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***Research Article** (Open Access)*

PROTECTIVE EFFECT OF METHANOLIC LEAF EXTRACT OF *ACHYRANTHES ASPERA* (Linn.) AGAINST CYCLOPHOSPHAMIDE INDUCED SUPPRESSION OF SPECIFIC IMMUNITY IN ALBINO RATS

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ABSTRACT
Ethnopharmacological relevance: Achyranthes aspera has occupied a pivotal role in ethnopharmacological studies. Following its traditional uses, crude extracts of different parts and their bioactive components have been extensively explored and reported to have protective pharmacological activities against various disease conditions. Cyclophosphamide is one of the widely used anti- cancer drug. Its metabolites exert toxicity to normal healthy cells of the body also, including the cells and organs of immune system. A combination therapy based on natural herbs can be useful in mitigating the toxic effects of chemotherapy.
Aim of the study: Upon reviewing the anti- microbial and anti- tumor properties of *Achyranthes aspera*, an effort has been made to explore the possibility of protective effect of leaf extract of *Achyranthes aspera* against cyclophosphamide induced toxicity on antigen- dependent specific immunity in albino rats.
Materials and methods: Group I (Vehicle control), II (CYP; 50 mg/kg body weight; i.p.), III (AAML; 400 mg/kg body weight; p.o.) and IV (AAML + CYP) rats were divided into subgroups (a) and (b) and were administered intraperitoneally with 0.2 ml of 1 % v/v CRBC suspension to induce specific immunity on 12th day of the experiment. On 16th day, rats in sub group (a) were assessed for antibody production using Haemagglutination titer assay, whereas rats in subgroup (b) were assesses for Delayed Type Hypersensitivity reaction after getting challenged with 0.1 ml of 1 % v/v CRBC suspension in left hind footpad.
Results: Administration of single dose of cyclophosphamide significantly lowered the HA titer as well as DTH activity in group II rats as compared to vehicle control ($p< 0.05$ and $p< 0.01$). Pre- treatment with leaf extract of *Achyranthes aspera* in cyclophosphamide treated rats significantly elevated the antibody titer value as well as percent change in hind foot thickness, when compared with cyclophosphamide only treated group rats ($p<0.05$ and $p< 0.001$). Phytochemical analysis of the extract revealed the presence of secondary metabolites, such as alkaloids, flavonoids, saponins, phenols, coumarins, glycosides, etc.
Conclusion: These findings revealed the protective effect of *Achyranthes aspera* leaves against cyclophosphamide induced suppression of humoral and cell mediated arms of specific immunity in albino rats.

***Keywords*:** *Chemotherapy, Humoral immunity, Cell- mediated immunity, Saponins, Achyranthes aspera, Cyclophosphamide*

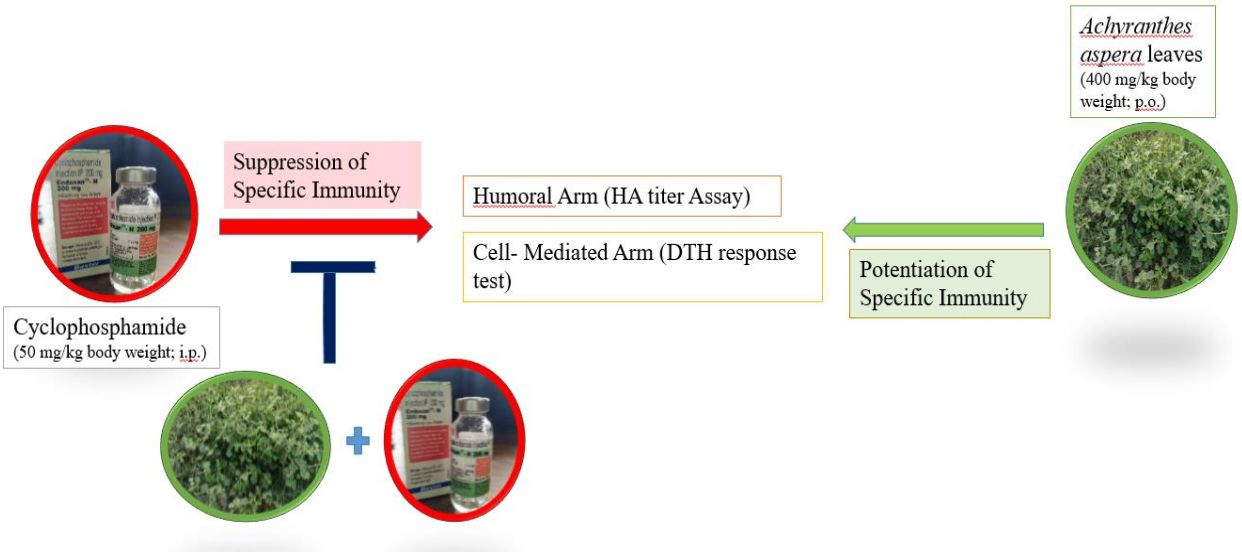
INTRODUCTION
Chemotherapy is a most commonly used modality for treatment of cancer. In chemotherapy, a single or combination of anti- neoplastic drugs are used to kill the rapidly dividing cancer cells. But, being non-selective in nature, they can also target the normal cells with high proliferation rate, such as, cells in bone marrow, digestive tract and hair follicles, thus leading to side effects like myelosuppression, immunosuppression, mucositis and alopecia (Prabhu and Guruvayoorappan, 2012). These side- effects

adversely affect the quality of life of individual as well as treatment performance. So, a search for alternative that can prevent the cytotoxicity of these drugs without altering their anti- neoplastic activities, becomes a mandate for modern era treatment strategies.

