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Effect of *Chlorophytum borivilianum* on adjuvant induced arthritis in rats

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Abstract

In this study, anti-arthritic effect of aqueous and alcoholic extracts of *Chlorophytum borivilianum* tubers on complete Freund's adjuvant (CFA) induced arthritis has been studied in wistar albino rats. During arthritic condition, aqueous and alcoholic extracts of *Chlorophytum borivilianum* significantly reduced the paw volume; inhibited body weight loss compared to vehicle treated control rats and thereby extracts of *Chlorophytum borivilianum* shows anti-arthritic activity.

Keywords: *Chlorophytum borivilianum*, Anti-arthritic activity, Freund's complete adjuvant, paw volume

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic disorder that is characterized by a symmetric polyarthritis finally leading to progressive joint destruction. The hallmarks of RA are joint inflammation and the proliferation of synovium that is accompanied by the erosion of the underlying cartilage and bone. The exact etiology of RA is still not clear. However, it is well established that T-cells play an important role in the initiation of the immune activation against self antigens. Autoreactive β lymphocytes produce auto-antibodies mainly against collagen type II, citrullinated proteins and glucose-6-phosphoisomerase. The autoreactive antibodies form immune-complexes with self-antigens in local joints, triggering the activation of complement and R signaling pathway, which leads to local inflammation and proliferation of synovium. In addition, Neutrophils and macrophages recruited to the local joints secrete (TNF-α) and IL-1, which contribute to cartilage and boneatumor necrosis factor- destruction. The modern drugs both steroidal and non-steroidal anti-inflammatory drugs are used for the amelioration of the symptoms of the disease, however they offer only temporary relief and also produce variable side effects [[1]].

In the indigenous system of medicine, *Chlorophytum borivilianum* belonging to family liliaceae is a very well known plant for its aphrodisiac as well as immunomodulatory activity in India. Roots holds very important position in Ayurveda and Unani system where it is mostly used to treat oligospermia, pre and post natal symptoms, arthritis, diabetes and dysuria [[2],[3][. It is scientifically reported for its antistress [[4]], aphrodisiac [[5]], antimicrobial [[6]], analgesic [[7]], anti-inflammatory [[8]], and immunomodulatory [[9]] activities. As its anti-arthritic activity of *Chlorophytum borivilianum* was not previously reported, hence present study was undertaken to prove this folk claim.

Materials and Methods

Plant material collection and authentication

Chlorophytum borivilianum roots were purchased from local vendor. The roots were dried, powdered to coarse size and stored in airtight container for further use. Plant species is authenticated by Botanist Dr. Prabha Bhogaonkar, Vidarbh institute of science and humanities, (V.M.V), Amravati. A specimen sample is deposited at Dept. of Botany, Vidarbh institute of science and humanities, (V.M.V), Amravati.

Extraction

The *C. borivilianum* tubers were powdered and defatted by petroleum ether. Marc then extracted with alcohol for 3 hour with mild heating. The aqueous extract was prepared by maceration process by treating 100g of fresh powder with 500ml of distilled water along with 10ml of chloroform as a preservative .The maceration process was carried for 7 days with occasional stirring. Both the extracts were filtered / condensed and evaporated to dryness under vacuum. This Alcoholic (AL) and Aqueous (AQ) extract further use for study.

Animals

For acute toxicity studies and anti-arthritic activities, wistar albino rats weighing between 150-200 g were selected. The animals were acclimatized to standard laboratory conditions (temperature 25 ± 2^{o}C) and maintained on 12 h light, 12 h dark cycle. Rats were fed with standard pellet and water *ad libitum*. The animal care and experimental protocol were in accordance with the Institutional Animal Ethical Committee (CPCSEA/IAEC/PC-01/04-2K8).

Freund's adjuvant Induced Arthritis [[10]]

Animals were randomly divided into eight groups of six animals each (n=6). Group I served as control received 2% tween 80, Group VIII received methotrexate (0.75 mg/kg p.o.) served as reference standard and Group II, III and IV received the Aqueous extracts in dose of 30, 100, 300 mg/kg body weight p.o. respectively. Group V, VI, VII received the Alcoholic extracts in dose of 30, 100, 300 mg/kg body weight p.o. respectively. All drugs prepared in 20%Tween 80 solution were administered orally.

Arthritis was induced by injecting a 0.1 ml of complete Freund's adjuvant (CFA) containing 1.0 mg of dry heat killed *Mycobacterium tuberculosis* bacteria homogenized in liquid paraffin into the left hind paw.

The swelling in hind paws were periodically examined in each paw from the ankle using digital plethysmometer (Panlabs, India.)

On 0th day, the left hind paw volume of all rats as a volume displacement was measured using digital plethysmometer (Panlabs, India.). CFA arthritis was induced in all rats on day 1. The aforementioned drug treatment was started on 1st day and continued for 21st day, the vehicle (20%Tween 80 solution) and all drug solutions were administered orally. The assessment of anti-arthritic activity was carried out by measuring change in paw volume edema on 7th and 21st day after injection.

The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and % inhibition of paw edema with respect to untreated group was calculated.

