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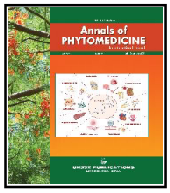

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Evaluation of immunomodulatory potential of *Chenopodium album* L. in cyclophosphamide immunosuppressed rats

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Abstract

Chenopodium album L. is a traditional food as well as a medicinal herb in India due to its anthelmintic, hepatoprotective and anticancerous properties. The objective of this current study was to investigate the immunostimulant potential of the hydroethanolic extract of *C. album* (CAHE) in rats. Adult Sprague Dawley Albino rats were randomly allocated into eight groups. Group I rats were given normal feed and water like other animal groups, group II rats were given cyclophosphamide @100 mg/kg body weight (b. wt.) as a standard immunosuppressant drug, group III was administered with levamisole @ 50 mg/kg b wt. as a standard immunostimulant, in group IV both cyclophosphamide and levamisole were administered, groups V and VI were administered with CAHE @ 200 and 400 mg/kg b. wt., respectively, only and in groups VII and VIII CAHE @ 200 and 400 mg/kg b. wt. was given along with cyclophosphamide @100 mg/kg b wt. on ninth and sixteenth day of investigation, respectively, for twenty eight days. Our study results indicated that CAHE significantly ($p \leq 0.05$) has higher total immunoglobulin, phagocytic index, hemagglutination test, delayed type hypersensitivity and immunoglobulin IgG and IgM estimation. Thus, it can be concluded from the present experiment that *C. album* holds promise as immunomodulatory agent by stimulating both humoral and cellular immune system.

1. Introduction

C. album belongs to the family Chenopodiaceae and is a native herb from Asia, Europe, and Africa. It has popular nutritive significance due to the high amount of protein and mineral content, especially iron (Fe). It has been used for medicinal values since ancient times (Lone *et al.,* 2018). It is used as a treatment for cough and arthritis in the traditional medicine system and is as well effective for diuretic action, antifungal, antiparasitic, hematopoiesis, antimicrobial and anti-inflammatory (Grozeva, 2011; Verma *et al.,* 2020). Herbal medicines are relatively cheaper and safer alternative treatments for various ailments (Mehrotra, 2021; Belachew *et al.,* 2017). The immune system plays a vital role as a defensive system against infectious agents causing diseases in man and animals. Health is directly correlated to the immune status of an animal. The immune system is a balanced state and protective factor, having adequate biological defence to fight infectious diseases or other harmful biological invasions while having adequate tolerance to avert allergy and autoimmune diseases (Ahmad *et al.,* 2022). Immunomodulatory agents are becoming very popular as natural health remedies, which tend to boost immunity and protect from invasion by pathogenic organisms causing diseases in man and animals (Wainwright *et al.,* 2022). Immunomodulation is a special type of mechanism that can modify the immune system of an organism by altering its mechanism. Any substance or agent that is capable of enhancing immune reactions is called an immunostimulant drug. It also includes nonspecific stimulation like granulocytes, macrophages, T-lymphocytes, and various effector substances (Jain *et al.,* 2022).Various factors that suppress the immune system by reducing resistance against infections are mainly attributed to environmental or chemotherapeutic factors (Shivaprasad *et al.,* 2006; Behl *et al.,* 2021). However, the immunomodulatory action of this plant extract has not been properly proven through previous systemic investigations, according to studies. Therefore, the current study has been undertaken to explore the immunomodulatory effect of various doses of hydroethanolic extract of *C. album* in a rat model.

2. Materials and Methods

Analytical-grade chemicals and reagents used in the present study were procured from Hi Media Laboratories Private Limited, Mumbai. Levamisole (Zydus Animal Health, India) and cyclophosphamide (Hi Media Laboratories, Mumbai). Serum IgM and IgG antibody estimation was done by ELISA complete kits (Koma Biotech, Seoul, Korea).