Traditional Indian medicinal literature, Rasayana, consists of a number of plants reputed to promote physical and mental health, improve defense mechanism of the body and enhance longevity. Botanical herbs like *Withania somnifera*, *Tinospora cordifolia* and *Asparagus* have been reported to provide immunoprotection in cancer chemotherapy without compromising antitumor effects of the cytotoxic drugs (Diwanay *et al*., 2004). Various secondary metabolites (Alkaloids, Glycosides, Saponins, Flavonoids, Coumarins and Sterols) isolated from the plants also exhibited a wide range of immunodulatory activity (Kumar *et al*., 2012).

Achyranthes aspera, commonly known as *Apamarga in Ayurveda, Prickly Chaff Flower in English, Latjira or Chirchira in Hindi*; is a stiff erect herb belonging to family Amaranthaceae. The plant has been reported to be globally available as a medicinal weed in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. It is found along field boundaries, road sides and waste places throughout India (Sharma and Chaudhary, 2015). Different parts of *Achyranthes aspera* has been used by traditional healers for treatment of pneumonia, cough, toothache, bowel complaints, piles, boil, skin eruption and reptile bites (Pandey *et al*., 2013). Extracts of leaves, stems, roots and seeds of *Achyranthes aspera* have been scientifically investigated for their anti- fertility (Reddy *et al*., 2016), hepatoprotective (Fahim and Sathi, 2018), anti- obesity and cardioprotective (Athesh *et al*., 2020), anti- diabetic (Mani *et al*., 2016), wound healing (Mondal *et al*., 2016), prothyroidic (Khan *et al*., 2021), anti- tuberculosis (Beg and Athar, 2020), antioxidant (Shakeel *et al*., 2015) and neuroprotective (Chitra *et al*., 2017) effects. *Achyranthes aspera* have also been reported to possess anti- microbial and immunomodulatory properties (Pavithra *et al*., 2020, Bhavya *et al*., 2021, Kolli *et al*., 2021). Root and leaf extracts of *Achyranthes aspera* were reported to exhibit *In- Vitro* anti- cancer activity against human liver and colon cancer cell lines and *In- Vivo* anti- tumor activity in Dalton's Lymphoma induced Balb/c mice model (Singh *et al*., 2017, Singh *et al*., 2021). Considering such wide spectrum of pharmacological potency of *Achyranthes aspera*, an effort has been made to investigate efficacy of its leaves in amelioration of cyclophosphamide induced suppression of specific immunity in male albino rats.

Graphic representation of the Plan of Work:



MATERIALS AND METHODS

2.1. Preparation of leaf extract.

The fresh and healthy plants of *Achyranthes aspera* were harvested from the local areas of Bihar and Jharkhand. The plant was identified and authenticated by Dr. (Mrs.) Malti Kerketta, Associate Professor, University Department of Botany, Dr. S.P.M. University, Ranchi, Jharkhand. The leaves were collected, cleansed and dried under shade. They were powdered by mixer grinder and stored in airtight containers for future use. The dried powder of leaf (100 g) were extracted with 300 ml of Methanol using Soxhlet apparatus for 48 hours (Raj and Gothandam, 2015). The solvent was evaporated at room temperature and the greenish-brown, semi- solid extract obtained was stored in refrigerator for further use. Percent yield of the extract obtained was calculated by following formula:

$$\%\ Yield\ of\ extract = \frac{Total\ amount\ of\ extract\ obtained\ (g)}{Amount\ of\ dried\ powder\ used\ (g)} \times 100$$

2.2. Determination of dose of extract.

Dose of methanolic leaf extract of *Achyranthes aspera* for the experiment was determined by reviewing previous research reports. Lethal dose was reported to be more than 2000 mg/kg body weight. Oral administration of $1/5^{th}$ of the lethal dose (400 mg/kg body weight) was taken as an effective dose for the present study (Zambare *et al*., 2011). The semi- solid extracts were dissolved in distilled water for appropriate dose preparation and administration to the experimental animals.

2.3. Phytochemical screening of extract.

The leaf extract of *Achyranthes aspera* was qualitatively screened for the presence or absence of major phytochemicals; such as, alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, amino acids and protein, saponins, tannins, terpenoids, quinones, resins and coumarins following standard laboratory protocols.

2.4. Experimental Drug.

A widely used anticancer drug, Cyclophosphamide (Endoxan- N; 200 mg) was used as the experimental drug. It was purchased from Azad Pharma, Ranchi, Jharkhand, in the form of white, crystalline powder contained in injection vial. The drug was stored in refrigerator till use. Cyclophosphamide powder was dissolved in distilled water for preparation of dose and final administration to the experimental animals. Cyclophosphamide solution was administered intraperitoneally at the dose of 50 mg/kg body weight to the experimental animals (Bin- Hafeez *et al*., 2001).

