in-plane C–O stretching vibration (mainly amide) combined with the ring stretch of phenyl ring. The most intense band was observed at 1042 cm\(^{-1}\) due to C–N stretching vibrations. The band shown at 805 cm\(^{-1}\) was caused by ring CH deformation, which probably gave structural evidence regarding polyphenols and saponins.

Table 1. GC-MS analysis of the crude extract of Capparis decidua.

| Compounds                                           | Retention time | Area % | Similarity index (%) | M.W.  | Molecular formula |
|-----------------------------------------------------|----------------|--------|-----------------------|-------|------------------|
| Methyl isothiocyanate                               | 7.944          | 6.81   | 88                    | 73.12 | C2H3NS           |
| 2-Carboxy-1, 1-dimethylpyrroldine (l–stachydrine)   | 11.573         | 7.50   | 95                    | 143.09| C7H13NO2         |
| 2-Isopropyl-5-methylphenol (thymol)                 | 15.808         | 5.48   | 81                    | 150.22| C10H14O          |
| 4-(Propan-2-yl) benzaldehyde (Cuminaldehyde)        | 19.714         | 6.96   | 98                    | 148.20| C10H12O          |
| 7-Hydroxy-2H-chromen-2-one                          | 19.955         | 1.34   | 83                    | 162.1 | C9H6O3           |
| Capric acid                                         | 20.501         | 2.26   | 93                    | 173.26| C10H20O2         |
| Caparratriene (sesquiterpene hydrocarbon)           | 21.893         | 2.19   | 87                    | 206.37| C15H26           |
| 3-Cyclopentylpropionic acid, 2-dimethylaminooethyl ester | 22.014     | 1.14   | 86                    | 213.32| C12H23NO2        |
| Fatty acid (palmitic)                               | 22.518         | 3.28   | 81                    | 256.43| C16H32O2         |
| Fatty acid (linoleic)                               | 22.892         | 4.54   | 84                    | 278.4 | C18H30O2         |
| 3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzyopyran-4-one (flavonoid) | 23.407     | 9.50   | 78                    | 286.24| C15H10O6         |
| Phytol((E)-3,7,11,15-tetramethylhexadec-2-en-1-ol)  | 23.901         | 8.81   | 86                    | 296.5 | C20H40O          |
| Tetramethyleicos-13-ene-17-ol-6,21-olide (terpenolides) | 25.214   | 2.12   | 95                    | 324.50| C21H40O2         |
| Glucocapparin                                       | 25.861         | 3.61   | 90                    | 333.3 | C8H15NO9S2       |
| 2,2'-Methylenebis[6-(1,1-dimethylethyl)-4-] ethylphenol | 28.437   | 2.42   | 85                    | 368   | C25H36O2         |
| Di-n-octylphthalate                                 | 28.996         | 3.03   | 93                    | 390.5 | C24H8O4          |
| β-Sitosterol                                         | 29.562         | 1.19   | 89                    | 414.71| C29H50O          |
| γ-Tocopherol                                        | 31.108         | 3.85   | 94                    | 416.68| C28H48O2         |
| 1,2-Benzenedcarboxylic acid, decylocyl ester        | 31.895         | 1.64   | 83                    | 419   | C26H42O4         |
| Simiarenol                                          | 37.061         | 5.81   | 81                    | 426.7 | C30H50O          |
| Lupeol                                              | 39.904         | 2.28   | 85                    | 426.7 | C30H50O          |
| n-Triacontanol                                       | 40.447         | 3.46   | 88                    | 438.81| C30H62O          |
GC-MS Analysis

Gas chromatography mass spectrometry (GC-MS) analysis was performed to identify and characterize major phytochemicals present in the crude extract of *Capparis decidua*, based on their elution order in HP-5MS column. The methanolic solution of Cd.Cr subjected to GC-MS analysis and the major constituents are presented in Table 1 and Figure 3 with their retention time (RT), concentration (peak area %) and structures. Main identified compounds were 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (9.50%), phytol ((E)-3,7,11,15-tetramethylhexadec-2-en-1-ol) (8.81%), 2-carboxy-1, 1-dimethylpyrrolodine (7.50%), 4-(propan-2-yl) benzaldehyde (6.96%), simiar-enol (5.81), 2-isopropyl-5-methylphenol (5.48%), linoleic acid (4.54%), γ-tocopherol (3.85%), glucocapparin (3.61%), n-triacontanol (3.46%), palmitic acid (3.28%), 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-] ethylenol (2.42%), lupeol (2.28%), capric acid (2.26%), caparatriene (2.19%) and β-sitosterol (1.19%).