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2.1 Plant collection and preparation of plant extracts

The plant was collected from the agriculture (field) in Pantnagar, Uttarakhand, India.Taxonomically, the plant specimens had been verified by the Botanical Survey of India (BSI) in Dehradun, Uttarakhand, vide No. BSI/NRC Tech./Herb (Ident.)/2016-17/89 dated May 11, 2016.The plant's leaves were dried to make a powder. The extract was prepared from the dried leaf powder, extracted in a 1:1 hydroethanolic solution by the cold maceration method (Handa *et al.,* 2006; Green, 2004).

2.2 Ethical committee clearance

Ethical approval for the studies on animals was obtained from the Animal Ethics Committee of the Institute, Vide No. "IAEC/CVASc./VPT/307 dated February 25, 2015.

2.3 Experimental animals

Studies were carried out using Sprague-Dawley albino rats two monthsold and weighing 200 g. They were obtained from the Central Drug Research Institute, Lucknow. The rats were kept in a standard animal husbandry conditionand were allowed to adlibtum standard pellet feedand water. The animals were acclimatized in the lab for one week prior to the experiments.

2.4 Experimental design

The experimental designs comprised eight groups, with each group having six rats. The duration of the investigation was 28 days. Group I rats were given normal feed and water like other animal groups; group II rats were given cyclophosphamide @100 mg/kg body weight (b. wt.) as a standard immunosuppressant drug; group III was administered with levamisole @ 50 mg/kg b wt. as a standard immunostimulant; in group IV both cyclophosphamide and levamisole were administered; groups V and VI were administered with CAHE @ 200 and 400 mg/kg b. wt., respectively, only and in groups VII and VIII CAHE @ 200 and 400 mg/kg b. wt. was given along with cyclophosphamide @100 mg/kg b wt. on ninth and sixteenth day of investigation, respectively, for twenty eight days. Rats of all groups were closely observed throughout the period of the experiment twice a day for clinical signs and mortality, if any, for 28 days. After the 28th day, tissue and blood specimens were collected from all animals, and serum was separated for various immunobiochemical studies.

2.5 Relative organ weight

The vital organs, which included the spleen, liver, kidneys, and thymus, were surgically removed after the treatment period, cleaned using tissue paper, and their weights recorded. After that, the relative organ wt. (g per 100 g of b. wt.) were calculated as mentioned below.

$$\text{Relative organ weight (g/gm)} = \frac{\text{Absolute organ weight}}{\text{Total body weight}} \times 100$$

2.6 Evaluation of humoral immune functions

2.6.1 Hemagglutination antibody (HA) titer assay

2.6.1.1 Preparation of SRBC

The blood of healthy sheep was collected in a sterile centrifuge tube containing sterile Alsever's solution. Alsever's solution and sheep blood were mixed together in a 1:1 ratio. Blood containing4 ml of Alsever's solution was washed three times in dulbecco phosphate buffer saline solution (DPBS) with the help of a centrifuge at 2000 rpm/10 min. For the purpose of counting RBCs, 1:10 and 1:100 dilutions of a final RBC pack were developed. The final working volume of sheep RBCs was adjusted to 5 x 109 cells/ml (Dai *et al.,* 2017).

2.6.1.2 Immunization of rats

Rats in each group were immunized by intraperitoneal injection of SRBCs in saline (100 µl/rat, approximately 0.5 x 10^9 cells/rat) 7 days priorto the completion of the experimental period (Shuklaa *et al.,* 2009).