2.5. Experimental Animal.

Male albino rats (100-120 g body weight) were used as experimental animal. They were purchased from local animal supplier in Ranchi, Jharkhand and were maintained in the animal house at the University Department of Zoology, Ranchi University, Ranchi, Jharkhand. Rats were reared in well- ventilated cages under standard laboratory conditions of hygiene, temperature (20- 25°C), photoperiod (12 hours light/dark cycle) and humidity. Albino rats were provided with standard rodent pellet diet and drinking water *ad libitum*. The handling, maintenance and sacrifice of albino rats were conducted following the guidelines of the Institutional Animal Ethics Committee, Ranchi University, Ranchi, Jharkhand, India (2/511/2022).

2.6. Experimental Design.

2.6.1. Grouping and dosing schedule of the animals.

Rats were kept for acclimatization for two weeks in ambient laboratory condition with the provision of food and water *ad libitum*, prior to the start of experiment. Rats were divided into four different groups of six individuals each.

Groups	Days																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 a	16 b	17
I (VC)	Distilled Water											Immunized with 1 % v/v CRBC suspension (i.p.)				Assessed for Haemagglutination Titer	Challenged with 1 % v/v CRBC suspension (s.c.)	Assessed for Delayed Type Hypersensitivity response
II (CYP)	Distilled Water													CYP (50 mg/kg b.wt; i.p.)				
III (AAML)	Methanolic Leaf Extract (400 mg/kg b.wt; p.o.)																	
IV (AAML + CYP)	Methanolic Leaf Extract (400 mg/kg b.wt; p.o.)													CYP (50 mg/kg b.wt; i.p.)				

Figure 1: Dosage administration and immunization schedule of rats in control and experimental groups.

Fig 1 showed the pattern of grouping and administration of extracts and experimental drug to the albino rats. The grouping of rats and dosing of the cyclophosphamide and plant extract was scheduled following Bin-Hafeez *et al*., **(2001)**. The experimental rats were maintained for 16 days. Rats in the vehicle control group (Group I) were given equivalent amount of distilled water for 14 days. Group II rats were intraperitoneally administered with a single dose of cyclophosphamide (50 mg/kg body weight) on 14th day of the experiment and were assessed after 48 hours of administration. In group III, rats were administered with methanolic leaf extract of *Achyranthes aspera* orally at the dose of 400 mg/kg body weight for 14 days. Group IV rats were pre-treated with methanolic extract of *Achyranthes aspera* leaves (400 mg/kg body weight; p.o.) for 14 days prior to the intraperitoneal administration of single dose of cyclophosphamide (50 mg/kg body weight) on 14th day.

2.6.2. Preparation of Antigen.

To induce specific immunity in control and experimental rats, Chicken Red Blood Cell (CRBC) suspension was used as an antigenic material. Preparation of antigen and immunization schedule was done following Hussain *et al.,* 2013. The blood collected from chicken was mixed with Alsever's solution in 1:1 proportion and was stored at 4℃ for future use.

The composition of Alsever's solution was as following:

Contents	***% W/V***
Dextrose	2.05
Sodium Chloride	0.42
Sodium Citrate	0.80
Citric acid	0.55

During experimentation, adequate amount of blood was taken from the stock solution and was allowed to stand at room temperature. After washing with normal saline (0.9 % W/V NaCl) 3-4 times, 100 µl of settled RBCs was dissolved in 10 ml of normal saline to make 1 % v/v CRBC suspension. The cell suspension was stored at 4℃ till administration. A definite volume of CRBC suspension was used for immunization and challenge to the albino rats.

2.6.3. Immunization Schedule.
Rats in each group were intraperitoneally administered with 0.2 ml of 1 % v/v CRBC suspension on 12th day of the experiment. Rats of each group were further divided into two sub-groups with three individuals in each. Rats in first sub-group (a) were assessed for Haemagglutination titer on 16th day of the experiment; whereas sub- group (b) rats were subcutaneously challenged with 0.1 ml of 1 % v/v CRBC suspension to assess Delayed Type Hypersensitivity activity (Fig 1).

2.7. Assessment of effects of leaf extract on specific immunity in cyclophosphamide treated rats.
The effect of cyclophosphamide and extract of *Achyranthes aspera* on humoral arm of specific immunity was studied by performing Haemagglutination (HA) titer assay; whereas Delayed type hypersensitivity (DTH) assay was used to determine their effects on the cell- mediated immune function.

2.7.1. Determination of humoral immune response against CRBC.
Measurement of antibody titer by haemagglutination reaction was performed using method of Wahab *et al*., 2014 with some modifications. On 16th day of the experiment, rats were anesthetized and blood samples from rats of sub- group (a) were collected via cardiac puncture and were centrifuged to separate serum. Microtiter plate of 96 well capacity was used to carry out the haemagglutination test. Microtiter wells were filled with 25 µl of normal saline. 25 µl of serum obtained from control and experimental group rats were added in the first well and were mixed thoroughly. Serial two- fold dilution of the serum was prepared by transferring 25 µl of the normal saline and serum solution in successive wells to get higher dilutions. 25 µl of 1 % v/v CRBC was added to each of these dilutions. The haemagglutination plate was incubated at 37℃ for one hour and was observed for agglutination. A mat formation at the bottom was visualized as positive haemagglutination reaction, whereas button formation indicated negative haemagglutination reaction. The antibody titer was expressed in terms of the rank of microtiter well with highest serum dilution showing visible haemagglutination.