Acute Toxicity Assay

Crude extract of *C decidua* (Cd.Cr) was administered as single dose in mice for evaluation of the acute lethal effects and the test animals did not exhibit any change in general behavior. No signs of toxicity were observed during initial 24 h and further no lethality was recorded after 48 h till 14 days, hence, indicated the safety of Cd.Cr up to the dose of 10 g/kg with no mortality.

Macrophage Phagocytic Response

The innate immune response is mainly generated and mediated by phagocytes (macrophages and neutrophils) and their derived cytokines. The phagocytic capacity of reticuloendothelial macrophages has positively been associated with the degree of clearance of systemic circulation from gelatin-stabilized carbon particles. Thus, practical competence of the reticuloendothelial system and its granulopoietic activity can be assessed by determining phagocytic index and rate of carbon clearance. The carbon clearance test was employed in pre-treated rats to evaluate the phagocytic potential of the reticuloendothelial system. A significant dose-dependent potentiation of macrophage phagocytic activity was seen in Cd.Cr-treated rats in comparison with normal control group. The rate of carbon clearance was enhanced in a dose-dependent manner (2.67 ± .15, 6.37 ± .47 and 12.5 ± .70) and the increase in phagocytic index (3.1 ± .04, 3.96 ± .06 and 5.28 ± .13) at the doses of 30, 100 and 300 mg/kg, respectively with highly significant effect at higher doses (Figure 4). Levamisole-treated group (positive control) was also found to significantly enhance the efficiency of phagocytic cells of reticular endothelial system of rats to clear carbon particles from systemic circulation, both in terms of carbon clearance (14.02 ± .64) and phagocytic index (5.21 ± .13).

Neutrophil Adhesion Test

The activation, marginalization, and chemokinesis of granulocytes (especially neutrophils) in systemic circulation were correlated with neutrophils’ interaction...
Table 2. The effects of oral treatment of the crude extract of *Capparis decidua* (Cd.Cr) on the neutrophil function.

| Treatment             | TLC (1000/mm³) | Neutrophil % | Neutrophil index | Neutrophil adhesion (%) |
|-----------------------|----------------|--------------|------------------|------------------------|
| Control (NS, 4 ml/kg) | 8.36 ± .08     | 37.08 ± .8   | 309.7 ± 4.41     | 12.52 ± .86            |
| Cyclophosphamide (25 mg/kg) | 5.51 ± .092   | 36.00 ± .85  | 198.4 ± 6.56***  | 11.82 ± 1.28           |
| Levamisole (100 mg/kg) | 14.18 ± .11    | 49.17 ± 1.13 | 696.9 ± 16.59*** | 40.75 ± 1.0***         |
| Cd.Cr (30 mg/kg)      | 8.5 ± .16      | 37.5 ± .52   | 318.71 ± 7.16    | 15.97 ± .81            |
| Cd.Cr (100 mg/kg)     | 11.02 ± .06    | 46.83 ± 1.19 | 515.8 ± 13.06*** | 27.47 ± .79**          |
| Cd.Cr (300 mg/kg)     | 13.88 ± .07    | 49.50 ± .58  | 686.8 ± 7.22***  | 38.35 ± 1.08***        |

Values are mean ± SEM, n = 6.

*P < .05, **P < .01 and ***P < .001 vs control group.
towards nylon pellets. Cd.Cr (30, 100 and 300 mg/kg)-
treated animals exhibited improved neutrophil adhesion
(15.97 ± .81, 27.47 ± .79 and 38.35 ± 1.08, respectively)
and also elevated neutrophil indices in dose-dependent
fashion, thus showed the stimulant efficacy of Cd.Cr
(Table 2) for neutrophils. The adhesion of neutrophils
(40.75 ± 1.0) to nylon pellets of standard immunostimulant
group was also highly significant from normal control
group and comparable to the group of highest dose of
Cd.Cr-treated animals (300 mg/kg).