2.6.1.3 Titration of hemagglutination serum antibodies

On the 29th day of the experiment, serum was separated from the samples of a previously immunized rat and decomplemented at 56^{o}C for 30 min. These serum samples were then kept at -20^{o}C until the estimation of the titer. To prevent non-specific agglutination, 1% bovine serum albumin (BSA) was added to DPBS which was used for preparing a 1% (v/v) SRBC suspension. A volume of 50 µl of 0.15 Molar phosphate buffer saline (PBS) was placed into all 96 wells microtiter plate (V bottom). Then, 50 µl of test serum was putin to the first well, thoroughly mixed and serially transferred into the next well up to the 12th well. Then, 50 µl of 1% SRBC (v/v) suspension was put in all the wells. The last row served as the SRBC control, which contained PBS but no test serum. The plate was mixed by tapping and incubated at 37^{o}C for 30 min, and then the hemagglutination pattern was observed. The antibody titer was evaluated by taking the reciprocal of the greatest dilution that produced complete hemagglutination (Sharma and Jain, 2018).

2.6.2 Immunoglobulin (Ig) estimation

Total serum immunoglobulin was measured using the zinc sulphate turbidity analysis (Mc Evan *et al.,* 1969).

2.6.3 Zinc sulphate turbidity test

250 mg of anhydrous $ZnSO_4$ were dissolved in 1litre of distilled water. In the control, a 0.1 ml serum sample was added to 6 ml of distilled water, while in the test, a 0.1 ml serum sample was added to 6 ml of $ZnSO_4$ solution. The solution was mixed well and kept for 60 min at room temperature. O.D. was recorded at 545 nm by a UV-VIS spectrophotometer (Bio Rad, USA), and turbidity was determined by the following equation:

Zinc sulphate ($ZnSO_4$) turbidity (ZST units) = Test – Control × 10

Total immunoglobulin (g/dl) = 0.04 + 0.98 ZST units

2.6.4 Estimation of antibody level IgG and IgM

Serum IgM and IgG antibody estimation was done by ELISA complete kits.

2.7 Evaluation of cellular immune function

2.7.1 Sensitization of rat

On the 18th day of exposure, animals were sensitized by subcutaneous administration of 50 µl SRBCs suspended in Freund's complete adjuvant (FCA). Again, SRBCs were separated from sheep blood for the preparation of SRBC-FCA emulsion, SRBCs and FCA were taken in equal proportion (2.5 ml of each appropriate for administration to

rats). In a vial containing 2.5 ml of FCA, a small quantity of SRBC suspension (100 to 500 µl) of the total 2.5 ml was added and mixed uniformly with the help of a 16 G needle fitted into a 5 ml syringe until the water in the oil emulsion resulted. The formation of emulsion was considered when a drop of it remained unspread by spreading on a cold surface.

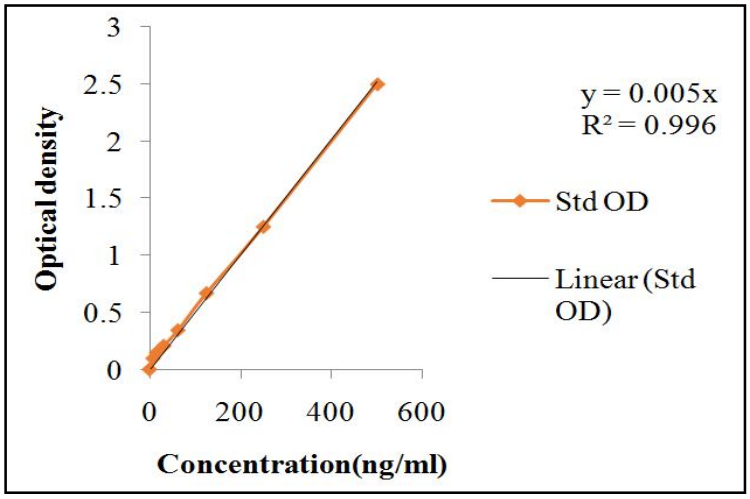

Figure 1: Standard calibration curve of IgM immunoglobulin.

