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Ameliorative effect of *Chenopodium album* in cyclophosphamide-induced oxidative stress and hematologic toxicity

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Abstract
Cyclophosphamide (CYP) is one of the commonly used anticancer drugs, but its use is limited by hematologic toxicity. *Chenopodium album* passing hepatoprotective, gastroprotective, cardioprotective, anti-inflammatory, and renal protective potential but its myeloprotective potential is underexplored. The aim of this study was to evaluate the ameliorative effect of Chenopodium album in cyclophosphamide-induced oxidative stress and hematologic toxicity in albino rats. CAHE @ 400 mg/kg body weight, po daily for 28 days and Cyclophosphamide @ 100 mg/kg body weight, po on 9th and 16th day given alone and with a combination of both in rats. At the end of the experiment, animals were humanly killed, and blood and liver samples were collected and stored for hematologic, and oxidative stress marker level estimations. Hematologic findings revealed hemoglobin and lymphocyte increases significantly ($P<0.05$) by the treatment of CAHE treatment significantly ameliorated oxidative stress parameter level. Treatment with CAHE significantly ($P<0.05$) reversed the hematologic toxicity and oxidative stress. However, more detailed studies are needed to explicate the mechanism underlying its protective effect.

Keywords: Cyclophosphamide, *Chenopodium album*, hematologic toxicity, oxidative stress

Introduction
Cyclophosphamide is an immunosuppressive alkylating agent, commonly used in cancer chemotherapy for the treatment of various medical problems, such as autoimmune diseases, lymphocytic leukemia, and neuroblastoma, and following organ transplantation [1]. Cyclophosphamide drug is not cell-cycle phase-specific and metabolizes to an active form of acrolein and phosphoramide [2]. Its cytotoxic effects are the results from acrolein and phosphoramide mustard which are chemically reactive metabolites that are activated by hepatic cytochrome p450 enzymes and alkylate DNA as well as protein, producing cross-links, resulting in the oxidative stress that mediated cellular damage [3]. CP cytotoxicity has been attributed to the toxic metabolites, acrolein, and phosphoramide produced during its metabolism. Acrolein can bind to reduced glutathione (GSH) leading to increased production of reactive oxygen species (ROS) and subsequently oxidative stress and lipid peroxidation, leading to the depletion of cellular defense mechanisms [4]. The phosphoramide metabolite forms cross-linkages within and between adjacent DNA strands at the guanine N-7 position. These modifications are permanent and eventually lead to programmed cell death [5]. CP has a pro-oxidant nature, which can cause oxidative stress, inflammation, and apoptosis in rats [6].
Medicinal herbs have attracted attention in recent years as an alternative source to pharmacologically active agents including antibiotics, chemotherapeutics, and other synthetic compounds [7]. An herb of immunomodulatory and antioxidant activities are becoming a viable novel approach for the treatment of immunological diseases and for protection from oxidative damage, among these herbs is *Chenopodium album* commonly known as White goosefoot, Bathua, and Wild spinach which belongs to the Amaranthaceae family. This is an annual weed with branched and erect stem, Flowers are aggregated into dense clusters in leaf axils and Leaves are alternate, starchy white underneath and twice as long as wide. They are usually oval to obovate shaped, more or less toothed or lobed edges [8].
Chenopodium album has acquired a special position in traditional medicine because of its unique active constituents, namely, minerals (Mg, Ca, K, P, Na Zn, Cu, Fe, Mn), vitamins (A,C, Carotenoid) and tannin, fiber, carbohydrate, protein, essential amino acids (lysine, isoleucine, leucine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine and

methionine), flavonoids (3-O-glycosides of caempferol, isoramnetin, and quercetin) [9, 10]. Which possess interesting protective effects against hypercholesterolemia, atherosclerosis, hepatotoxicity, nephrotoxicity, oxidant stress, and anti-parasitic [11-13]. In response to these findings, our study was designed to investigate the impact of hydroethanolic extract of *Chenopodium album* (CAHE) against hematological disturbance and hepatic oxidative damage induced by Cyclophosphamide administration in rats.

