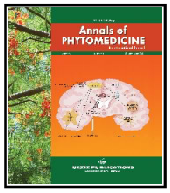
Annals of Phytomedicine 12(1): 360-366, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.24

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Investigation of beneficial impact of *Eulophia herbacea* Lind. tubers extract as an immunomodulatory and adaptogenic agent

Snehal K. Bhavsar◆, Anil U. Tatiya, Saurabh Maru* and Dhanshri S. Patil

Department of Pharmacognosy, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur-425405, Dhule, Maharashtra, India
**NMIMS School of Pharmacy and Technology, Shirpur-425405, Dhule, Maharashtra, India*

Article Info

Article history
Received 12 February 2023
Revised 27 March 2023
Accepted 28 March 2023
Published Online 30 June-2023

Keywords
Eulophia herbacea Lind.
Swimming endurance
Immunomodulator
Carbon clearance

Abstract

Eulophia herbacea Lind. tuber extract (EEH) was tested for adaptogenic and immunomodulatory activities in mice. The anoxia stress tolerance test and swimming endurance were used to measure the adaptogenic activity, while the neutrophil adhesion test, the carbon clearance test, and the cyclophosphamide-induced myelosuppression response were used to assess the immunomodulatory activity. Swimming and anoxia time have both increased significantly. On the other hand, it demonstrated a considerable improvement in the neutrophil adhesion test, a significant increase in the phagocytic index in the carbon clearance assay, and a significant defense against myelosuppression caused by cyclophosphamide. The findings of this study suggest that *E. herbacea* may have potential apoptogenic and immunomodulatory action as well as therapeutic benefits for the early detection of illnesses.

1. Introduction

An intensive area of research into alternative systems of medicine for both acute and chronic illnesses led to the identification of several herbs with disease-fighting capabilities.

Many plants have been used, either individually or in combination in Ayurveda formulations to modulate the immune system. Common names for the orchid *E. herbacea* (Orchidaceae) include Kutri-kand, Kukad-kand, and unmarked. The Himalayas, Bengal, and western portions of the Deccan peninsula are home to the genus *Eulophia*, which is a group of perennial terrestrial orchids with fleshy tubers and infrequent pseudobulbs (Patil,1992). The tubers of *E. herbacea* belonging to the family Orchidaceae (WOI., 1992) shows multiple activities with good growth suppressive effect against human nasopharynx (KB), leukemia K562, lungs (HOP62) human cancer cell lines making it a potential biomolecule against human cancer cells. It has been shown to be the most effective at shielding DNA from the harm that free radicals may do. Also shows hypolipidemic and antidiabetic activity.

A diet rich in micronutrients and antioxidants is known to have immune activity changes brought on by environmental toxins and dietary choices (Bafna and Mishra, 2010). The body's defense mechanism can be modulated by using herbs as immunomodulators in the native medical system. *Eulophia* tubers have proteins, carbohydrates, mucilage, tannins, and amino acids (Bhavsar and Tatiya, 2015). The immune system has been demonstrated to be influenced by the following plant derivative active components, polysaccharides, lectins, peptides, flavonoids, and tannins, in numerous experimental models. Stress can manifest in our bodies in a variety of ways. One or more of the typical symptoms of stress include insomnia, depression, anxiety, agitation, tiredness, and lethargy (Mamarasulov *et al.,* 2020). The ability of an adaptogen to combat stress in all its forms may be its most important characteristic. There is no correlation between the sort of stress and how the body responds to it (Singh *et al*., 2005).

Adaptogens are biologically active substances derived from plants that appear to promote physical endurance for working even in challenging environmental settings and under adverse conditions in various experimental models. They also appear to improve the organism's generalized resistance to numerous unpleasant assaults that threaten internal balance (Bhattacharya *et al*., 2000; Kannur *et al*., 2006).

The chemical profile indicates *E. herbacea* as a good source of immunomodulatory as well adaptogenic agents. Tubers have been used to cure worms, rheumatism, and tumors of the scrofulous glands of the neck, utilized to treat pimples. Menstrual problems, urinary symptoms, and spermatorrhoea are treated with tuber decoction. Salep is derived from tubers, and pseudobulbs are taken as a tonic. Menstrual problems, urinary symptoms, and spermatorrhoea are treated with the tuber's decoction. Salep is found in tubers, and pseudobulbs are taken as a tonic. It is also used as antirheumatic, antifutigue, and in skin diseases (Bhavsar and Tatiya, 2022).

Regarding the literature on the adaptogenic and immunomodulatory properties of *E. herbacea* tubers extract, there are no available scientific reports. To validate its function as an immunostimulant, the current investigation was carried out to evaluate the adaptogenic and immunomodulatory activities of methanol extract derived from the tubers of *E. herbacea* in connection to its folklore medicinal qualities.