Adaptive Immune Response Against
Sheep Erythrocytes

Humoral Immune Response. For the modulation of humoral
response, haemagglutinating antibody (HA) titre and immu-
noglobulins (IgG and IgM) were measured 7, 14, 21 and 28
days after the exposure to antigen (SRBCs), as shown in
Figure 5. Oral treatment of Cd.Cr for 1 week, followed by
SRBCs immunization, elicited humoral response that re-
mained significantly high as compared to the control group.
The improvement in humoral response was found to be dose-
dependent and highly significant (P < .01) as compared to
the control group at respective day. The results, at day 28 after
initial exposure, regarding humoral markers of Cd.Cr (300
and 500 mg/kg) groups, were 62.08 ± 3.36, 137.01 ± 4.10
and 292.91 ± 15.59; and 94.69 ± 3.75, 138.63 ± 5.00 and
336.50 ± 16.38 for HA titre, IgG (ng/ml) and IgM (ng/ml)
levels, respectively. Standard immunostimulant group,
treated with levamisole, also potentiated the humoral
response, for weeks, and showed comparable results as those
of Cd.Cr (500 mg/kg).

Cell-Mediated Immune Response by Delayed-type Hypersensitivity
(DTH) Reaction. Oral treatment of Cd.Cr for 7 days led to
significant (P < .05) and dose-dependent stimulation of DTH
reaction as compared to the control group (Table 4). Percent
change in paw edema is a direct measure of delayed-type
hypersensitivity reaction which is associated with cell-
mediated response, and it was found to be increased per-
sistently, Cd.Cr-administered rats, over 72 h of SRBCs in-
jection in the hind paw, that was comparable to levamisole
group (Table 3). A significant elevation in cytokines level
was seen among control and extract groups over the period of
3 weeks of second exposure to antigen, showing consider-
able stimulation of T cell-mediated immune response. The
control group also showed slight paw inflammation that was
reduced to normal after 3 days (9.27 ± 1.35, 5.60 ± .73 and
2.83 ± .65, after 24, 48 and 72 h). Cd.Cr (500 mg/kg) group
showed remarkable and highly significant (P < .001) levels
of DTH response (48.96 ± 2.06, 43.24 ± 1.83 and
37.52 ± 1.82) which were comparable to the levamisole
group (47.34 ± 3.25, 37.81 ± 2.85 and 32.28 ± 1.92). Serum
cytokines (INF-γ and IL-2) levels were also found elevated in
Cd.Cr-treated rats as compared to the control group (P < .05),
after day 10, 14, 18 and 21 of second exposure of antigen in
hind paw (Table 4). The results of cellular response, after day
28, of Cd.Cr (500 mg/kg) group, were 338.46 ± 5.52 and
402.8 ± 6.76 pg/mL; ie significantly different (P < .001) than
control animals; 103.65 ± 3.63 and 112.24 ± 4.97 pg/mL for
INF-γ and IL-2, respectively.
Cyclophosphamide-Induced Myelosuppression

Cyclophosphamide, at the dose of 30 mg/kg, produced myelosuppression with significant reduction in leukocytes, erythrocytes, platelets and hemoglobin. The administration of Cd.Cr at 30, 100 and 300 mg/kg orally daily for 14 days resulted in the dose-dependent restoration of hematopoietic stem cell activity (Table 5). Cyclophosphamide-induced cytotoxicity was also confirmed by significant declined \( P \leq .001 \) activities of major antioxidant enzymes including GSH-Px (535.7 ± 79.64 U/mL), SOD (33.26 ± 5.29 U/mL) and CAT (15.78 ± 3.14 U/mL), and increased levels of MDA (22.82 ± 1.25 μm/L), exhibited by intoxicated animals as compared to control group (Figure 6). Pretreatment with Cd.Cr (30, 100 and 300 mg/kg, p.o.) significantly \( P < .05 \) restored the serum GSH-Px, SOD and CAT activities in comparison with intoxicated group. The levels of MDA was found significantly reduced \( P < .05 \) by oral treatment of

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**Figure 5.** The effects of aqueous methanolic extract of *Capparis decidua* (Cd.Cr) on humoral immune response; (A) HA titre, (B) IgG and (C) IgM, of rats sensitized with sheep erythrocytes (SRBCs). Values are mean ± SEM, \( n = 6 \), \***P < .001, *P < .05, **P < .01 \) when compared with control group.
levamisole and Cd.Cr at various doses in treatment groups. The results indicated significant preventive ability of Cd.Cr against the chemotherapies-related myelosuppression and oxidative stress leading to secondary immunodeficiency diseases.

