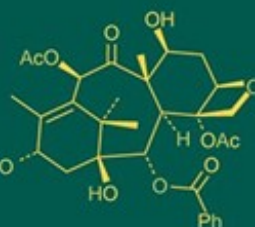
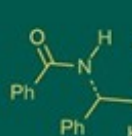
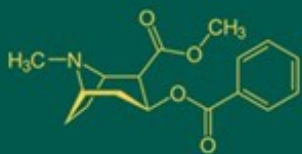


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Phytochemical analysis and protective effects of *Cuminum cyminum* hydroethanolic extract against cyclophosphamide induced body weight loss in Sprague–Dawley rats

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ABSTRACT

Cuminum cyminum is a fast-growing annual herbaceous plant, belonging to the family Apiaceae of the genus *Cuminum*. Cumin seeds have carminative, aromatic, stomachic, stimulant and astringent properties. Cyclophosphamide (CYP) is a highly effective chemotherapeutic and immunosuppressive drug, but the drawbacks of its use include weight loss and lymphoid toxicity. The phytochemistry of a 60:40 hydroethanolic extract of *Cuminum cyminum* seeds (CCHE) was screened and its effect on the body and organ weights changes induced by CYP were assessed in female Sprague-Dawley rats ($n = 6/\text{group}$; eight groups). The yield of the extract was found to be 16.10% (w/w). The alkaloids, glycosides, carbohydrates, tannins, flavonoids, phenolics and terpenoids were detected in the plant using qualitative screening, but not the proteins or saponins. On day 28, CYP (100 mg/kg, days 9 and 16) caused a decrease of 13.0% in body weight compared to control and decreased thymus weight. Co-administration of CCHE (200 or 400 mg/kg) improved body weight at day 14 when compared to CYP alone. While CCHE enhanced spleen and thymus weights, it also significantly affected liver, lung, and brain weights as well as weight gain at 400 mg/kg, which needs to be explored further. These findings suggest potential modulation of the CYP-related loss of weight and lymphoid organs.

Keywords: *Cuminum cyminum*, Cyclophosphamide (CYP), Immunomodulation, Phytochemical

Introduction

Cuminum cyminum L. Commonly called cumin (family Apiaceae), it is widely grown across the Indian sub-continent and Mediterranean basin. Cumin seeds are traditionally used as culinary spice and carminative, but preliminary studies show that the seeds contain a diverse array of secondary metabolites that may possess antioxidant, cytoprotective and immune-modulatory effects.

Cumin seeds are known for their potent biological properties. Some of the key biological activities attributed to cumin seeds are its antifungal (Skrinjar *et al.*, 2009) [1], anti-carcinogenic (Gagandeep *et al.*, 2003) [2], anti-thrombotic (Dhandapani *et al.*, 2002) [3], antidiabetic, antioxidant (Ferrie *et al.*, 2011) [4] and antibacterial (Morton, 1976) [5] properties. Cumin seeds have been used traditionally for toothache, dyspepsia, diarrhea, epilepsy and jaundice (Eikani *et al.*, 1999; Nostro *et al.*, 2005) [6, 7] and possess diuretic, carminative and antispasmodic properties (Janahmadi *et al.*, 2006; Singh *et al.*, 2002) [8, 9]. The plant extract has different chemical constituents like Alkaloids, Anthraquinone, Coumarin, Flavonoid, Glycoside, Protein, Resin, Saponin, Tannin and steroid (Rai *et al.*, 2012) [10]. Organic acids (citric, aspartic, tartaric, propionic, malic, ascorbic, oxalic, maleic and fumaric) were also isolated from *Cuminum cyminum* seed. Volatile oil content in cumin fruits is 2.5-4.5 % and fixed oil content is up to 10 % (Shivakumar *et al.*, 2010) [11]. Previous investigations of Indian grown *Cuminum cyminum* fruit oil revealed that it contained a significant amount of trans-dihydricarvone (31.11%), γ -terpinene (23.22%) and p-cymene (15.8%) and very little of cuminaldehyde (0.58%) and α -phellandrene (12.01%) (Chaudhary *et al.*, 2014) [12].