Corresponding author: Dr. Snehal K. Bhavsar
Assistant Professor, Department of Pharmacognosy, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur-425405 , Dhule, Maharashtra, India.
E-mail: snehalbhavsar10@gmail.com
Tel.: +91-8888212381

2. Materials and Methods

2.1.1 Plant material and extraction

In the months of July and August, the orchidaceous *E. herbacea* tubers were harvested from the subtropical highland Toranmal region of Maharashtra, India. Dr. S. R. Khirsagar, a Botanist at Shri Shivaji Vidya Prasarak Sanstha's Late. Karamveer Dr. P. R. Ghogrey Science College in Dhule, Maharashtra, India, identified and verified them. A prepared herbarium specimen was provided to the Institute under the reference number RCPIPER/ PCOG/MH2016-29. Using a Soxhlet system, methanol was extracted from the air-dried *E. herbacea* tubers. Filtered and evaporated under decreased pressure, the crude extract produced a viscous, dark mass with a yield of 4.71% (w/w).

2.1.2 Drugs

Accurately weighed amounts of the hydroalcoholic extract were suspended in 2% gum acacia. In models using adaptogenic substances, siotone capsules were the norm. Levamisole was utilized in immunomodulatory models, immunosuppressant cyclophosphamide.

2.1.3 Phytochemical analysis

Preliminary phytochemical analysis done by performing different chemical test (Singh, 2020). EEH showed positive tests for steroids and terpenoids.

2.1.4 Experimental animals

Swiss albino mice (25-30g) of either sex were used in the study. They were kept in cages, six to a cage, at typical temperatures of 26.2°C, 44-56% relative humidity, and 12 h light: 12 h dark cycles every day for a week prior to and during the studies. Water and the usual rat pellet diet were given to each animal.

2.1.5 Acute toxicity studies (LD_{50})

According to OECD recommendation No. 423, testing for EEH extract toxicity was conducted. First, a limit test was carried out. The toxicity investigation employed Swiss albino mice measuring 20-25 g.

Three mice received extract doses of 100, 500, 1000, and 2000 mg/kg in succession.

After each hour of dose delivery, the individual animal was checked for signs of toxicity and odd behavior for up to 14 days. Additionally, death in mice was noted.

The study also measured variables such as body weight, food intake, and water consumption.

2.2 Apoptogenic activity

2.2.1 Swimming endurance test in mice

Group I acted as control, which only received one vehicle at 10 ml/kg p.o. At a dose of 100 mg/kg p.o., Group II got the Siotone capsule as Standard. Group III received 100 mg/kg p.o. of EEH, and Group IV 200 mg/kg p.o. of EEH. Group V received 400 mg/kg p.o. of EEH, A week of therapy was given to the mice. On the seventh day, an hour after receiving the medicine, all the mice underwent a swimming endurance test. The mice were allowed to swim on their own inside a 20 cm in diameter by 30 cm high perplex glass filled with water that was maintained at a temperature of 26°C. The endpoint was pursued up until their exhaustion and the time of their death by drowning. Each group's average swimming duration was computed (Nataraj *et al*., 2011; Kannur *et al*., 2006).

2.2.2 Anoxia stress tolerance in mice

Group I served as Control which received only vehicle 10 ml/kg p.o. Group II received the Siotone capsule as Standard at a dose of 100 mg/kg p.o., Group III received EEH at a dose100 mg/kg p.o., Group IV EEH at dose 200 mg/kg p.o., Group V got a EEH at a dose 400 mg/kg EEH p.o. for three weeks, the animals received the above-described care. Each mouse was individually placed in a hermetic jar with a 300 ml capacity to create stress at the end of the first, second, and third weeks, or on the seventh, fourteenth, and twenty-first day, one hour after the treatment. This was done to measure the anoxia tolerance time. The moment, the animal began to experience its first spasms, it was promptly removed from the vessel and, if necessary, revived. The time interval between the animal's entrance into the hermetically sealed vessel and the onset of the first convulsion was used to determine the animal's anoxia tolerance. Convulsion's endpoint appeared to be very acute, and eradication took a minute longer than expected (Pawar and Hugar, 2011).

2.3 Immunomodulatory activity

2.3.1 Neutrophil adhesion test

Group I functioned as the Control group and only received vehicle 10 ml/kg p.o. A 25 mg/kg p.o. dosage of Standard. Levamisole was administered to Group II. EEH was administered to Group III at a dose of 100 mg/kg p.o., 200 mg/kg p.o. of EEH extract is used in group IV. A 400 mg/kg p.o. dosage of EEH was administered to group V. The above treatment was given orally to the animals for 14 days. Blood was drawn from the retro-orbital plexus on the fourteenth day of medication therapy and then tested for differential leucocyte counts and total leucocyte counts (TLC). Following preliminary counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37°C. The incubated blood samples were tested once more for TLC and DLC. A second TLC and DLC examination was performed on the incubated blood samples. The blood sample's neutrophil index is determined by TLC and the proportion of neutrophils (NI). The calculation of per cent neutrophil adhesion is provided below (Ghule *et al*., 2006; Dashputre and Naikwade, 2010).