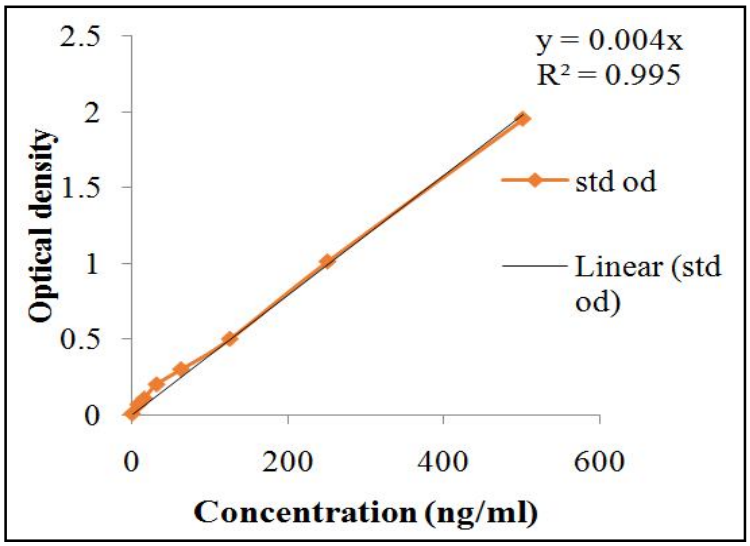

Figure 2: Standard calibration curve of IgG immunoglobulin.

2.7.2 Challenge

Sensitized rats were challenged after 10 days (*i.e.,* on the 28th day) by administering 100 µl of SRBCs (1.5 x 109 cells/ml) into the right hind foot pad by using a tuberculin syringe attached to a 26 G needle. Before administering SRBCs in to the foot pad, animals were mildly anesthetized and foot pad thickness was measured.

2.7.3 Assessment of reaction

The foot pad swelling was recorded by Vernier Calipers at 0 h (before the injection of SRBC) and then at 12 h and 24 h after the challenge (Swarnkar *et al.,* 2019).

2.8 Evaluation of non-specific immune function activity

2.8.1 Phagocytic activity of macrophages

Indian ink with a rate of 0.3 ml/30 g body weight as a source of colloidal carbon was injected intravenously into the tail vein of rats. Each animal's blood sample was taken by the retro-orbital sinus at intervals of 0 and 15 min, following the Indian ink administration. A 50 µl of rat blood sample was mixed properly with 3 ml of 0.1% sodium carbonate solution (Na_2CO_3), and this solution's O.D. was measured at 650 nm. The phagocytic index (K) was measured by the following formula:

$$K = (\text{Log } OD_1 - \text{Log } OD_2)/15$$

where, optical density values are represented by OD_1 and OD_2 at zero and 15 min intervals, respectively (Cheng *et al.,* 2005).

2.8.2 Evaluation of neutrophil adhesion

According to Wilkinson's (1978) methodology, a neutrophil adhesion examination was conducted. The samples of blood were taken from the orbital plexus from anaesthetized (ether) rats of all the groups on

the 29th day. TLC and DLC were determined. After the primary number of cells were counted, animal blood specimens were incubated for 15 min at 37°C with nylon fiber (80 mg/ml of the blood sample), and again, TLC and DLC were determined for each incubated sample. The value of the multiplication of neutrophil per cent and TLC is considered the neutrophil index. Neutrophil adhesion per cent was calculated by employing the following equation:

$$\text{Neutrophil adhesion (\%)} = \frac{\text{NIu} - \text{NIt}}{\text{NIu}} \times 100$$

where, NIu represents the neutrophil index of untreated blood samples and NIt represents treated blood samples neutrophil index.

2.9 Statistical analysis

The results were statistically analyzed by using SPSS statistical software for one-way and two-way analysis of variance (ANOVA). The mean ± SE (standard error) is used to express the results, where p values <0.05 were taken to be statistically significant.

3. Results

Treatment with a higher dose of CAHE in group VI showed significant (p<0.05) increased in the liver, kidney, spleen, and thymus relative organ weight when compared to control group animals. Lower doses of CAHE in group V do not alter the relative organ weight at a significant level, but in comparison to cyclophosphamide immunosuppressed animal group II, they were found to be significant (Table1).