The present study investigates the phytochemical profile of a 60% hydroethanolic extract of *Cuminum cyminum* seeds (CCHE) and evaluates its protective efficacy against CYP-induced systemic wasting and organ toxicity in female Sprague–Dawley rats, benchmarking its performance against the reference immunos-timulant levamisole.

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Materials and Methods

Plant material and extract preparation

Cumin (*Cuminum cyminum*) seeds were bought from a registered local market and the seeds were cleaned, dried in a dark place and milled to a coarse powder.

Preparation of plant extract

Seeds were dried and ground to obtain a coarse powder for the preparation of the hydroethanolic extract. 500 grams of *Cuminum cyminum* seed powder was allowed to soak in 2000 mL hydroethanol (60:40) in

stoppered container for 24 hours with frequent agitation at room temperature. The hydroethanolic extract was collected, filtered and then concentrated in rotatory vacuum evaporator at a temperature of 40 °C and finally stored for further analysis. The concentrated hydroethanolic extract was further subjected to phytochemical screening.

Qualitative phytochemical screening

The resultant extracts were subjected to phytochemical testing according to standard tests.

S.no.	Phytochemical	Procedure	Interpretation
1.	Alkaloids	Extract + 5 ml dilute HCl and filtered	Mayer's reagent Appearance of white precipitate
2.	Glycosides	Extract + dil HCl and filter	3 ml chloroform + ammonia solution Pink colour
3.	Carbohydrate	1 ml of extract	Molisch reagent + sulphuric acid Red/ Violet colour
4.	Saponin	2 ml of extract	Distilled water and shake Persistent foam
5.	Tannin	2 ml of extract	Ferric chloride Brownish blue/ black colour
6.	Flavonoids	0.2 g extract + 2 ml methanol and heated	Mg metal + conc.HCl Orange to red colour
7.	Phenolic compounds	2 ml extract	2 ml of 5% ferric chloride Blue colour
8.	Terpenoids	2 ml extract+ 1 ml chloroform	Conc sulphuric acid Reddish brown precipitate
9.	Proteins and amino acids	2 ml extract	Millon's reagent Red colour

Quantitative phytochemical assays

Total phenolic content

Total phenolic content (TPC) was quantified through the Folin–Ciocalteu method (Kaur *et al.*, 2002) [13]. Initially, 0.2 mL of CCHE solution (1000 µg/mL) was diluted to a total volume of 3.0 mL using distilled water and allowed to equilibrate for 3 minutes. The reaction was initiated by adding 0.5 mL of commercial Folin–Ciocalteu phenol reagent; after an additional 3-minute wait, 2.0 mL of 20% (w/v) aqueous sodium carbonate was mixed in. The resulting solution was kept in the dark at room temperature for 30 minutes to facilitate color development. Optical absorbance was then measured at 650 nm using a UV-Vis spectrophotometer zeroed against an assay blank. Phenolic concentrations were extrapolated from a 5-point gallic acid calibration curve and reported as milligrams of gallic acid equivalents per gram of dry extract.

Total flavonoid content

Total flavonoid content (TFC) was evaluated via the aluminum chloride colorimetric assay. Initially, a 50 µL aliquot of the extract solution (1 mg/mL) was made up to 1.0 mL with methanol, then diluted with 4.0 mL of distilled water. Addition of 0.3 mL of 5% sodium nitrite (NaNO₂) was done following a 5-minute hold. Then, 0.3 mL of 10% aluminum chloride (AlCl₃) was incorporated into the mixture and allowed to react for 6 minutes. Subsequently, 2.0 mL of 1 mol/L NaOH was added before bringing the total volume to 10.0 mL with distilled water. After a final 15-minute incubation period, optical density of the resulting complex was measured at 510 nm. Flavonoid levels were calculated against a rutin standard calibration curve and reported as milligrams of rutin equivalents per gram of dry extract.