2.7.2. Determination of cell- mediated immune response against CRBC.
The cell- mediated immune response against CRBC antigen was assessed in various control and experimental group rats by Delayed type hypersensitivity (DTH) elicited by foot pad reaction test. The test was performed following the method of Wahab *et al.,* 2014 with some modifications. On 16th day, rats of sub-group (b) were challenged with subcutaneous administration of 0.1 ml of 1 % v/v CRBC in the left hind footpad, in addition to immunization with intraperitoneal administration of CRBC on 12th day. Before administration of challenge dose, the thickness of right and left hind footpad was measured by Vernier Caliper. The right hind footpad was injected with same volume of normal saline for assessing any non-specific swelling. After 24 hours of challenge, the footpad thickness was measured to check for any increase or decrease. The pre and post- challenge difference in the thickness of footpad was expressed in mm and was considered as an index of cell- mediated immunity.

2.8. Statistical analysis.
The values were expressed as mean ± SD (Standard Deviation). Data were statistically analysed using Student's *t*-test to determine significant differences in data of various groups. Values were considered statistically significant when $p<0.05$, $p<0.01$ and $p<0.001$. Group II, III and IV were statistically compared with group I values to determine effects of cyclophosphamide, leaf extract and pre-treatment with leaf extract along with cyclophosphamide administration in rats. Statistical difference between group II and IV indicated the possible protective effect of leaf extract against cyclophosphamide induced alterations.

RESULTS

3.1. Yield of extract.
The percent yield of obtained leaf extract of *Achyranthes aspera* was 13.26 %.

3.2. Phytochemical screening of extract.

Table 1 showed the qualitative analysis of methanolic leaf extract of *Achyranthes aspera* for the presence or absence of different phytochemicals. The phytochemicals, such as, alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, tannins, terpenoids, quinones, resins and coumarins gave positive result for the leaf extract of *Achyranthes aspera*; whereas Ninhydrin test for amino acid and proteins showed negative result for the extract tested. The richness of phytochemicals in the extract might be responsible for the protective and ameliorative effects exerted by the it.

Table 1: Qualitative screening of methanolic leaf extract of Achyranthes aspera to show the presence (+) or absence (-) of different phytochemicals.

S.No.	Phytochemical	Protocol followed	Methanolic Leaf extract of *Achyranthes aspera*
1.	**Alkaloids**	Wagner's Test	+
2.	**Carbohydrates**	Molisch's Test	+
3.	**Cardiac Glycosides**	Keller Kelliani's Test	+
4.	**Flavonoids**	Alkaline Reagent Test	+
5.	**Phenols**	Ferric Chloride Test	+
6.	**Amino Acids and Proteins**	Ninhydrin Test	-
7.	**Saponins**	Foam Test	+
8.	**Tannins**	Braymer's Test	+
9.	**Terpnoids**	Salkowski's Test	+
10.	**Quinones**	Hydrochloric Acid Test	+
11.	**Resins**	Turbidity Test	+
12.	**Coumarins**	Sodium Hydroxide Test	+

3.3. Effect of leaf extract pre-treatment on humoral immune response.

Table 2 and Fig 2 showed the effect of cyclophosphamide and *Achyranthes aspera* leaf extract treatment on Haemagglutination titer of albino rats. Haemagglutination titer for group I rats was 10.67 ± 2.08, which was decreased to 3.33 ± 1.15 in group II rats after intraperitoneal administration with single dose of cyclophosphamide (50 mg/kg body weight) on 14th day of the study. This decrease of 68.79 % in heamagglutination titer of group II rats was significant, when compared to group I rats, showing inhibitory effect of cyclophosphamide on production of antibodies against the introduced antigen, CRBCs ($p<0.05$). In leaf extract control group (III), oral daily dose treatment with methanolic leaf extract of *Achyranthes aspera* at the dose of 400 mg/kg body weight resulted in a haemagglutination titer of 12.00 ± 2.64. No significant difference was observed between titer values of group I and III rats, indicating the absence of toxic effect of extract treatment. A numerically higher value of titer in leaf extract treated group as compared to vehicle control one showed the potentiating effect of extract on the antibody production against CRBC (Table 2; Fig 2). In group IV, pre-treatment with leaf extract of *Achyranthes aspera* for 14 days prior to administration of cyclophosphamide resulted in a 130.33 % increase in titer value as compared to cyclophosphamide only treated group II rats. In group IV, haemagglutination titer was 7.67 ± 1.53, which was significantly higher than the group II rats ($p<0.05$). There was no any significant difference in titre value of rats in group I and IV, indicating the protective efficacy of leaf extract pre- treatment against cyclophosphamide induced suppression of antibody production against CRBC.

Table 2: Effect of methanolic extract of leaves of Achyranthes aspera treatment against cyclophosphamide induced alterations in humoral arm of specific immunity in albino rats. Values were Mean ± S.D (n=3), a showed significant difference from Group I, b showed significant difference from Group II, *p<0.05.