Discussion

The immune system consists of an interactive network of cells and mediators that work together to recognize, repel and eradicate pathogens and abnormal host cells. Immune mechanisms integrate together to generate immune responses optimally which together can pull specific elements from different effector organs against the microbes or toxic materials.24 The efficiency of immune competent cells can be increased, using immunomodulatory agents from natural origin (animal or plant), to enhance the immune response of body against pathogens; though, systemic studies are required on these compounds to validate their therapeutic claims regarding their pathogens; though, systemic studies are required on these compounds to validate their therapeutic claims regarding their

Table 3. The effects of the crude extract of Capparis decidua (Cd.Cr) on the delayed-type hypersensitivity reaction in sheep erythrocyte-induced immunogetic rats.

| Treatment                  | 24 h          | 48 h          | 72 h          |
|----------------------------|---------------|---------------|---------------|
| Control (NS, 4 ml/kg; p.o.) | 9.8 ± 1.54    | 5.97 ± 1.2    | 4.33 ± 1.52   |
| Dexamethasone (5 mg/kg; p.o.) | 2.72 ± 0.17***| 3.07 ± 0.38*  | 3.52 ± 2.44   |
| Levamisole (100 mg/kg; p.o.) | 44.51 ± 3.95***| 36.02 ± 1.89***| 31.67 ± 1.88***|
| Cd.Cr (100 mg/kg; p.o.)     | 13.88 ± 1.03  | 13.04 ± 0.98  | 12.37 ± 1.0   |
| Cd.Cr (300 mg/kg; p.o.)     | 26.09 ± 1.82***| 24.13 ± 1.69***| 21.72 ± 1.74***|
| Cd.Cr (500 mg/kg; p.o.)     | 39.02 ± 2.88***| 35.56 ± 2.39***| 32.07 ± 2.67***|

Values are mean ± SEM, n = 6.

*p < .05, **p < .01 and ***p < .001 vs control group.

and the antioxidant assays have revealed the presence of phenolic compounds like flavonoids, tocopherols and phenolic acids as the pharmacologically important phytoconstituents which are thought to be effective as immunomodulatory, anti-inflammatory, cardioprotective, anti-apoptotic, anti-aging and antiangiogenic agents.26 Determination of total phenolic and flavonoid contents showed the presence of flavonoids and phenolics in the crude extract while, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method is used for the evaluation of antioxidant activity. DPPH molecule contains the free radical, capable of accepting electron, and in the presence of antioxidant these radicals would be scavenged and absorbed. The radical scavenging activity of Cd.Cr on the free radicals may be due to the hydrogen donation ability of polyphenols and flavonoids present in the crude extract.27

FTIR spectroscopy has emerged dramatically as a characterization tool of plant metabolites and is now considered as one of the most reliable, sensitive, rapid, non-destructive and economical techniques for the identification of functional groups.28 FTIR and GC-MS analysis of Cd.Cr revealed the presence of various groups of phytoconstituents, especially alkaloids, flavonoids, polyphenols, sterols and fatty acids, and confirmed the results of phytochemical screening and the antioxidant assay. The FT-IR analysis used for the identification of the functional groups of the pharmacologically active components present in crude extract by interpreting the obtained bands in the region of infrared, ie aromatic ring, C-O and –OH (flavonoids and polyphenols). These functional groups represent the primary, secondary and tertiary hydroxyl groups, and on the basis of these chemical bonds, a compound can be determined.29 Phenolic compounds with two aromatic rings bonded by a C3 unit (central pyran ring), identified by FT-IR, indicate the presence of the flavones, glycosides, flavanols (catechins) and chalcones.28 The GCMS analysis showed most important phytoconstituents including isothiocyanate which exhibit anticancer properties,30 capric acid (decanoic acid) reported to upregulate human TNF-α, IL-1β, IL-6 and IL-8,30 β-sitosterol and phytosterol utilized for immunomodulation,31 tocopherol famous for antioxidant and anticancer potential, lupeol down-regulates proinflammatory signaling in macrophages, linoleic and
Table 4. The effects of oral treatment of Cd.Cr on the levels of interferon-gamma (INF-γ) and interleukin-2 (IL-2) in immunogenic albino rats.

| Groups            | Day 10          | Day 14          | Day 18          | Day 21          |
|-------------------|-----------------|-----------------|-----------------|-----------------|
|                   | INF-γ (pg/ml)   | IL-2 (pg/ml)    | INF-γ (pg/ml)   | IL-2 (pg/ml)    |
| Control (NS 4 ml/kg) | 131.17 ± 6.09  | 155.81 ± 5.54   | 135.46 ± 4.96   | 141.47 ± 5.73   |
| Dexamethasone (0.5 mg/kg) | 53.53 ± 2.52** | 46.81 ± 3.97*** | 60.99 ± 3.48**  | 49.56 ± 3.50*** |
| Levamisole (100 mg/kg) | 499.54 ± 4.26***| 546.58 ± 21.8***| 480.17 ± 5.01***| 540.27 ± 11.4***|
| Cd.Cr (100 mg/kg)   | 182.32 ± 4.95*  | 270.96 ± 6.65***| 157.98 ± 3.09*  | 258.34 ± 6.74***|
| Cd.Cr (300 mg/kg)   | 325.89 ± 6.85** | 378.75 ± 6.08***| 309.29 ± 4.91***| 362.68 ± 7.06***|
| Cd.Cr (500 mg/kg)   | 488.77 ± 5.46***| 549.8 ± 13.9*** | 470.48 ± 4.04***| 542.2 ± 16.3*** |