Experimental design

Animals

Healthy, nulliparous and non-pregnant female Sprague–Dawley rats (approximately 8 weeks old, weighing 150–200 g) were obtained from the National Laboratory Animal Centre at CSIR-Central Drug

Research Institute (CDRI), Lucknow, India. The animals were maintained in polypropylene cages lined with autoclaved paddy husk under standard barrier housing conditions. Standard laboratory rodent pellet feed and potable water remained accessible *ad libitum*. Before starting the experimental protocols, rats were habituated to the laboratory environment for 14 days. All experimental procedures received formal ethical clearance from the Institutional Animal Ethics Committee and adhered strictly to the regulatory guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

Experimental design

Rats were randomly assigned into eight groups having six rats in each. Group I served as control, group II was administered with cyclophosphamide @ 100 mg/kg b. wt. orally on 9th and 16th day of study, group III was administered with levamisole @ 50 mg/kg b. wt. subcutaneously and in group IV both cyclophosphamide and levamisole was administered, group V and VI were administered with hydroethanolic extract of *Cuminum cyminum* (CCHE) @ 200 and 400 mg/kg b. wt. respectively orally daily and in group VII and VIII, hydroethanolic extract of *Cuminum cyminum* (CCHE) @ 200 and 400 mg/kg b. wt. respectively orally was given along with cyclophosphamide @ 100 mg/kg b. wt. orally on 9th and 16th day of study respectively, for a period of 28 days.

Somatic and gravimetric measurements

The general physical condition and behavioral patterns of the rats were monitored twice each day throughout the 28-day regimen, looking specifically for clinical indications of systemic toxicity such as coat condition (ruffled fur), ocular discharge, diarrhoea, tremors, or early mortality. Weekly body weight progression was tracked by weighing each rat individually at Day 0 and subsequently on days 7, 14, 21, and 28 using a precision balance.

On day 29, after an overnight fasting period (with *ad libitum* water maintained), the animals were sacrificed using diethyl ether inhalation followed by cervical dislocation. Key visceral and lymphoid tissues namely the liver, kidneys, spleen, brain and thymus were promptly

harvested and trimmed free of surrounding adipose and adventitial tissues, blotted on tissue paper and immediately weighed on.

$$\text{Relative Organ Weight (\%)} = \left(\frac{\text{Absolute Organ Weight}}{\text{Total Body Weight}} \right) \times 100$$

Statistical analysis

Statistical analysis was carried out by employing analysis of variance (one-way ANOVA) at 5 % level of significance. Data were processed through SPSS statistical software. Differences were judged statistically significant at $p < 0.05$.

Results and Discussion

Maceration of *Cuminum cyminum* seed powder (500 g) with a hydroethanolic mixture (60:40) produced 80.5 g of a light brownish-black, semi-crystalline residue. This recovered mass translates to an overall extractive yield of 16.10%.

Preliminary phytochemical screening of the hydroethanolic extract (CCHE) confirmed the presence of a wide spectrum of secondary metabolites, testing positive for polyphenols, flavonoids, alkaloids, tannins, terpenoids, glycosides, and reducing carbohydrates. Both saponins and proteinaceous elements were absent (Table 1).

Table 1. Phytochemical analysis of CCHE

S.no.	Phytochemical constituents	Inference
1.	Alkaloids	+
2.	Glycosides	+
3.	Carbohydrate	+
4.	Saponin	-
5.	Tannin	+
6.	Flavonoids	+
7.	Phenolic compounds	+
8.	Terpenoids	+
9.	Proteins and amino acids	-

Quantitative spectrophotometric assays further supported these observations, demonstrating notable concentrations of antioxidant-active compounds: total phenolic content was estimated at 87.21 ± 3.77 mg GAE/g of dry extract, accompanied by a total flavonoid content of 39.20 ± 2.30 mg RE/g of dry extract.

Throughout the 28-day study, oral supplementation with CCHE alone at both 200 mg/kg and 400 mg/kg was well tolerated. The animals exhibited neither mortality nor any overt signs of neurobehavioral impairment, gait abnormalities or diarrhea reflecting an absence of systemic toxicity across the evaluated dose range. In sharp contrast, cyclophosphamide treated group provoked evident clinical distress, manifested as reduced feed consumption, lethargy, and sustained body mass loss. This weight deficit emerged statistically significant ($p < 0.05$) by day 14, compared to healthy controls. Body weight further lowered by day 21 representing an overall 15.0% growth deficit relative to control group animals.