Groups	**I (VC)**	**II (CYP)**		**III (AAML)**	**IV (AAML + CYP)**		
HA titer (Rank of wells with dilution)	**10.67 ± 2.08**	**Value**	**% change from group I**	**12.00 ± 2.64**	**Value**	**% change from group**	
						II	**I**
		3.33 ± 1.15^{a*}	**68.79 % (Decrease)**		**7.67 ± 1.53^{b*}**	**130.33 % (Increase)**	**28.12 % (Decrease)**

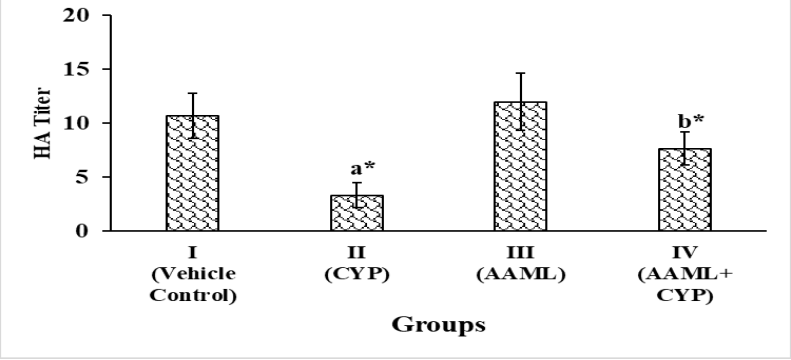


Figure 2: Effect of oral administration of methanolic extract of Achyranthes aspera (400 mg/kg body weight) on humoral arm of specific immunity, studied in terms of Haemagglutination titer, in cyclophosphamide treated rats.

3.4. Effect of leaf extract pre-treatment on cell- mediated immune response.

Table 3 and Fig 3 showed the Delayed Type Hypersensitivity (DTH) response in control and experimental group rats, measured in terms of percent change in hind footpad thickness. In group I rats, hind footpad thickness was increased by 38.44 ± 4.44 % after 24 hours of challenge with 1 % v/v CRBC. Administration of single dose of cyclophosphamide resulted in an increase of only 12.48 ± 2.73 % in the thickness of left hind footpad of rats in group II, which was significantly lower than the group I rats ($p<0.01$). In group III, rats were treated with methanolic leaf extract of *Achyranthes aspera* at the dose of 400 mg/kg body weight for 14 consecutive days and resulted in an increase of 52.65 ± 4.28 % in footpad thickness of rats after getting challenged by CRBC on 16th day of the experiment. The change in thickness of left hind footpad of rats in group III was significantly high as compared to vehicle control group rats, suggesting for an increased DTH response in leaf extract treated group ($p<0.05$). In group IV rats, pre-treatment with methanolic leaf extract of *Achyranthes aspera* for 14 days prior to administration of cyclophosphamide on 14th day resulted in a DTH activity of 50.26 ± 3.65 %, which was significantly higher than the group II rats ($p<0.001$). The percent change in hind footpad thickness of rats in group IV was significantly higher than its value in Vehicle control group ($p<0.05$) (Table 3; Fig 3).

Table 3: Effect of methanolic leaf extract of Achyranthes aspera treatment against cyclophosphamide induced alterations in cell-mediated arm of specific immunity in albino rats. Values were Mean ± S.D (n=3), a showed significant difference from Group I, b showed significant difference from Group II, *p<0.05, #p<0.01, $p<0.001.

Groups	Treatment and Dose (mg/kg body weight)	Pre-challenge Left Hind Foot Thickness (mm)	Post-challenge Left Hind Foot Thickness (mm)	% Change in Left Hind Foot Thickness (%)
I	Vehicle Control (Distilled Water)	**3.13 ± 0.21**	**4.33 ± 0.21**	**38.44 ± 4.44**
II	Cyclophosphamide (CYP) (i.p., 50)	**2.90 ± 0.26**	**3.27 ± 0.38**	**12.48 ± 2.73$^{a\#}$**
III	AAML (p.o, 400)	**2.97 ± 0.25**	**4.53 ± 0.47**	**52.65 ± 4.28^{a*}**
IV	AAML (p.o, 400) + CYP (i.p., 50)	**3.03 ± 0.38**	**4.57 ± 0.67**	**50.26 ± 3.65$^{a*b\$}$**

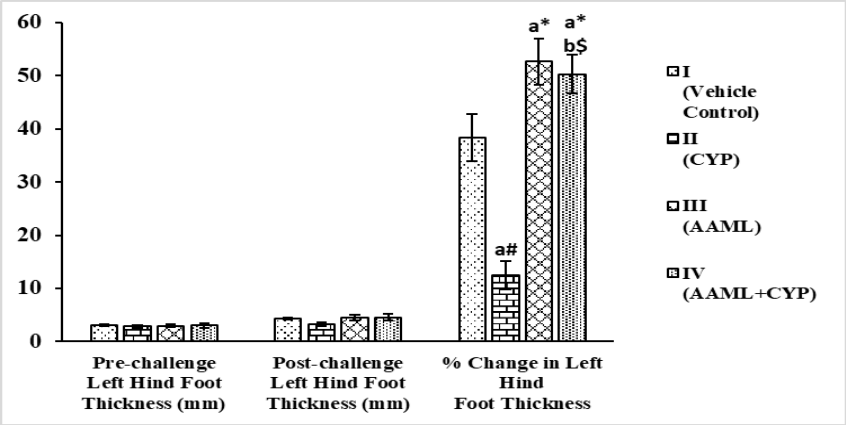


Figure 3: Effect of oral administration of methanolic leaf extract of Achyranthes aspera (400 mg/kg body weight) on cell-mediated arm of specific immunity, studied in terms of Delayed-type hypersensitivity reaction, in cyclophosphamide treated rats.