Material and Methods

Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade and procured from Hi Media Lab. Pvt. Ltd., Mumbai. Autospan diagnostic kits from Arkray Healthcare Pvt. Ltd. Gujrat, India were used for the determination of creatinine and blood urea nitrogen.

Experimental animals

Twenty four 8-weeks old male Sprague dawley rats were used as experimental animals in the present investigation. The rats were obtained from the National Laboratory Animal Center (NLAC) of CSIR-Central Drug Research Institute, Lucknow and were housed in standard cages, given free access to a standard diet and water, and maintained on a 12 h dark/light cycle at 23 ± 2 °C. All animal procedures and treatments were in accordance with the guidelines of the Institutional Animal Ethics Committee of G.B. Pant University, Uttarakhand. Accredited by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi vide approval number IAEC/CVASc./VPT/307.

Plant material

Aerial parts of *Chenopodium album* collected from the agricultural field of G.B. Pant University, Pantnagar. The plants were taxonomically authenticated by the Botanical Survey of India (BSI), Dehradun vide No. BSI/NRC Tech./Herb (Ident.)/2016-17/ 89.

Extraction and fractionation

The extract was prepared by extraction in a hydroethanolic solution in a ratio of 1:1. The extracts were prepared by cold maceration method as described by Handa *et al.* (2008) [14], Green (2004) [15].

Experimental design

The rats were randomly allocated into five groups of 6 rats each as follows:

Group I (Control): Rats received 1 ml Distilled water, po

Group II (CYP): Rats received Cyclophosphamide @ 100 mg/kg b wt, po on 9th and 16th day.

Group III (CAHE): Plant extract @ 400 mg/kg b wt, po daily for 28 days.

Group IV (CAHE+CYP): Cyclophosphamide @ 100 mg/kg b wt, po on 9th and 16th day + Plant extract @ 400 mg/kg b wt, po daily for 28 days.

The doses of CAHE and CYP were selected based on previous studies [16] and the dose was adjusted according to body weight changes.

Collection and preparation of samples

At the 29th day, all rats were sacrificed under anesthesia and blood and kidney samples were collected. Serum was separated from blood samples and used for assaying creatinine and urea. The kidneys were washed in cold phosphate buffered saline (PBS) and homogenized (10% w/v) in cold PBS, centrifuged and the clear homogenate was used for biochemical assays.

Hematological examination

Erythrocyte count (RBCs), hemoglobin (Hb) concentration, packed cell volume (PCV), and blood indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and total leukocytes (TLC) were determined by automated hematology analyzer [17].

Oxidative stress

Malondialdehyde (MDA)

This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm, an MDA standard was used to construct a standard curve against which readings of the samples were plotted [18].

Catalase (CAT)

Determination of tissues CAT enzyme activity: it assayed by the method of Sinha which based on the formation of chromic acetate from dichromate and glacial acetic acid in the presence hydrogen peroxide, chromic acetate that produced was measured colorimetrically at 570 nm, one enzyme unit was defined as the amount of enzyme which catalyzed the oxidation of 1 μmole H2O2 per minute under assay conditions [19].

Glutathione peroxidase (GPX)

Tissues GPx enzyme activity: it was measured as IU/gm wet tissue by the reaction between glutathione remaining after the action of GPx and 5, 5-dithiobis-(2- nitrobenzoic acid) to form a complex that absorbs maximally at 412 nm [18].

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). All statistical comparisons among groups were determined by one-way ANOVA. A P value<0.05 was considered significant.