Values are mean ± SEM, n = 6.

*P < .05 significant, **P < .01 more significant, ***P < .001 highly significant; ns: non-significant, when compared with control group.
palmitic acid (fatty acids) possess significant anti-inflammatory actions. 17

The initial response of innate mechanisms is the phagocytosis of antigenic substances by neutrophils, monocytes, macrophages and mast cells. Phagocytic cells recognize the non-self substances using mannose receptors, toll like receptors, complement receptors and Fc receptors, which bind with limited molecular patterns of pathogens, complement proteins after non-selective opsonization and immunoglobulins after selective opsonization. 31 For the induction of phagocytosis, two cellular responses are critical; ie actin polymerization leads to the formation of pseudopodia for the ingestion of pathogen and cytokines (especially tumor necrosis factor-α and interleukin-12), that initiate the inflammatory process. 32 There may be many possible actions on regulator and effector components of mononuclear phagocyte system by which Cd.Cr caused stimulation of the phagocytic functions, but mannose and toll like receptors’ upregulation may be the most important mechanism. Increased expression of cytokines and opsonization of carbon particles may also be involved in the stimulation of reticuloendothelial system.

Neutrophils show immediate response of recruitment and activation to cell injury and inflammation which is driven by the release of proinflammatory mediators, including interleukins, tumor necrotic factor-α, granulocyte and granulocyte-macrophage colony-stimulating factor, by resident tissue macrophages and mast cells. 33 The expression of highly regulated cytokines enhances the adhesion of neutrophils to the endothelium. This action is majorly due to the enhanced affinity of the surfaces of both cells,

![Table 5. The effects of aqueous methanolic extract of Capparis decidua (Cd.Cr) on hematological parameters of rats treated with cyclophosphamide.](image)

| Treatment                  | TLC (10^3/mm^3) | RBCs (10^6/mm^3) | Platelets (10^5/mm^3) | Hemoglobin (g/dl) |
|----------------------------|-----------------|-----------------|----------------------|------------------|
| Control (NS, 4 ml/kg; p.o.) | 8.36 ± 0.08     | 7.78 ± 0.12     | 3.95 ± 0.09          | 13.35 ± 0.26     |
| Intoxicated (Cyclophosphamide, 30 mg/kg; p.o.) | 4.51 ± 0.099*** | 6.46 ± 0.04*    | 2.52 ± 0.07**       | 9.95 ± 0.27***   |
| Levamisole (100 mg/kg; p.o.) | 9.10 ± 0.077**** | 8.04 ± 0.07**** | 4.14 ± 0.05****     | 14.62 ± 0.24**** |
| Cd.Cr (30 mg/kg; p.o.)      | 5.04 ± 0.079    | 6.77 ± 0.05     | 2.74 ± 0.03          | 10.08 ± 0.22     |
| Cd.Cr (100 mg/kg; p.o.)     | 5.89 ± 0.07**   | 6.97 ± 0.08     | 3.13 ± 0.07**       | 11.32 ± 0.20**   |
| Cd.Cr (300 mg/kg; p.o.)     | 8.48 ± 0.15***  | 7.833 ± 0.07*** | 3.77 ± 0.05***      | 12.68 ± 0.24***  |

Values are mean ± SEM, n = 6.
*P < .05, **P < .01 and ***P < .001 vs control group.
#P < .05, ##P < .01 and ###P < .001 vs intoxicated group.