DISCUSSION

Cyclophosphamide has been widely used in chemotherapy since the late 1950s. It is an alkylating agent having high therapeutic index and broad spectrum of activities against a variety of cancers and immune-related disorders like Systemic Erythematosus Lupus, Rheumatoid Arthritis, Multiple Sclerosis and in organ transplantation. Metabolic activation of cyclophosphamide by the hepatic cytochrome P450 to active metabolites like phosphoramide mustard and acrolein, is responsible for its anti- tumor potential as well as cytotoxicity to the normal cells. Cyclophosphamide induced immunosuppression leads to significant morbidity and mortality, which becomes a major limiting factor in clinical chemotherapy without efficacious remedies (Yu *et al*., 2014 and Nitharwal *et al*., 2013). Recently, many plant- based extracts and bioactive components have been scientifically explored for their potency in alleviation of cyclophosphamide- induced immunosuppression, such as- *Acacia nilotica* (Ahmad *et al*., 2012), *Decalepis hamiltonii* (Shathish *et al*., 2012), naturally acetylated hemicellulose from bamboo shavings (Huang *et al*., 2016), polysaccharides from *Lycium ruthenicum* (Gong *et al*., 2015), *Ganoderma atrum* (Yu *et al*., 2014) and *Lonicera japonica* (Zhou *et al*., 2018), Gallic acid (Shruthi *et al*., 2018) and pomegranate peel (Wu *et al*., 2019).

The haemagglutination titer assay has been used a marker of humoral immune response, as it measured the amount of antibody formed against the introduced antigen in the individual. In the present study, single dose of cyclophosphamide significantly reduced the HA titer value of rats, which were significantly elevated in case of leaf extract pre-treated group rats ($p<0.05$) (Table 2 and Fig 2). In accordance to the above finding, Raj and Gothandam, 2015 reported a decrease in antibody titer after treatment with cyclophosphamide (50 mg/kg body weight; p.o.) and a significant increase in the circulating HA titer value in methanolic extract of *Amorphophallus commutatus* treated group. They argued that the extract significantly stimulated the humoral immune response by increasing the antibody produced against SRBC. Similarly, Wahab *et al*., 2014 reported the ameliorative effect of *Averroha carambola* on humoral immune response to sheep erythrocytes in cyclophosphamide treated mice. They suggested that the immunomodulatory activity of the leaves of *Averrhoa carambola* might be due to presence of phytoconstituents like carambolaflavone. In the same context, Devi *et al*., 2020 reported a significant elevation in HA titer of Swiss Albino mice treated with ethanolic extract of rhizome of *Kaempferia parviflora* along with cyclophosphamide administration as compared to cyclophosphamide only treated group. They further explained that the increase in proliferation and transformation of B lymphocytes might be responsible for the increase in antibody titer value and the immunomodulatory effect of the plant can be attributed to the presence of flavonoid compounds.

The DTH reaction is a type IV hypersensitivity reaction according to the Coombs and Gell classification and it provides a functional in- vivo assessment of the cell- mediated immunity. The DTH reaction is mediated through activation of T cells leading to release of lymphokines and subsequently inducing the activation and accumulation of macrophages that boost phagocytic activity and inflammation, resulting in the net increase in the thickness of the footpad in previously immunized animals (Nfambi *et al*., 2015). In the present study, a significant higher value of % change in hind footpad thickness of rats in *Achyranthes aspera* leaf extract treated group as compared to vehicle control as well as cyclophosphamide treated group rats showed an increased activity of cell- mediated immune response against CRBC (Table 3 and Fig 3). The finding of present study was in accordance with the study made by Kar *et al*., 2019, who reported a significant decrease in paw edema at 24 and 48 hours of sheep antigen challenge to cyclophosphamide treated albino rats as compared to control animals. According to them, oral treatment with hydro-alcoholic extract of *Gymnema sylvestre* in cyclophosphamide treated rats elicited a significant increase in DTH response, suggesting an activation of cellular immunity. This immunomodulatory potency can be attributed to the presence of phytochemicals like alkaloids, triterpenoids, flavonoids, steroids, tannins and phenolic compounds. Similarly, Devi *et al*., 2020 also reported a significant increase in footpad thickness of mice of *Kaempferia parviflora* alone treated and *K. parviflora* treatment in immunosuppressed group, when compared to cyclophosphamide control group. In the present study, qualitative phytochemical screening of methanolic leaf extract of *Achyranthes aspera* showed presence of flavonoids, phenols, alkaloids, saponins, terpenoids and coumarins (Table 1).

CONCLUSIONS

Leaf extract of *Achyranthes aspera* modulated the specific immunity through increased production of antibody and elevation of DTH response in normal as well as cyclophosphamide treated immunocompromised albino rats. Thus, the leaf extract not only possessed protective efficacy against cyclophosphamide induced suppression of humoral as well as cell- mediated arm of specific immunity, but also potentiated the specific immune response against introduced antigen. These potential might be attributed to the presence of bioactive metabolites and can be further analysed to develop novel therapeutic alternatives in chemotherapy.

List of Abbreviations:

AAML- *Achyranthes aspera* methanolic leaf extract
CRBC- Chicken Red Blood Cell
CYP- Cyclophosphamide
DTH- Delayed- Type Hypersensitivity
HA- Haemagglutination
SRBC- Sheep Red Blood Cell
VC- Vehicle Control

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REFERENCES

Ahmad S, Mika D and Guruvayoorappan C (2012). Chemoprotective and immunomodulatory effect of *Acacia nilotica* during cyclophosphamide induced toxicity. *Journal of experimental therapeutics & oncology* **10** (2) 83–90.