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

where, NIu = Neutrophil index of the untreated blood sample.

NIt = Neutrophil index of the treated blood sample.

2.3.2 Carbon clearance test

Group I served as Control which received only vehicle 10 ml/kg p.o., Group II received Standard levamisole at a dose 25 mg/kg p.o. Group III received EEH at a dose100 mg/kg p.o. Group IV EEH was given at dose 200 mg/kg p.o., Group V received EEH at a dose of 400 mg/kg p.o. Animals were treated as shown above orally for 5 days. At the end of 5 days, carbon ink suspension (0.1 ml) was administered into the tail vein 48 h after the last treatment. At 0 and 15 min, blood samples were taken from the retro-orbital sinus (in 5 µl of EDTA solution). A 25 µl sample was combined with 2 ml of a 0.1% sodium carbonate solution, and its absorbance at 660 nm was measured (Jaythirtha and Mishra, 2004; Sumanth and Chowdary, 2009).

The phagocytic index was calculated using the following equation:

Phagocytic index = k (sample)/ k (control)

where K = (Log OD1- Log OD2) /15

OD1 = optical densities at 0 min and,

OD2 = optical densities at 15 min, respectively.

2.3.3 Cyclophosphamide-induced myelosuppression

Group I functioned as the control, receiving just one vehicle at a dose of 10 ml/kg. Group II was given 30 mg/kg p.o. of standard cyclophosphamide. Group III received 100 mg/kg p.o. of EEH. Group IV received 200 mg/kg p.o. of EEH, Group V got 400 mg/kg p.o. dose of EEH. Animals received the above treatment orally for 14 days. On days 11, 12, and 13, all animals, save those in the negative control group, received an oral dose of cyclophosphamide solution one hour after the extract was administered. On day 14, blood samples were taken, and the total white blood cell (WBC) count was calculated. (Gokhale *et al*., 2003).

3. Results

3.1 Adaptogenic activity

3.1.1 Swimming endurance

The immune system is significantly affected by stress (Solomon *et al*., 1985). Rodents become immobilized after an initial period of strong activity when forced to swim in a confined space. Immobility denotes behavioral hopelessness, which resembles a depressive state of mind (Thiebot *et al*., 1992; Sumanth *et al*., 2007). Therefore, the appearance of immobility indicates a state of exhaustion, fatigue, and decreased stamina, with the endpoint being the point at which the mice were unable to continue swimming and began drowning (Giri *et al*., 2011). EEH was found to significantly lengthen mice's swimming performance time at doses of 200 mg/kg and 400 mg/kg, respectively. It was discovered that the outcome of the higher dose (400 mg/kg) of EEH was comparable to the standard. In comparison to untreated animals, it was found that EEH boosted swimming endurance with increased swimming time. Results are given in Table 1 Figure 1. It is abundantly obvious that the extracts can improve mice's overall performance and physical endurance.

Table 1: Effect of EEH on swimming endurance time

Treatment	Dose (mg/kg)	Swimming survival time (min.) mean ± SEM
Control	– –	188 ± 25
Standard	100	370.02 ± 4.7[a]
EEH	100	330 ± 5.3[a]
EEH	200	348 ± 7.1[a]
EEH	400	368 ± 3.4[a]

All values expressed, mean ± SEM, n=6; Data was analyzed by one-way ANOVA followed by Dunnet test [a]p< 0.001**.

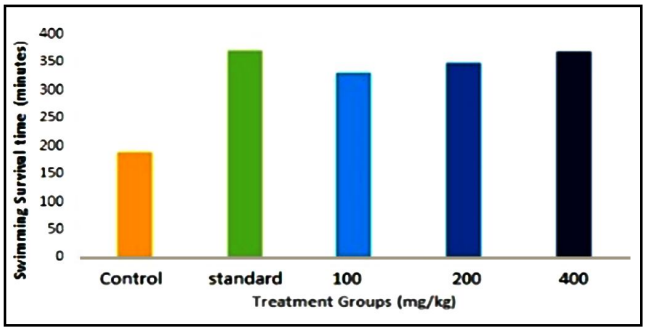


Figure 1: Effect of EEH on swimming survival time.