Concurrent treatment with CCHE effectively counteracted cyclophosphamide-induced lowering of body weight. By day 14, rats co-administered with the extract at 200 mg/kg and 400 mg/kg exhibited superior body mass improvement relative to the CYP-alone group. This improvement in body weight persisted through study completion. Body weight on day 28 was significantly higher in Group VII and Group VIII, outperforming the unsupplemented CYP group. Together, these somatic trends indicate that CCHE provides dose-dependent resilience against chemotherapy-associated wasting (Table 2).

Organ weight measurements taken at the end of the study showed clear differences among the groups ($p < 0.05$). Compared to control group, cyclophosphamide treated group showed significant decrease in both immune and metabolic organs weight (Rezvanfar et al., 2008) [14].

Values bearing different superscripts (a, b, c, d, e, f) differ significantly @ 5% level ($p < 0.05$)

Table 2. Effect on body weight (g) following oral administration of hydroethanolic extract of *Cuminum cyminum* for 28 days in rats

Groups	Treatments	Dose and route	Animal body weight (g)				
			0 day	7 day	14 day	21 day	28 day
I.	Control	1 ml of distilled water, po	196 ± 2.10^a	200 ± 2.58^a	208 ± 1.66^a	213 ± 2.10^a	216 $\pm 2.10^a$
II.	CYP	100 mg/kg b wt, po	186 ± 2.10^{bc}	193 ± 2.10^{abc}	186 ± 2.10^d	181 ± 3.07^c	188 $\pm 1.66^d$
III.	Levamisole	50 mg/kg b wt, sc	185 ± 2.23^{bc}	195 ± 2.23^{abc}	203 ± 2.10^{ab}	205 ± 2.23^b	208 $\pm 3.07^b$
IV.	CYP+Levamisole	100 mg/kg b wt, po and 50 mg/kg b wt, sc respectively	190 ± 2.5^b	198 ± 1.66^{ab}	200 ± 3.65^b	201 ± 1.66^b	205 $\pm 2.23^b$
V.	CCHE-1	200 mg/kg b wt, po	180 ± 2.58^c	190 ± 2.58^c	196 ± 2.10^b	198 ± 1.66^b	201 $\pm 1.66^{bc}$
VI.	CCHE-2	400 mg/kg b wt, po	185 ± 2.2^{bc}	193 ± 2.10^{abc}	196 ± 2.10^b	198 ± 1.66^b	195 $\pm 2.23^{cd}$
VII.	CYP+CCHE-1	100 mg/kg b wt, po and 200 mg/kg b wt, po respectively	183 ± 2.10^{bc}	191 ± 1.66^{bc}	191 ± 4.01^c	188 ± 3.07^c	195 $\pm 2.23^{cd}$
VIII.	CYP+CCHE-2	100 mg/kg b wt, po and 400 mg/kg b wt, po respectively	185 ± 2.23^{bc}	193 ± 2.10^{abc}	191 ± 1.66^c	185 ± 2.23^c	190 $\pm 2.58^d$

(continued on next page)

Table 3. Effect on absolute organ weights (g) following oral administration of CCHE for 28 days in rats.