Table1: Effect on relative organ weights (g) following oral administration of CAHE for 28 days in rats

Groups	Treatments	Relative organ weight (g)			
		Liver	Kidney	Spleen	Thymus
I	Control	3.26 ± 0.18^{d}	0.66 ± 0.03^{b}	0.27 ± 0.01^{b}	0.17 ± 0.01^{b}
II	CYP	3.11 ± 0.25^{d}	0.80 ± 0.08^{b}	0.27 ± 0.01^{b}	0.14 ± 0.01^{b}
III	Levamisole	3.75 ± 0.16^{b}	1.04 ± 0.06^{a}	0.36 ± 0.01^{a}	0.18 ± 0.01^{b}
IV	CYP + levamisole	4.72 ± 0.07^{a}	1.03 ± 0.04^{a}	0.26 ± 0.01^{b}	0.18 ± 0.02^{b}
V	CAHE-1	3.36 ± 0.06^{d}	0.73 ± 0.03^{b}	0.32 ± 0.01^{c}	0.26 ± 0.01^{a}
VI	CAHE-2	4.31 ± 0.12^{a}	0.77 ± 0.02^{b}	0.33 ± 0.02^{c}	0.27 ± 0.01^{a}
VII	CYP + CAHE-1	3.39 ± 0.07^{d}	0.66 ± 0.01^{b}	0.34 ± 0.06^{a}	0.17 ± 0.01^{b}
VIII	CYP + CAHE-2	3.01 ± 0.06^{c}	0.65 ± 0.05^{b}	0.33 ± 0.01^{c}	0.18 ± 0.00^{b}

The data were represented as mean ± S.E. (n=6/ group); CYP stand for cyclophosphamide, CAHE stands for hydro ethanolic extract of *C. album*. The comparison of values of different super scripts (a, b, c, and d) within a column differs significantly (p<0.05).

We evaluated the serum concentrations of immunoglobulins, hemagglutination titre, total immunoglobulin, IgM, and IgG for the evaluation of the humoral immune system (Table 2).There was a significant (p<0.05) increase in HA titre levelin CAHE-2 treated rats as compared to control group rats. Treatment with CAHE in CYP immunosuppressed rats showed a significant rise in HA titrein both CYP + CAHE-1 and CYP + CAHE-2 groups when compared to CYP alone treated rats.

Table 2: Humoral immune function test

Groups	Treatments	Humoral immune function test			
		Hemagglutination titre	Total immunoglobulin	IgM (ng/ml)	IgG (ng/ml)
I	Control	5.33 ± 0.42^{d}	2.04 ± 0.02^{c}	372.78 ± 0.87^{c}	448.30 ± 2.64^{b}
II	CYP	0.67 ± 0.21^{h}	0.85 ± 0.02^{f}	249.85 ± 1.94^{e}	343.93 ± 2.39^{d}
III	Levamisole	7.67 ± 0.21^{bc}	3.47 ± 0.01^{a}	438.30 ± 0.92^{a}	624.51 ± 4.00a
IV	CYP + levamisole	1.50 ± 0.62^{g}	1.55 ± 0.08^{d}	396.85 ± 0.41^{b}	399.20 ± 1.84^{c}
V	CAHE-1	7.33 ± 0.21^{c}	2.82 ± 0.08^{b}	402.33 ± 0.95^{b}	450.63 ± 0.92^{b}
VI	CAHE-2	8.50 ± 0.22^{a}	1.88 ± 0.11^{g}	345.53 ± 0.46^{e}	636.76 ± 0.90^{a}
VII	CYP + CAHE-1	3.67 ± 0.49^{f}	1.51 ± 0.10^{d}	253.80 ± 1.58^{d}	211.55 ± 1.17^{e}
VIII	CYP + CAHE-2	4.33 ± 0.67^{e}	1.25 ± 0.06^{e}	252.30 ± 0.50^{d}	406.66 ± 1.78^{c}

The data were represented as mean ± S.E. (n=6/ group); CYP stances for cyclophosphamide, CAHE stands for hydroethanolic extract of *C. album*. The comparison of values of various superscripts (a, b, c, d and e) within a column differs significantly (p<0.05).