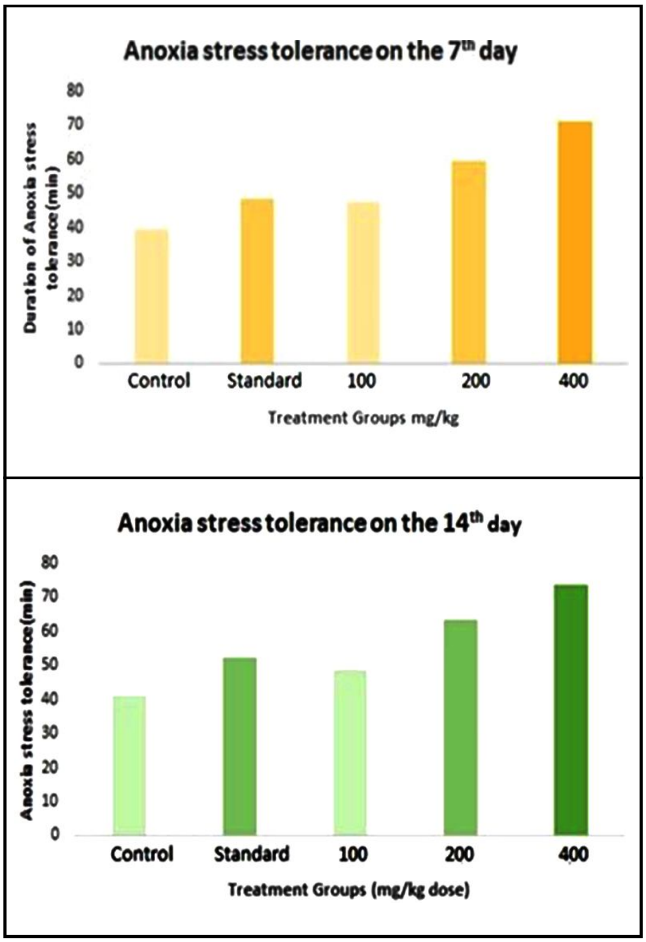
3.1.2 Anoxia stress tolerance

One of the most helpful parameters for determining a drug's adaptogenic effect is anoxia. Any drug's primary antistress impact may be an increase in adaptability during anoxia (Sonkar *et al*., 2011). The occurrence of convulsion was the test's endpoint. The effects of a hypobaric environment on mice for a predetermined amount of time, the mitochondria of the heart and brain neuro-transmitters are drastically diminished (Singh *et al*., 2001). On days 14 and 21, treated groups showed a significant increase in anoxia stress tolerance time. After receiving treatment for one week, there was an increase in anoxia tolerance time. The results are given in Table 2 and Figure 2.

Table 2: Effect of EEH on anoxia stress tolerance

Treatment	Dose (mg/kg)	Anoxia time (min.) Mean ± SEM		
		7th day	14th day	21st day
Control	– –	39.25 ± 2.5	41.28 ± 3.0	43.31 ± 2.5
Standard	100	48.09 ± 5.3	52.34 ± 5.1	54.75 ± 2.9
EEH	100	47.49 ± 4.1	48.8 ± 4.0	51.68 ± 4.7
EEH	200	59.50 ± 1.1[a]	63.36 ± 2.3[a]	65.35 ± 3.2[a]
EEH	400	70.93 ± 2.7[a]	73.92 ± 3.2[a]	74.05 ± 4.0[a]

All values expressed, mean ± SEM, n=6; Data were analyzed by one-way ANOVA followed by Dunnet test [a]p<0.001**.



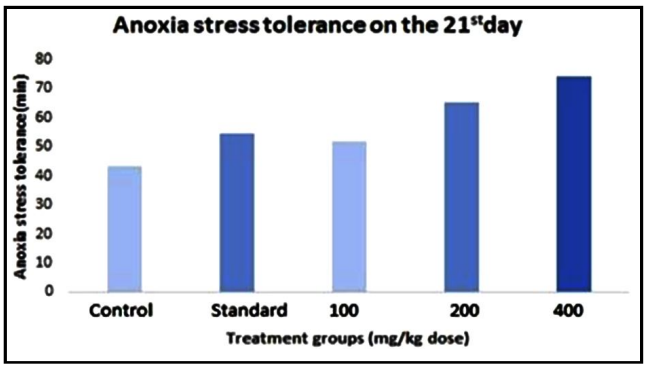


Figure 2: Effect of EEH on anoxia stress tolerance onthe 7th, 14th, and 21st day.

The study's findings revealed that the EEH considerably increased the mean time until a convulsion, confirming its anti-stress function.

Statistic evaluation

One-way analysis of variance (ANOVA) was used to determine the statistical significance, and then the Bonferroni correction was applied. $p>0.05$ was regarded as significant, and the values were reported as mean ± SEM.

3.2 Immunomodulator activity

3.2.1 Neutrophil adhesion test

Neutrophil counts decreased after blood was incubated with nylon fibers (NF) because the neutrophils stuck to the fibers. The EEH-treated groups were shown to have higher neutrophil adhesion rates than the control group. However, as compared to the control, doses of 200 and 400 mg/kg showed a substantial increase in neutrophil adhesion, pointing to a potential immunostimulant effect of EEH Table 3 and Figure 3. This may aid in strengthening the body's defenses against microbial diseases (Benacerraf, 1978).