Groups	Treatments	Absolute organ weight (g)						
		Liver	Kidney	Spleen	Lungs	Heart	Brain	Thymus
I.	Control	6.02 ± 0.10 ^e	1.29 ± 0.01 ^d	0.60 ± 0.01 ^d	1.40 ± 0.01 ^d	0.97 ± 0.01 ^{ab}	1.30 ± 0.02 ^c	0.34 ± 0.01 ^d
II.	CYP	5.16 ± 0.01 ^f	1.14 ± 0.01 ^e	0.44 ± 0.01 ^f	1.16 ± 0.01 ^f	0.94 ± 0.01 ^{ab}	1.01 ± 0.01 ^f	0.24 ± 0.01 ^f
III.	Levamisole	7.17 ± 0.07 ^b	1.43 ± 0.01 ^b	0.73 ± 0.01 ^b	1.36 ± 0.01 ^{de}	0.92 ± 0.02 ^b	1.26 ± 0.01 ^{cd}	0.41 ± 0.009 ^a
IV.	CYP +levamisole	7.80 ± 0.01 ^a	1.52 ± 0.01 ^a	0.68 ± 0.01 ^c	1.45 ± 0.02 ^c	0.98 ± 0.01 ^a	1.23 ± 0.02 ^d	0.29 ± 0.01 ^e
V.	CCHE-1	6.97 ± 0.01 ^c	1.38 ± 0.01 ^c	0.75 ± 0.01 ^{ab}	1.34 ± 0.01 ^e	0.86 ± 0.01 ^c	1.35 ± 0.02 ^b	0.37 ± 0.004 ^{bc}
VI.	CCHE-2	6.88 ± 0.01 ^c	1.28 ± 0.02 ^d	0.79 ± 0.01 ^a	1.45 ± 0.02 ^c	0.84 ± 0.01 ^c	1.40 ± 0.01 ^a	0.39 ± 0.007 ^{ab}
VII.	CYP+CCHE-1	6.28 ± 0.01 ^d	1.25 ± 0.01 ^d	0.54 ± 0.01 ^e	1.75 ± 0.01 ^a	0.94 ± 0.01 ^{ab}	1.16 ± 0.01 ^d	0.33 ± 0.007 ^d
VIII.	CYP+CCHE-2	5.97 ± 0.02 ^e	1.17 ± 0.01 ^e	0.64 ± 0.01 ^c	1.69 ± 0.01 ^b	0.92 ± 0.008 ^{ab}	1.09 ± 0.01 ^e	0.35 ± 0.004 ^{cd}

Values in the table are mean ± S.E. ($n = 6$); CYP= cyclophosphamide, CCHE= hydroethanolic extract of *Cuminum cyminum*, CYP was administered on 9th and 16th day only. Values bearing different superscripts (a, b, c, d, e, f) differ significantly @ 5% level ($p < 0.05$)

Rats that received only CCHE showed clear signs of immune stimulation. Their spleen weights increased at the low dose and also at the high dose. Similarly, thymus weights also improved. These values were comparable to, or even slightly higher than, those achieved with the standard immune-booster levamisole. When CCHE was given

alongside CYP, it effectively counteracted chemotherapy-induced organ atrophy. The high dose of CCHE offered even greater protection than low dose of CCHE against lymphoid involution, maintaining thymus weight and increasing spleen weight ($p < 0.05$ compared to CYP alone). Liver weight was also protected from CYP damage in Group VII and Group VIII, compared to the CYP-alone group. Overall, these findings show that CCHE provides substantial systemic cytoprotection across multiple organ systems (Table 3 & 4).

Table 4. Effect on relative organ weight (g) following oral administration of hydroethanolic extract of *Cuminum cyminum* for 28 days in rats.

Groups	Treatments	Relative organ weight (g)						
		Liver	Kidney	Spleen	Lungs	Heart	Brain	Thymus
I.	Control	2.85 ± 0.14 ^c	0.59 ± 0.003 ^c	0.27 ± 0.01 ^{de}	0.64 ± 0.006 ^c	0.45 ± 0.01 ^c	0.60 ± 0.005 ^{cd}	0.16 ± 0.005 ^d
II.	CYP	2.78 ± 0.05 ^c	0.62 ± 0.03 ^{bc}	0.24 ± 0.01 ^e	0.64 ± 0.03 ^c	0.52 ± 0.02 ^{ab}	0.56 ± 0.02 ^d	0.13 ± 0.005 ^e
III.	Levamisole	3.51 ± 0.12 ^{ab}	0.70 ± 0.02 ^{ab}	0.36 ± 0.01 ^{bc}	0.66 ± 0.02 ^c	0.45 ± 0.02 ^c	0.61 ± 0.02 ^{cd}	0.20 ± 0.005 ^{ab}
IV.	CYP +levamisole	3.73 ± 0.13 ^a	0.73 ± 0.02 ^a	0.32 ± 0.01 ^c	0.72 ± 0.03 ^{bc}	0.47 ± 0.02 ^{bc}	0.59 ± 0.03 ^{cd}	0.13 ± 0.008 ^e
V.	CCHE-1	3.61 ± 0.17 ^{ab}	0.71 ± 0.03 ^a	0.39 ± 0.01 ^b	0.79 ± 0.03 ^c	0.44 ± 0.02 ^c	0.70 ± 0.03 ^b	0.19 ± 0.008 ^{bc}
VI.	CCHE-2	3.80 ± 0.11 ^a	0.70 ± 0.02 ^{ab}	0.43 ± 0.01 ^a	0.80 ± 0.02 ^b	0.46 ± 0.007 ^{bc}	0.77 ± 0.02 ^a	0.21 ± 0.005 ^a
VII.	CYP+CCHE-1	3.32 ± 0.09 ^b	0.66 ± 0.01 ^{abc}	0.28 ± 0.006 ^d	0.92 ± 0.03 ^a	0.49 ± 0.01 ^{abc}	0.61 ± 0.01 ^{cd}	0.17 ± 0.007 ^{cd}
VIII.	CYP+CCHE-2	3.50 ± 0.12 ^{ab}	0.68 ± 0.02 ^{ab}	0.37 ± 0.01 ^b	0.99 ± 0.03 ^a	0.54 ± 0.02 ^a	0.63 ± 0.01 ^{bc}	0.20 ± 0.006 ^{ab}