Athesh K, Sivasubramanian R, Jothi G and Brindha P (2020). Evaluation of anti- obesity potential of aqueous extract of *Achyranthes aspera* Linn. In high fat diet induced obese rats. *Clinical Phytoscience* **6** 69. DOI: https://doi.org/10.1186/s40816-020-00217-5.

Beg Md A and Athar F (2020). Pharmacokinetic and molecular docking studies of *Achyranthes aspera* phytocompounds to exploring potential anti- tuberculosis activity. *Journal of Bacteriology & Mycology*: Open Access **8** (1) 18-27. DOI: 10.15406/jbmoa.2020.08.00268.

Bhavya S, Ganapathi U, Mariavincent MB and Elanchezhiyan M (2021). Anti-hepatitis B activity of methanolic extracts of *Achyranthes aspera* leaves. *European Journal of Molecular & Clinical Medicine* **8** 11.

Bin-Hafeez B, Ahmad I, Haque R and Raisuddin S (2001). Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. *Journal of Ethnopharmacology* **75** (1) 13-18. DOI: 10.1016/s0378-8741(00)00382-2.

Chitra V, Manasa K, Mythili A, Tamilanban T and Gayathri K (2017). Effect of hydroalcoholic extract of *Achyranthes aspera* on Haloperidol- induced Parkinson's disease in Wistar rats. *Asian Journal of Pharmaceutical and Clinical Research* **10** (9) 318-321. DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i9.19285.

Devi AR, Kariyil BJ, Raj NM, Akhil GH and Balakrishnan-Nair DK (2020). Immunomodulatory effect of *Kaempferia parviflora* against cyclophosphamide induced immunosuppression in Swiss Albino mice. *Pharmacognosy Magazine* **16** S13-21 DOI: 10.4103/pm.pm_233_19.

Diwanay S, Chitre D and Patwardhan B (2004). Immunoprotection by botanical drugs in cancer chemotherapy. *Journal of Ethnopharmacology* **90** (1) 49–55. DOI: https://doi.org/10.1016/j.jep.2003.09.023.

Fahim NF and Sathi ZS (2018). Assessment of hepatoprotective activity of roots and barks of *Achyranthes aspera* in Carbon tetrachloride induced hepatotoxicity in rats. *Malaysian Journal of Halal Research* **1** (2) 23-26. DOI: http://doi.org/10.26480/mjhr.02.2018.23.26.

Gong Y, Wu J and Li ST. (2015). Immuno-enhancement effects of *Lycium ruthenicum* Murr. polysaccharide on cyclophosphamide-induced immunosuppression in mice. *International journal of clinical and experimental medicine* **8** (11) 20631–20637.

Huang JQ, Pang MR, Li GY, Wang N, Jin L and Zhang Y (2017). Alleviation of cyclophosphamide-induced immunosuppression in mice by naturally acetylated hemicellulose from bamboo shavings. *Food and Agricultural Immunology* **28** (2) 328-342. DOI: 10.1080/09540105.2016.1272553.

Hussain A, Shadma W, Maksood A and Ansari SH (2013). Protective effects of *Picrorhiza kurroa* on cyclophosphamide-induced immunosuppression in mice. *Pharmacognosy Research* **5** (1). DOI: 10.4103/0974-8490.105646.

Kar PP, Rath B, Ramani YR and Maharana CS (2019). Amelioration of cyclophosphamide induced immunosupression by the hydro-alcoholic extract of *Gymnema Sylvestre* leaves in albino rats. *Biomedical & Pharmacology Journal* **12** (1) 251-258. DOI: http://dx.doi.org/10.13005/bpj/1635.

Khan Md A, Samdershi D, Kujur P, Sana H, Neetu K and Besra S (2021). Studies on effects of aqueous leaf extract of *Achyranthes aspera* on thyroid function in male albino rats. *Biospectra* **16** (1) 59-62.

Kolli S, Nitin M, Inamdar S and Kumar DA (2021). Immunomodulatory activity of methanolic and aqueous extracts of whole plant of *Achyranthes Aspera* Linn. in swiss albino mice and wistar albino rats. *RGUHS Journal of Pharmaceutical Sciences* **11** (1) 15-21.

Kumar D, Arya V, Kaur R, Bhat ZA, Gupta VK and Kumar V (2012). A review of immunomodulators in the Indian traditional health care system. *Journal of microbiology, immunology and infection* **45** (3) 165–184. DOI: https://doi.org/10.1016/j.jmii.2011.09.030.

Mani P, Vijayaraj R, Kumar KN, Senthil J, Kumar GD and Jayaseelan T (2016). Green synthesis of silver nanoparticles from ethanolic seed extract of *Achyranthes aspera* (linn.) and its anti-inflammatory activities. *International Journal of Pharmacy & Therapeutics* **7** (1) 42-48.

Mondal S, Ghosh D, Ganapaty S, Reddy MS and Ramakrishna K (2016). Evaluation of Healing Potential of *Achyranthes aspera* L. (Amaranthaceae) seeds in excision, incision, dead space and burn wound model-An *in-vivo* Study. *Pharmacognosy Journal* **8** (3). DOI: 10.5530/pj.2016.3.20.