Table 3: Effect of EEH neutrophil adhesion test

Treatment	Dose (mg/kg p.o.)	TLC × 10^6 cells/µl	Neutrophil %		Neutrophil adhesion (%)
			UB	NFTB	
Control	--	3.4 ± 0.86	50.8 ± 2.6	47.4 ± 3.1	6.69 ± 1.34
Standard	25	6.0 ± 1.9	69.0 ± 1.3	60.2 ± 2.6	12.75 ± 0.81[a]
EEH	100	3.0 ± 1.8	50.6 ± 4.9	48 ± 1.7	5.13 ± 0.74[a]
EEH	200	3.2 ± 0.81	61.8 ± 1.2	54.8 ± 4.4	11.32 ± 1.02[a]
EEH	400	4.52 ± 0.83	56.6 ± 1.4	49.4 ± 2.5	12.72 ± 0.88[a]

Data were evaluated by one-way ANOVA followed by the Dunnet test; UB: Untreated Blood; NFTB: Nylon fibertreated blood; Values are reported as mean ± SEM, n=6; and a p 0.001**.

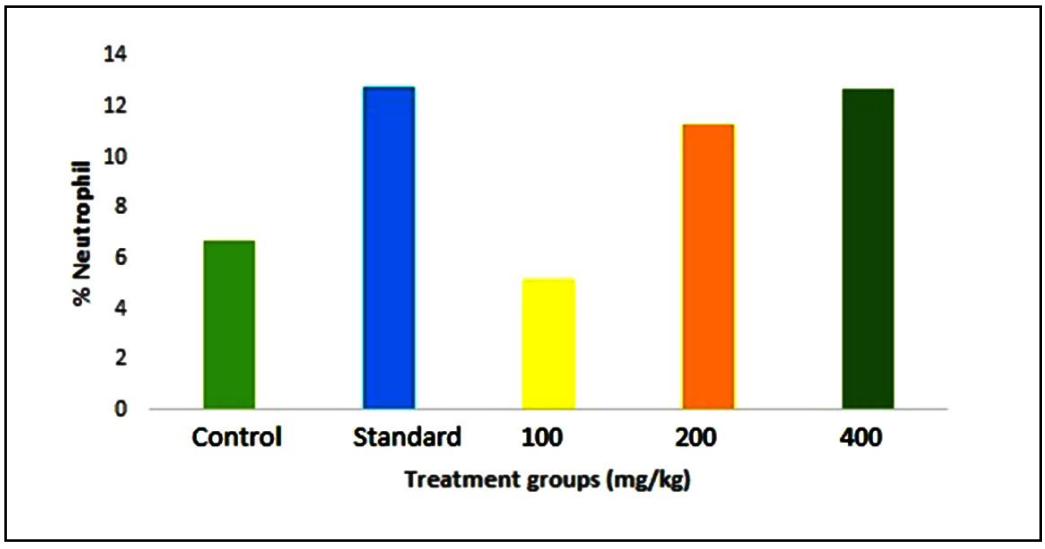


Figure 3: Effect of EEH on neutrophil adhesion test.

3.2.2 Carbon clearance test

Ingesting and eliminating microorganisms, cancerous cells, inorganic particles, and tissue debris is a process known as phagocytosis that occurs in some body cells collectively known as phagocytes (Miller *et al*., 1991). In humans, several clinical diseases are linked to phagocytic abnormalities (White and Gallin, 1986). The removal of carbon atoms from the blood circulation served as a gauge for the reticulum-endothelium system's phagocytic activity. The rate at which RES cells cleared carbon after receiving oral doses of EEH (100, 200, and 400 mg/kg) for five days showed a dose-related increase. The results are given in Table 4, Figure 4.

Table 4: Effect of EEH on carbon clearance test

Treatment	Dose (mg/kg)	Phagocytic index Mean ± SEM
Control	––	0.0058 ± 0.0003
Standard	25	0.034 ± 0.009[a]
EEH	100	0.007 ± 0.001
EEH	200	0.020 ± 0.005
EEH	400	0.032 ± 0.0076[b]

Values are expressed as mean ± SEM., n=6., Data was analyzed by one way ANOVA followed by Dunnet test [a]p<0.005**, [b]*p*<0.05*.