Results

The erythrogram result in Table 1 and 2, revealed that Hb concentration, PCV, TEC count, and TLC were significantly reduced in response to cyclophosphamide, compared with the control group of rats. Conversely, they were significantly increased upon CAHE treatment in the CYP & CAHE group compared with CYP treated rats. Additionally, the leukogram result showed that CYP significantly lowered the total leukocyte and lymphocyte counts and increased (P<0.05) the neutrophil, basophil, monocyte, and eosinophil counts as compared to the control group. On the other hand, treatment of CYP & CAHE group improved the alterations significantly (P<0.05) in the leukogram induced by CYP except for the neutrophil, monocyte, and basophil count which was changed insignificantly in comparison with the CYP-treated group. In CAHE group significantly increases hemoglobin, lymphocyte, and monocyte as compared with the control group except that the eosinophils, basophils, and total leucocyte count (Table1 & 2).

The Result of erythrocytic indices in Table 3, revealed, that MCV, MCH, and MCHC were significantly reduced in CYP-treated rats as compared with the control one. No significant alteration occurs in the CYP+CAHE group except MCHC as compared to CYP treated rats. In the CAHE group MCV, MCH and MCHC levels significantly increased as compared with control and CYP treated rats.

The result of oxidative stress markers Levels in Table 4 revealed that MDA Levels were significantly (P<0.05) higher in cyclophosphamide treated group II as compared to the control group. No significant alteration occurs in CAHE treatment group III and group IV but the level of MDA in group IV reduces significantly as compared to group II. The Levels of CAT and GPX were significantly (P<0.05) lower in cyclophosphamide treated group II and cyclophosphamide with CAHE group IV as compared to the control group. No significant alteration occurs in CAHE treatment group III except GPX.

Table 1: The effect of hydroethanolic extract of *Chenopodium album* (CAHE) on hematological parameters

Groups	Treatments	Heamoglobin (g/dl)	PCV (%)	TEC (× 10^6/µl)	TLC (×10^2/µl)
I.	Control	12.07±0.13^a	46.23±0.43^a	7.13±0.14^a	8.00±0.10^b
II.	CYP	10.25±0.20^b	36.43±0.86^d	5.55±0.20^d	3.12±0.08^d
III.	CAHE	13.87±0.05^c	43.98±0.47^e	6.58±0.06^b	8.35±0.15^b
IV.	CYP+CAHE	12.52±0.19^a	40.08±1.57^f	6.82±0.08^e	4.57±0.25^c

Values in the table are mean ±S.E., Values having different superscripts (a, b, c, d, e, f, g) differ significantly (P<0.05) when compared within a column.

Table 2: The effect of hydroethanolic extract of *Chenopodium album* (CAHE) on differential leucocyte count (%)

Groups	Treatments	Lymphocyte	Neutrophil	Monocyte	Eosinophil	Basophils
I.	Control	71.82 ±0.19^e	22.62 ±0.93^f	3.64 ± 1.04^b	1.37±0.20^c	0.57 ±0.20^b
II.	CYP	48.63±3.60^d	41.07 ±3.94^a	4.82 ± 0.46^c	4.18 ±0.64^a	1.30 ±0.22^c
III.	CAHE	73.40±1.60^a	18.00±1.78^e	5.89 ± 0.47^a	1.63 ±0.31^c	1.10 ±0.32^c
IV.	CYP+CAHE	63.33±1.26^c	27.68±1.10^b	5.65 ± 1.10^a	3.48 ±0.55^d	1.52 ±0.35^a

Values in the table are mean ±S.E., Values having different superscripts (a, b, c, d, e, f, g) differ significantly (P<0.05) when compared within a column.

Table 3: The effect of hydroethanolic extract of *Chenopodium album* (CAHE) on Erythrocytic indices

Groups	Treatments	MCV (Femto litre)	MCH (Picogram)	MCHC (%)
I.	Control	64.97±1.68^c	19.62±0.41^b	30.23±0.4^b
II.	CYP	59.87±1.75^f	18.62±0.92^g	28.22±0.90^c
III.	CAHE	66.83±0.79^h	21.07±0.18^a	31.55±0.37^a
IV.	CYP+CAHE	58.84±2.37^f	18.39±0.46^g	31.43±1.09^a

Values in the table are mean ±S.E, Values having different superscripts (a, b, c, d, e, f, g) differ significantly (P<0.05) when compared within a column.