CAHE treatment indicated that significant increase intotal immunoglobulin level after the 28th day of the experiment. The maximum significant decrease in total Ig levelwas found in CYP group II, but in levamisole treated group III and low doses CAHE treated group V showed a significant rise in Ig level concentration in comparison to control grouprats.

There was a significant increase in IgM and IgG immunoglobulin in CAHE-2 treated rats as compared to control. The IgG level in CAHE-2 group VI was strongly elevated, similar to the levamisole group rats. Treatment with CAHE in CYP immunosuppressed rats in group VIII also showed a significant rise in IgM and IgG levels when compared to CYP alone treated rats.

Table 3: Cellular immune functions test

Groups	Treatments	DTH	
		After 12 h	After 24 h
I	Control	0.27 ± 0.01^{cB}	0.27 ± 0.01^{cB}
II	CYP	0.17 ± 0.00^{cB}	0.17 ± 0.00^{cB}
III	Levamisole	0.49 ± 0.01^{bA}	0.49 ± 0.01^{bA}
IV	CYP + levamisole	0.39 ± 0.13^{bB}	0.39 ± 0.13^{bB}
V	CAHE-1	0.73 ± 0.10^{aB}	0.73 ± 0.10^{aB}
VI	CAHE-2	0.28 ± 0.09^{cB}	0.28 ± 0.09^{cB}
VII	CYP + CAHE-1	0.73 ± 0.04^{aA}	0.73 ± 0.04^{aA}
VIII	CYP + CAHE-2	0.77 ± 0.06^{aA}	0.77 ± 0.06^{aA}

The data were represented as mean ± SE (n = 6/ group); CYP stances for cyclophosphamide, CAHE stands for hydroethanolic extract of *C. album*. The comparison of values of different superscripts (a, b, and c) within a column and values (A, B, and C) with in row differs significantly ($p<0.05$).

Cellular immunity was measured by delayed type hypersensitivity (DTH) test. There was a significant ($p<0.05$) foot pad thickness (mm) increased after 12 h in group VIII, followed by in group VII, as compared to control animals. Similarly, after 24 h, foot pad skin swelling was ($p<0.05$) significantly reduced in CYP group II and significantly higher in CYP immunosuppressed CAHE treatment groups VII and VIII as compared to control. CAHE-1 showed a significant increase in the thickness of the foot pad as compared to CAHE-2 after 12 h and 24 h (Table 3).

The evaluation of nonspecific immune functions was measured by the neutrophil adhesion test and the phagocytic activity index (Table 4). The impact on the phagocytic activity index was measured by a carbon clearance examination. A significant ($p<0.05$) rise in phagocytic activity index was found in CYP immunosuppressed rats treated with a high dose of CAHE in group VII in comparison to CYP group II.

A significant reduction in per cent neutrophil adhesion was observed in the CYP group II and CYP + CAHE-1 group VII. Levamisole treated group III and lower doses of CAHE-1 group V revealed a significant ($p<0.05$) rise in per cent neutrophil adhesion as compared to animals of the control group which indicates a reversal of the cyclophos-phamide induced effect on neutrophil adhesion by levamisole and CAHE.