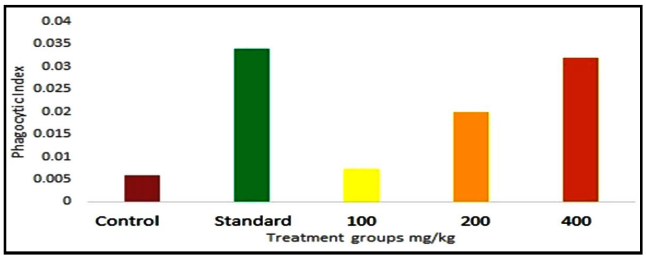


Figure 4: Effect of EEH on carbon clearance test.

An increase in carbon clearance is a sign of increased *in vivo* phagocytic activity and the elimination of foreign particles by the granulopoiesis system (Mukherjee *et al*., 2010). By showing a dose-related improvement in the rate of carbon clearance by the cells of the reticulum-endothelium system, EEH appeared to improve the phagocytic function.

3.2.3 Cyclophosphamide-induced myelosuppression

Hemoglobin, RBC, WBC, and platelet counts were all significantly decreased by cyclophosphamide at a dose of 30 mg/kg, intravenously. Compared to cyclophosphamide treatment alone, the combination of cyclophosphamide and methanol extract of *E. herbacea* (100-400 mg/kg, p.o.) led to a restoration of bone marrow activity.

Table 5: Effect of EEH on cyclophosphamide-induced myelosuppression

Treatment	Dose (mg/kg)	WBC×10³ cells/µl	RBC×10⁶ cells/µl	Hb (%)	Platelets × 10³cells/µl
Control	--	3.9 ± 0.33	6.94 ± 0.26	12.1 ± 0.48	10.46 ± 1.5
CY control	30	2.64 ± 0.48	5.05 ± 0.57	9.52 ± 0.37[b]	4.81 ± 1.6
EEH	100	4.02 ± 0.48	4.67 ± 0.69[b]	8.92 ± 1.16[a]	12.88 ± 4.04
EEH	200	3.22 ± 0.83	6.26 ± 0.39	9.66 ± 0.60[b]	14.66 ± 6.4
EEH	400	9.54 ± 2.3[b]	7.64 ± 0.71[b]	10.74 ± 1.1	12.89.8 ± 3.11

Values are expressed as mean ± SEM., n=6., Data was analyzed by one way ANOVA followed by Dunnet test [a]*p*<0.005**, [b]*p*<0.05*.

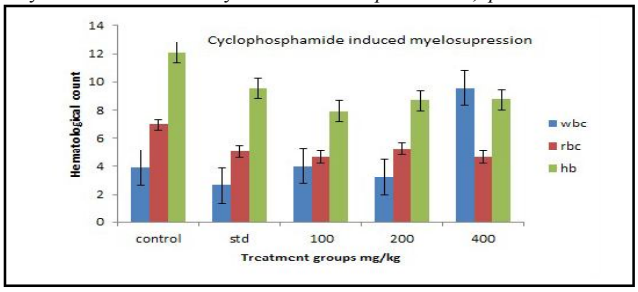


Figure 5: Effect of EEH on cyclophosphamide-induced myelosuppression.

EEH treatment greatly raises the number of total WBCs, RBCs, hemoglobin, and platelets while also reversing the myelosuppressive effects of cyclophosphamide.

4. Discussion

In this investigation, we discovered that *E. herbacea* hydroalcoholic extract (EEH) has an immunomodulatory effect in cellular and humoral immunity experimental models. Low doses of the extract 100 mg/kg orally were shown to be the most beneficial, whereas high doses of EEH 400 mg/kg, were only somewhat effective at controlling the immune system when taken orally. The study was carried out in four different ways, each of which provides information on the effects on different immune systems. Different plant products can influence immunological responses by stimulating or suppressing them, and they may be used as supportive therapy in conjunction with conventional medications in immune-compromised patients (Wagner and Blandt, 1984; Dubey, *et al.,* 2020). The number of macrophages that reach the site of inflammation and the margination of polymorphonuclear lymphocytes in blood arteries are both described by neutrophil adherence to nylon fibers (Shinde *et al*., 1999). EEH (200 and 400 mg/kg, p.o.) was administered at low and high dosages, and both showed a substantial improvement in neutrophil adhesion to nylon fibers. It is possible that this resulted in an upregulation of the b2 integrins, which are located on the surface of neutrophils and allow them to adhere firmly to the nylon fibers (Srikumar *et al*., 2005). Therefore, it was concluded that EEH stimulates neutrophils to migrate to the site of inflammation. To assess how well medications affected the reticuloendothelial system, the carbon clearance test was performed. The diffuse reticuloendothelial system (RES) is made up of phagocyticcells. The removal of particulates from the bloodstream depends heavily on RES cells. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate at which carbon is removed from the blood by macrophages is governed by an exponential equation (Gokhale and Damreand, 2003). It is hypothesized that the fact that doses of EEH significantly increased the phagocytic index may be related to an increase in reticuloendothelial system activity brought on by EEH treatment given to animals beforehand. One of the often-used methods for determining the serological reactions in animals given vaccinations is the mouse lethality test. For whatever reason, stress alters the body's regular functions, impairs labor productivity, raises weariness, lowers locomotion, and worsens mental sadness (Singh *et al*., 2001). In an animal experiment, stress had the opposite effect on EEH. Steroids and triterpenoids may have contributed to the increased swimming endurance time in mice compared to the control group (Pratap *et al.,* 2021).