Values in the table are mean ± S.E. ($n = 6$); CYP= cyclophosphamide, CCHE= hydroethanolic extract of *Cuminum cyminum*. CYP was administered on 9th and 16th day only. Values bearing different superscripts (a, b, c, d, e, f) differ significantly @ 5% level ($p < 0.05$)

Cyclophosphamide (CYP) is a very effective chemotherapy agent but its use in medicine is limited due to significant side effects in many organs due to its breakdown into toxic byproducts primarily acrolein and phosphoramidate mustard. These chemicals damage cellular DNA and cause intense oxidative stress, destroying rapidly dividing cells which leads to severe weight loss and weakened immune organs. The toxicological profile which we observed in female Sprague Dawley rats after two oral doses of CYP included progressive weight loss and marked hypoplasia of the spleen, thymus, liver, kidneys and brain. The sharp decrease in splenic and thymic mass illustrate the high sensitivity of primary and secondary lymphoid organs to the effects of alkylating agents, which suppresses both T-cell maturation and humoral lymphocyte expansion (Kim *et al.*, 2023) [15].

This extract is also rich in polyphenols, flavonoids, natural alkaloids and terpenoids. These antioxidants in help the body combat harmful free radicals (ROS) and boost the levels of natural antioxidant en-

zymes such as SOD, catalase, and glutathione. CCHE helps to decrease oxidative stress in cells, protecting sensitive tissues from toxic by-products of chemotherapy. When rats were given CCHE alone, their spleen and thymus glands were larger than those of the normal controls, and as large as those of rats fed the standard immune boosting drug, levamisole. This means that the natural substances found in cumin directly stimulate immune cell formation and repair of tissues. Liver weight of CCHE-treated rats indicates good protection of the liver from CYP-induced injury. This discovery is in line with previous research that indicated that cumin extracts are beneficial for the liver because they prevent oxidation of fats and tissues caused by the toxic drugs.

6. Conclusion

In healthy female Sprague-Dawley rats, hydroethanolic extract of *Cuminum cyminum* seeds, at doses of 200 and 400 mg/kg reversed cyclophosphamide-induced decrease in the body weight and relative weights of spleen and thymus, with the higher dose showing more preservation of the lymphoid organs. The extract has high phenolic and flavonoid content, which could be responsible for these effects,

but this has not been directly tested. Extract also raised liver, lung and brain weight and reduced weight gain at 400 mg/kg. The data obtained in the present study would be useful in the development of new modern drugs with different plant extract combinations which can be used potentially in the cure of many ethno-medicinal diseases. Hence the plant extract can be used in treatment of many diseases. But, more detailed analysis of the chemical composition of these medicinal plants is needed. These plant extract can be incorporated in other foods as nutraceuticals for effective and proper metabolism as well as for the maintenance of good physiological state in man and animals.

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