Table 4: Nonspecific immune function test

Groups	Treatments	Nonspecific immune functions test	
		Phagocytic index	Neutrophil adhesion
I	Control	0.06 ± 0.00^{a}	45.92 ± 0.36^{d}
II	CYP	0.02 ± 0.00^{d}	25.65 ± 1.00^{h}
III	Levamisole	0.06 ± 0.00^{a}	59.12 ± 4.41^{c}
IV	CYP + levamisole	0.03 ± 0.00^{c}	30.22 ± 0.86^{f}
V	CAHE-1	0.06 ± 0.00^{a}	71.70 ± 1.71^{a}
VI	CAHE-2	0.06 ± 0.01^{a}	28.71 ± 2.25^{g}
VII	CYP + CAHE-1	0.02 ± 0.00^{d}	62.89 ± 0.80^{b}
VIII	CYP + CAHE-2	0.04 ± 0.00^{b}	40.18 ± 2.48^{e}

The data were represented as mean ± SE (n=6/ group); CYP stances for cyclophosphamide, CAHE stands for hydroethanolic extract of *C. album*. The comparison of values of different superscripts (a, b, c, d, and e) within a column differs significantly ($p<0.05$).

4. Discussion

Herbs, herbal formulations, and their extracts have been utilized to preserve health in line with the natural environment since the beginning of human civilization. Because of safety, the traditional method of administering and using botanicals and galenicals remains to be popular. Herbal medicines provide safety and security for human health without any negative side effects (Makhuvele *et al.,* 2020; Tiwari *et al.,* 2023). Increasing protective immune functions

without any harmful toxic effects is a major challenge. The study revealed that significant results from both specific and non-specific immune function test. Our studies using HA and total immunoglobulin for humoral immune response found a significant ($p<0.05$) enhancement in humoral immunity in rats treated with CAHE for both HA titer and total immunoglobulin level in comparison to cyclophosphamide immunosuppressed rats. A higher dose of CAHE produced a better HA titre than levamisole, indicating a more potent immunomodulatory response (Amhamed *et al.,* 2018). Our data are consistent with those of Suseelan *et al.* (2001), who reported that the extract of *Chenopodium* showed HA titre against bacterial strain of Gram-negative and Gram-positive. Humoral immunity encompasse-santigen interaction with B cells, resulting in subsequent proliferation and distinction into plasma cells that secrete antibodies. The humoral immune responses react with antigens and neutralizing them or enhancing their elimination by the occurrence of cross-linking resulting formed clusters that are engulfed by phagocytic cells. Antigen requisite fragments and immunoglobulins are important role in humoral immunity, which is a major part of glycoproteins and amino acid chains. Most of these essential components are naturally present in CAHE. Copper is an essential trace metal that is also present in CAHE, it required for the regulation of cerruloplasminenzymes that play an important role in the humoral immune response (Amhamed *et al.,* 2018). Other phytoconstiuents of CAHE like fatty acids, zinc, manganese, and selenium and vitamins like B6, B12 and C, are also essential for the B-lymphocytes maturation in the bone marrow (Poonia and Upadhayay, 2015; Sangeeta *et al.,* 2023). Meanwhile, our data are consistent with those of Fan *et al*. (2019), who reported that the treatment with the aqueous extract of *Chenopodium quinoa* L. enhanced the total immunoglobulin and IgM immunoglobulin levels in cyclophosphamide immunosup pressed mice.

Flavonoids have immunomodulatory potential in the immune system. It is responsible for the regulation of nuclear transcription factor activity, innate and acquired immune cells, the production of antibodies, and cytokines secretion (Baranowski *et al.,* 2022; Bamne *et al.,* 2023). The results suggest that flavonoids can help in reduction of pro-inflammatory immune reactions. Quercetin is an abundant polyphenol in nature, found in rich quantity in *C. album* (Odegaard and Chawla, 2013; Seetharamu *et al.,* 2023). This flavonoid effectively reduces the MHC class II and important co-stimulatory molecules, whereas increasing the synthesis of chemokines that promote inflammation (Li *et al.,* 2016). Quercetin also regulates the suppression of antigen specific T-cell activation by decreasing the functional activity of LPS stimulated dendritic cells (Hosseinzade *et al.,* 2019).