The mitochondria of heart and brain neurotransmitters drastically decrease when mice are subjected to a hypobaric environment for a predetermined amount of time. The anoxia time was prolonged in the current study by EEH in a dose-dependent manner. The impact on the pituitary-adrenal gland may be the cause of the effect, which is likely associated to an increase in brain resistance to anoxia and a decrease in cerebral oxygen consumption in acute anoxia (Singh *et al*., 2001). There was no mouse mortality in the acute toxicity trial. Additionally, no harmful side effects were noticed.

5. Conclusion

The adaptogenic and immunomodulatory properties of EEH were examined. The pharmacological findings indicate that administering EEH to experimental animals can increase their capacity for non-specific stress; consequently, an extract from *E. herbacea* tubers demonstrated significant adaptogenic effects and by using cellular and humoral immunity, stimulate the immune system in animal experiments.

Acknowledgments

Authors are thankful to R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur and NMIMS School of Pharmacy, Shirpur for providing facilities.

Authors are thankful to the Institute of Animal Health and Veterinary Biologicals Rasalpura Mhow (M.P.) for animals and animal activity.

Conflicts of interest

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

References

Benacerraf, B. (1978). A hypothesis to relate the specificity of T lymphocytes and the activity of I region specific Ir genes in macrophages and borrower lymphocytes. Journal of Immunology., **120**:1809-1832.

Bhattacharya, S. K.; Bhattacharya, A. and Chakraborty, A. (2000). Adaptogenic activity of siotone, a poly herbal formulation of ayurvedicrasayans. Indian Journal of Experimental Biology., **38**:119-128.

Bhavsar, S.K. and Tatiya, A.U.(2022). Extraction and chemoprofiling study of *Eulophia herbaceae* Lind. tubers for colorectal cancer Ann. Phytomedicine., Special Issue **1**:S1-S10

Dashputre, N. L. and Naikwade, N. S. (2010). Immunomodulatory activity of *Abutilon indicum* Linn on albino mice. Int. J. Pharm. Sci. Res., **1**(3): 178-184.

Dubey, P.; Jayant S. and Srivastava, N. (2020) Preliminary phytochemical screening, FTIR and GC-MS analyses of aqueous, ethanolic and methanolic extracts of stem of *Tinospora cordifolia* (Willd.) Miers for search of antidiabetic compounds Ann. Phytomed., **9**(2):183-197.

Ghule, B.; Ghante, P.; Nakhat P. and Yeole, P., (2006). Immunostimulant effects of *Capparis zeylanica* Linn. leaves. Journal of Ethno-pharmacology., **108**(2):311-315.

Gokhale, A. and Damreand M. (2003). Investigations into the immunomodulatory activity of *Argyreia speciosa*. Journal of Ethnopharmacology., **84**(1):109-114.

Jayathirtha, M. and S. Mishra, (2004). Preliminary immunomodulatory activities of methanol extract of *Ecliptaalba* and *Centella asiatica*. Ann. Phytomed., **11**:361-365.

Kannur, D.M.; Hukkeri, V. and Akki, K. (2006). Adaptogenic activity of *Caesalpinia bonducella* seed extracts in rats. Journal of Ethnopharmacology, **77**(7-8):327-331.

Mamarasulov, B.; Davranov, K. and Jabborova, D. (2020). Phytochemical, pharmacological, and biological properties of *Ajuga turkestanica* (Rgl.) Brig (Lamiaceae). Ann. Phytomed., **9**:44-57.

Miller, L.E.; H. R. Ludke, J.E.; Peacock and R.H. Tomar, (1991). Manual of Laboratory Immunology. Lea and Febiger, London., pp:1-18.

Mukherjee, D.; Khatua, T.; Venkatesh, P.; Saha, B.P. and Mukherjee, K. (2010). Immunomodulatory potential of rhizome and seed extracts of *Nelumbo nucifera* Gaertn. Journal of Ethnopharmacology, **128**(2): 490-494.

Nataraj, G.; Nanjappaiah, H. and Shivkumar, H. (2011). Evaluation of Adaptogenic potential of *Hibiscus cannabinus* in acute stress induced mice. Pharmacologyonline., **2**:508-513.

Patil, D.A. (1992). The Flora of Dhule and Nandurbar Districts, Bishan Singh and Mahendar Pal Singh publisher and distributor, Dehradun, pp:566.