Table 4: The effect of hydroethanolic extract of *Chenopodium album* (CAHE) on oxidative stress

Groups	Treatments	MDA	CAT	GPX
I.	CONTROL	0.78±0.04^{bc}	19.94±0.58^a	22.49±0.83^a
II.	CYP	1.59±0.15^a	11.90±0.56^c	10.01±0.47^d
III	CAHE	0.76±0.06^c	20.54±0.56^a	17.57±0.39^b
IV	CYP+CAHE	1.16±0.11^b	15.20±0.30^b	15.38±0.41^c

Values in the table are mean ±S.E, Values having different superscripts (a, b, c, d, bc) differ significantly (P<0.05) when compared within a column.

Discussion

Cyclophosphamide is a chemotherapeutic agent effective against various cancers with limitation in use resulting from ROS production that leads to peroxidative damage and toxicity particularly in hepatic tissue that leads to oxidative stress [20]. However, its use has been limited due to severe toxicity several studies have focused on the use of natural products that possess antioxidant activity to protect against Cyclophosphamide-induced hepatotoxicity [21, 22]. Cyclophosphamide treated rats groups showing a significantly decreased level of Hb, PCV TEC granulocyte, and lymphocyte as compared with the control group animal. In a group of CAHE with Cyclophosphamide level of the hematological parameter normalize significantly. Treatment of CAHE proves ameliorative activity in albino rats its due to CAHE have highest nutritional value in which present several elements like Ca, Fe, Zn and vitamin c. that is responsible for the alteration of hematologic toxicity [8].

In this study, we also aimed to evaluate the levels of oxidative stress indicators, MDA, CAT and GPX in rats for ameliorative effect of *Chenopodium album* in cyclophosphamide-induced oxidative stress. The data presented in this study showed that cyclophosphamide increased lipid peroxidation in hepatic tissues as expressed by increased tissue levels of MDA and lower levels of CAT and GPX in cyclophosphamide treatment group. The plant alone group showed similar results as control group. In the cyclophosphamide with CAHE treatment group level of oxidative parameter not significantly normalize but it increases to normal level. Our results were in agreement with

many authors [23, 24].

CAT and GPX constituted a mutually supportive team of defense against reactive oxygen species. Lipid peroxidation (MDA) is caused by free radicals such as NO. MDA, the product of lipid peroxidation, has toxic effects on cell membranes and cells [25]. The increased lipid peroxidation leads to inactivation of the enzymes by crosses linking with MDA; this will cause an increased accumulation of superoxide, H2O2, and hydroxyl radicals which could further stimulate lipid peroxidation. [26]. Cyclophosphamide can cause cell injury due to increased lipid peroxidation resulting from the pressure of the large body mass. Cell injury causes the release of cytokines, especially tumor necrosis factor-alpha (TNF-a) which generates ROS from the tissues which in turn causes lipid peroxidation [27]. Mitochondrial dysfunction can also lead to apoptosis or necrosis depending on the energy status of the cell. Finally, ROS and lipid peroxidation products also activate stellate cells, thus resulting in fibrosis [26, 28]. Previous studies documented that pretreatment of quercetin that is present in CAHE significantly improved the hepatic levels of catalase and GSH and decrease the hepatic MDA levels suggesting that quercetin was able to protect the liver from the deleterious effects of melphalan through scavenging the free radicles and enhancing the hepatic antioxidant enzymes SOD and catalase [29].

Conclusion

The current finding shows a clear defensive mechanism of a hydroethnolic extract of *Chenopodium album* (CAHE) against hematologic toxicity and oxidative stress induced by cyclophosphamide and suggesting that *Chenopodium album* may be used to enhance hematinic activity. Hence, the consumption of *Chenopodium album* in diet can be a good source of functional nutrients that protect the body against cyclophosphamide-induced hematologic toxicity and oxidative stress.

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