Nfambi J, Bbosa GS, Sembajwe LF, Gakunga J and Kasolo JN (2015). Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in wistar albino rats. *Journal of Basic and Clinical Physiology and Pharmacology* **26** (6) 603–611. DOI: 10.1515/jbcpp-2014-0104.

Nitharwal RK, Patel H, Karchuli MS and Ugale RR (2013). Chemoprotective potential of *Coccinia indica* against cyclophosphamide-induced toxicity. *Indian Journal of Pharmacology* **45** (5) 502-7. DOI: 10.4103/0253-7613.117783.

Pandey NK, Sharma HP, Patnaik A and Jain P (2013). A review on potential magic folk herbal medicinal plant: *Achyranthes aspera* L. *International Journal of Medicinal Plants* .Photon **105** 350-363.

Pavithra S, Mohana B, Mani M, Saranya PE, Jayavel R, Prabhu D and Kumaresan S (2020). Bioengineered 2D ultrathin sharp-edged MgO nanosheets using *Achyranthes aspera* leaf extract for antimicrobial applications. *Journal of Inorganic and Organometallic Polymers and Materials.* DOI: https://doi.org/10.1007/s10904-020-01772-7.

Prabhu VV and Guruvayoorappan C (2012). Evaluation of immunostimulant activity and chemoprotective effect of mangrove *Rhizophora apiculata* against cyclophosphamide induced toxicity in BALB/c mice. *Immunopharmacology and Immunotoxicology* **34** (4) 608–615. DOI: 10.3109/08923973.2011.642883.

Raj S and Gothandam KM (2015). Immunomodulatory activity of methanolic extract of *Amorphophallus commutatus* var. *wayanadensis* under normal and cyclophosphamide induced immunosuppressive conditions in mice models. *Food and Chemical Toxicology*. DOI: http://dx.doi.org/10.1016/j.fct.2015.04.026.

Reddy CV, Kamble AK and Patil SJ (2016). Evaluation of antifertility activity of *Achyranthus aspera* leaves in female mice. *International Journal of Pharmaceutical Sciences and Research* **7** (9) 3794-3801. DOI: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (9).3794-01.

Shakeel S, Sharma AK, Sohail Md and Sharma V (2015). *Achyranthes aspera* have the potential antioxidant property in scavenging free radicals produced as a result of oxidative stress induced by arsenic. *World Journal of Pharmacy and Pharmaceutical Sciences* **4** (06) 562- 573.

Sharma V and Chaudhary U (2015). An overview on indigenous knowledge of *Achyranthes aspera. Journal of Critical Reviews* **2** (1) 7-19.

Shathish K, Reena D and Guruvayoorappan C (2012). Chemoprotective effect of *Decalepis hamiltonii* against cyclophosphamide induced toxicity. *Journal of experimental therapeutics & oncology* 9 (4) 291–301.

Shruthi S, Vijayalaxmi KK and Shenoy KB (2018). Immunomodulatory effects of gallic acid against cyclophosphamide- and cisplatin-induced Immunosuppression in swiss albino mice. *Indian Journal of Pharmaceutical Sciences* **80** (1) 150-160. DOI: 10.4172/pharmaceutical-sciences.1000340.

Singh S, Verma SK and Singh SK (2017). *In-vitro* anticancer activity of *Achyranthes aspera* root extract against different human cancer cell lines. *Biolife* **5** (1) 119-122. DOI:10.17812/blj.2017.5119.

Singh RK, Verma PK, Kumar A, Kumar S and Acharya A (2021). *Achyranthes aspera* L. leaf extract induced anticancer effects on Dalton's Lymphoma via regulation of PKCα signaling pathway and mitochondrial apoptosis. *Journal of Ethnopharmacology* **274**. DOI: 10.1016/j.jep.2021.114060.

Wahab S, Hussain A, Ahmad MP, Rizvi A, Ahmad MF and Farooqui AHA (2014). The ameliorative effects of *Averroha carambola* on humoral response to sheep erythrocytes in non-treated and cyclophosphamide immunocompromised mice. *Journal of Acute Disease* 115-123. DOI: 10.1016/S2221-6189(14)60027-5.

Wu Y, Zhu CP, Zhang Y, Li Y and Sun JR (2019). Immunomodulatory and antioxidant effects of pomegranate peel polysaccharides on immunosuppressed mice. *International Journal of Biological Macromolecules* **15** (137) 504-511. DOI: 10.1016/j.ijbiomac.2019.06.139.
Yu Q, Nie SP, Wang JQ, Liu XZ, Yin PF, Huang DF, Li WJ, Gong DM and Xie MY (2014). Chemoprotective effects of *Ganoderma atrum* polysaccharide in cyclophosphamide-induced mice. *International Journal of Biological Macromolecules* **64** 395-401. DOI: 10.1016/j.ijbiomac.2013.12.029.
Zambare M, Bhosale UA, Somani RS, Yegnanarayan R and Talpate KA (2011). Effect of treatment with *Achyranthes aspera* (Agadha) ethanol extract on various haematological and biochemical parameters in alloxan induced diabetic rats. *International Journal of Pharmaceutical Frontier Research* **1** (1) 42-52.
Zhou X, Dong Q, Kan X, Peng L, Xu X, Fang Y & Yang J. (2018). Immunomodulatory activity of a novel polysaccharide from *Lonicera japonica* in immunosuppressed mice induced by cyclophosphamide. *PLoS ONE* **13** (10). DOI: https://doi.org/10.1371/journal.pone.0204152.