Delayed type hypersensitivity (DTH) is a type IV cell-mediated cellular immunity. The test provides a functional determination of the cell-mediated immune response**.** In which intradermally inoculate an antigen and evaluate the skin reaction response. After intradermal inoculation of the antigen, which depends on antigen-specific memory T-cells and obtained results were due to the recruitment of mononuclear cells (MNCs) and neutrophils(Kamboh *et al.,* 2018). When T cells are stimulated, lymphokines are released, which activate and aggregate macrophages, increase vasodilatation, create vascular permeability, and promote inflammation (Nfambi *et al.,* 2015). It also promotes phagocytic activity and raises the level of lytic enzymes for active neutralization of microorganisms (Hosseinzade *et al.,* 2019).The increment in footpad thickness that was subjected to CAHE in this experiment could be attributed to the capability of the plant extract to stimulate lymphocyteaugmentation in the production of antibodies in the previously immune compromised animals due to potentially increasing cell-mediated immunity (Banji *et al.,* 2012).

The removal of harmful microorganisms or solid particles is accomplished mainly through phagocytosis. Neutrophils, monocytes and macrophages carried out a multiple essential functions including chemotaxis, phagocytosis, and intra and extracellular obliteration of antigen fragments. After ingesting a particle or cell, the phagosome combines together with a lysosome and forms a phagolysosome. The harmful pathogens are 'digested' by enzymes and peroxides (Muller *et al.,* 2005). The present study found that CAHE treatment in animals caused increased clearance and uptake of colloidal particles from the blood stream due to flavonoids like quercetin andrutin, which are present in plant extract (Yýlmaz *et al.,* 2019). Thus, CAHE treatment appeared to increase the phagocytic process, as evident by the enhancement of carbon particle clearance. Our results are also in line with the literature in that the *Chenopodiumam brosioides* L. extract increased a significant antiradical scavenging activity; fish splenocytes responded potentially with greater cell viability than control group (Reyes *et al.,* 2019). Neutrophils perform an essential function in cell-mediated immunity. They quickly identify foreign bodies and eliminate foreign invaders by phagocytosis (Hirayama *et al.,* 2017). Neutrophils adhering to nylon fibers is sign of 'migration of cells in the blood' that reach towards the inflammation site 'such a result is likely attributable to the upregulation of special types of β2 integrin proteins (found on the surface of neutrophils and lymphocytes), which were extremely adhesive to nylon fibers (Pravansha *et al.,* 2012; Gupta *et al.,* 2010). This study confirms a considerable increment in the adhesion of neutrophils due to foreign particles. The increased adhesion of neutrophils could be attributed to the presence of several kinds of phytochemical, macro and micronutrients in CAHE (Kamboh *et al.,* 2018). Various experiments on *C. album* have confirmed that it contains vitamins, fatty acids, essential amino acids, and trace metals, all of which have an essential role in the regulation of neutrophils in the cell-mediated immune response (Gqaza *et al.,* 2013; Pachauri *et al.,* 2017).

5. Conclusion

The present findings of the study revealed the administration of a hydroethanolic extract of *C. album* (CAHE), is responsible for both humoral immunity and cell-mediated immunity in rats. The plant extract significantly increases the immune response by increasing immunoglobulin levels and lymphocyte proliferation. CAHE exhibited protective effects against cyclophosphamide-induced immunotoxi-city, suggesting its role as a potential safeguard against chemotherapy-induced immune suppression. In some tests, lower doses of CAHE were found to be more efficacious than the higher dose. This could be due to the phytochemicals and essential nutrients found in the plant. Thus, CAHE has a potential therapeutic value in certain immunosuppressing clinical conditions. It may be further investigated as a potential therapeutic candidate for autoimmune diseases such as Graves' disease, rheumatoid arthritis, multiple sclerosis, and anticancer activity.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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