Figure 6. The effects of different doses of the crude extract of Capparis decidua (Cd.Cr) and levamisole on (A) glutathione peroxidase (GSH-Px), (B) superoxide dismutase (SOD), (C) catalase (CAT) and (D) malondialdehyde (MDA) in cyclophosphamide-intoxicated Albino rats. Values are mean ± SEM, n = 5-6, *P < .05, **P < .01 and ***P < .001 vs intoxicated group and #P < .05, ##P < .01 and ###P < .001 vs control group.
Adaptive immunogenic response to antigens is somewhat delayed but specific and orchestrated to either inactivate or eliminate the potentially harmful pathogens. Historically, sheep red blood cells (SRBCs) have been utilized as the gold standard antigen because of the generation of highly specific and reproducible adaptive immune response mainly primary antibody response. Neutralization of viruses, opsonization of pathogens, antibody-dependent cytolyis and initiation of complement-mediated lysis are the hallmark of humoral immune response that involves the synthesis of antigenspecific antibodies. The major contributors for generation and regulation of humoral response are dendritic cells, macrophages, B cells and helper T cells. The improvement in this response, by oral administration of Cd.Cr for 7 days, is evidenced by the increased HA titre and immunoglobulins (both IgG and IgM) against SRBCs. Cd.Cr-treated groups showed the stimulation of antibody production that was found to be persistent after 28 days of exposure to the antigen. Macrophages, T lymphocytes, and their products (cytokines and lymphokines) are the major effector components of cell-mediated immunity, directly involved in the immune-inflammatory reaction, also known as delayed-type hypersensitivity (DTH) reaction. For this purpose, sensitized T cells need to interact with presented antigen and release the proinflammatory mediators like histamine, products of arachidonic acid metabolism and eventually interferon-γ and interleukin 2 leading to DTH. Thus, INF-γ and IL-2 were also determined to establish scientific ground for the development of DTH in SRBCs-induced antigenic rats. The levels of INF-γ and IL-2 were found to be persistently elevated after 14 days of second exposure to the antigen, thus justified the increased DTH reaction in animals, as shown by paw edema. Furthermore, significantly high levels of INF- γ and IL-2 in Cd.Cr-treated animals showed improved efficiency of cell-mediated response towards sheep erythrocytes, as compared to the control group. This increment in cytokines levels may be attributed to increased secretions of proinflammatory mediators and/or enhanced expression of specific genes responsible for their synthesis.

Chemotherapy-induced myelosuppression is one of the significant problems that lead to secondary immune deficiencies altering the intrinsic immune response and leading to enhanced host susceptibility towards opportunistic infections. Hematopoietic toxicity especially leukocytopenia is the main limitation for the therapeutic application of cytotoxic drugs in organ transplants and cancer. One of the major limitations of cytotoxic drugs is the production of large amount of reactive oxygen species (ROS) by directly damaging lysosomes and other ROS containing bodies, which can cause severe damage to the endogenous antioxidant system and lead to significant decline of antioxidant enzyme activities including SOD, CAT and GSH-Px. Thus, the antioxidant enzyme activity can be correlated with the degree of oxidative stress and MDA is one of the most sensitive biomarkers of oxidative stress to cellular lipids. The findings of cyclophosphamide-induced myelosuppression model indicated that Cd.Cr has the potential to inhibit the cytotoxic effects by elevating the GSH-Px, SOD and CAT activities, and decreasing the serum MDA levels, indicating that Cd.Cr could enhance the antioxidant capacity of cyclophosphamide-intoxicated animals. The restoration of hematological parameters and antioxidant enzyme activities in Cd.Cr-treated rats revealed the effectiveness of C decidua against drug (cyclophosphamide)-induced myelosuppression and oxidative stress hence validating its preventive role against secondary immunodeficiencies.

Conclusions

The results of current study provide primary evidence that the crude extract of C decidua not only stimulated the adaptive immune response, as indicated by augmented humoral and cell mediated immunity, but also the innate immunity which was demonstrated by improved phagocytic index and neutrophil adhesion to nylon pellets. C decidua also possesses ameliorative potential against cyclophosphamide-induced myelosuppression in rats through its antioxidant actions. Hence, the study validates that the plant native to the Cholistan desert, C decidua, possesses immunomodulatory potential and justifies its folkloric use against various infectious diseases and malignancies. However, there is a need for further investigations regarding isolation, characterization and biological evaluation of the major constituent(s) and elucidation of detailed molecular mechanism(s) responsible for immunomodulatory potential.

Acknowledgments

Authors are grateful to the department of Biochemistry and Biotechnology, IUB, for providing antigen preparation facility, and Hakeem Lukman, herbal practitioner, Bahawalpur, for providing information about folklore use of Capparis decidua.

Author Contribution

The study was planned and in vivo experimental activities performed by Hafiz Muhammad Farhan Rasheed under the supervision of Professor Dr. Qaiser Jabeen. The authors read the final manuscript in detail.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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