Pawar, V. and Hugar, S. (2012). Adaptogenic activity of *Trigonellafoeum* (Linn.) seeds in rodents exposed to anoxia and immobilization stress. Asian Pacific Journal of Tropical Biomedicine, **2**(1):S208-S211.

Pratap, G. P.; Jyothi, B.; Husain, M. K.; Nagaraj, V. and Sudarsanam, G. (2021). Pharmacognostical and phytochemical studies of *Mollugo nudicaulis* Lam.: A controversial plant origin ayurvedic drug. Ann. Phytomed., **10**:44-52.

Shinde, U.A.; Phadke, A.S.; Nair, A.M.; Mungantiwar, A.A.; Dikshit, V.J. and Saraf, M.N. (1999). Preliminary studies on the immunomodulatory activity of *Cedrus deodara* wood oil. Fitoterapia, **70**:333-339.

Singh, S.; Bajpai M. and Mishra, P. (2020) Pharmacognostical evaluation of indigenous herbal drug: *Carissa carandas* Linn. fruit. Ann. Phytomed., **9**(2):263-270

Singh, B.; Saxena, B. K. Chandan, B. K.; Gupta, D. K.; Bhutani, K. K. and Anand, K. K. (2001). Adaptogenic activity of novel, withanolide - free aqueous fraction from roots of *Withania somnifera* Dun. Phytotherapy Research, **15**:311-318.

Singh, B.; Chandan, B.K. and Gupta, D.K. (2003). Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of *Withania somnifera* Dun. (Part II). Phytotherapy Research, **17**:531-536.

Singh, B.; Chandana, B.K..; Sharma, N.; Singha, S.; Khajuriaa, A. and Gupta, D.K. (2005). Adaptogenic activity of glyco-peptido-lipid fraction from the alcoholic extract of *Trichopus zeylanicus* Gaerten (part II). Phytomedicine, **12**:468-481.

Singh, R. and Rao, H.S. (2008). Hepatoprotective effect of the pulp/ seed of *Aegle marmelos* Correa ex Roxb against carbon tetrachloride-induced liver damage in rats. Int. J. Green Pharm., **2**:232-234.

Solomon, G.F.; Amkraut A.A. and Rubin, R.T. (1985). In stress, psychological and physiological interactions, burchfield SR (ed.). Hemisphere Publishing Corp: Washington., pp:99-110.

Sonkar, R. and R. Mishra, (2011). Adaptogenic activity of triphala megaext. Indian Journal of Research in Pharmaceutical and Biomedical Sciences, **76**(6):467.

Srikumar, R.; Narayanaperumal, J.P. and Rathisamy, S.D. (2005). Immunomodulatory activity of Triphala over neutrophil functions. Biol. Pharm. Bull., **28**:1390-1403.

Sumanth, M. and Mustafa, S. S. (2007). Antistress, adaptogenic and immunostimulating activity of roots of *Boerhaavia diffusa* in mice. International Journal of Pharmacology, **3**:416-420.

Sumanth, M. and G. Chowdary, (2009). Antistress and immunomodulatory activity of aqueous extract of *Momordi cacharantia*. Phcog Mag.: Research Article, **5**(9):69-73.

Sumanth, M., A. Gupta and N.S. Kumar, (2008). Immunomodulatory and antioxidant activity of polyherbal formulation. International Journal of Pharmacology, pp:287-91.

Thiebot, M.H.; Martin, P. and Puech, A. J. (1992). Animal behavioral studies in the evaluation of antidepressant drugs. Br. J. Psychiatry Suppl., **15**: 44-50.

Verma, S. and S.P. Singh, (2008). Review: Current and future status of herbal medicines. Veterinary World, **1**(11):347-50.

Wagner, H. and S. Bladt, (1996). Plant Drug Analysis, a Thin Layer Chromatography 2nd ed. Springer-Verlag Berlin, new York., pp:305-326.

Wagner, H. (1984). In: Hiroshi Hikino, N.R. Farnsworth (Eds.), Economic and Medicinal Plant Research, vol. I. Academic Press, London, pp:113-153.

Wealth of India. (1992). A Dictionary of Indian Raw Materials and Industrial Product Raw Material Series. Publications and Information Directorate, CSIR, New Delhi, Revised Series: Vol. **3**:412-13.

White, C.J. and Gallin, J.I. (1986). Phagocyte defects. Clinical Immunology and Immunopathology, **36**(3):50-61.

Citation **Snehal K. Bhavsar, Anil U. Tatiya, Saurabh Maru and Dhanshri S. Patil (2023). Investigation of beneficial impact of EulophiaherbaceaLind.tubers extract as an immunomodulatory and adaptogenic agent. Ann. Phytomed., 12(1):360-366. http://dx.doi.org/10.54085/ap.2023.12.1.24.**