Table 1: Changes in paw volume in CFA induced arthritis in rats on day 7th

All values are in Mean±SEM; p<0.01 = Significant, ** p<0.001 = More significant vs. Control; n = 6, NS: Non-significant*

Contol	AQ-30	AQ-100	AQ-300	AL-30	AL-100	AL-300	Metho-0.75
1.51	1.44	1.39	1.41	1.56	1.4	1.23	1.18
1.55	1.41	1.44	1.5	1.6	1.41	1.29	1.2
1.62	1.49	1.48	1.43	1.46	1.38	1.3	1.24
1.43	1.54	1.54	1.39	1.44	1.52	1.33	1.26
1.49	1.52	1.55	1.46	1.5	1.4	1.27	1.14
1.59	1.6	1.61	1.36	1.51	1.38	1.23	1.12
1.531	1.5	1.501	1.425	1.511	1.415	1.275	1.19
0.028	0.028	0.032	0.02	0.024	0.021	0.016	0.022
	NS	NS	*	NS	*	**	**

Table 2: Changes in paw volume in CFA induced arthritis in rats on day 21st

All values are in Mean±SEM; p<0.01 = Significant, ** p<0.001 = More significant vs. Control; n = 6, NS: Non-significant*

Control	AQ-30	AQ-100	AQ-300	AL-30	AL-100	AL-300	Metho-0.75
1.34	1.28	1.37	1.22	1.37	1.28	1.22	1.1
1.43	1.33	1.3	1.26	1.42	1.25	1.17	1.16
1.4	1.37	1.27	1.19	1.44	1.33	1.15	1.19
1.46	1.39	1.39	1.3	1.35	1.35	1.24	1.23
1.29	1.41	1.42	1.34	1.38	1.24	1.28	1.21
1.38	1.43	1.4	1.31	1.3	1.29	1.26	1.27
1.383	1.368	1.358	1.27	1.376	1.29	1.22	1.193
0.025	0.022	0.024	0.023	0.02	0.017	0.02	0.024
	NS	NS	**	NS	*	**	**

Body weight

The loss of body weight was monitored with 1 gm precision balance (Gill real weight, India.).

Table 3: Changes in body weight in CFA induced arthritis in rats

Days	Control	AQ-30	AQ-100	AQ-300	AL-30	AL-100	AL-300	Metho-0.75
0 day	212.6 ± 2.31	165.34 ± 3.5	166.4 ± 4.21	154.32 ± 12.4	207.34 ± 5.21	194.24 ± 6.12	166.24 ± 2.45	188.9 ± 1.32
7th day	192.45 ± 2.34	169.92 ± 6.21	172.58 ± 4.2	159.34 ± 4.5	211.71 ± 4.1	199.89 ± 3.4	173.34 ± 9.1	194.6 ± 4.8
14th day	178.09 ± 7.51	170.21 ± 3.10	172.98 ± 4.8	161.76 ± 5.1	212.09 ± 8.61	201.56 ± 9.8	174.93 ± 7.2	196.89 ± 9.4
Mean change in weight	34.51	4.87*	6.58**	7.44**	4.75*	7.32**	8.69**	7.99**

**p<0.01 ** p<0.001 Control; n = 6*

Statistical Analysis

The experimental results are represented as Mean ± SEM (Standard Error of Mean). Statistical analysis was performed by one-way ANOVA followed by Dunnet's't' test. P< 0.01 was considered significant.

Results

From the acute toxicity study, the LD50 cut-off dose for aqueous extract as well as alcoholic extract were found to be safe up to the dose of 2000 mg/kg. Based upon this three therapeutic doses (30, 100, 300 mg/kg) were selected for the study.

The aqueous extract (300mg/kg) inhibited the rat paw oedema by 43.61% and alcoholic extract (300mg/kg) by 46.29% whereas methotrexate produce 48.50 % inhibition of rat paw oedema after 21 days (Table 2).

Usually the loss of body weight observed during the arthritis condition but pretreatment with aqueous and alcoholic extract showed increase in the body weight (Table 3).

Discussion

The CFA mono-arthritis of the knee is induced by intra articular injection of complete Freund's adjuvant, suspension of heat-killed *Mycobacterium butyricum* or *Mycobacterium tuberculosis*. This chronic monoarthritis is characterized by joint inflammation, cartilage destruction and bone erosion, which persist for at least several weeks. The CFA mono-arthritis model represents a modification of the classical adjuvant-induced poly-arthritis [[11]].

In the present study, rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease. The determination of paw

swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs.

From the results observed in the current investigation, it is observed that the aqueous and alcoholic extract of *Chlorophytum borivilianum* possesses potentially useful anti-arthritic activity.

Conclusion

Chlorophytum borivilianum is a medicinal plant which may have beneficial effects in the prevention and treatment of arthritis. Active components of *Chlorophytum borivilianum* include steroidal saponins and polyphenolics. Saponins may have anti-arthritic effects associated with their anti-protozoal activity. It has been postulated that saponins may have anti-arthritic properties by suppressing intestinal protozoa which may have a role in joint inflammation. *Chlorophytum borivilianum* phenolics are also anti-oxidants and free-radical scavengers, which may aid in suppressing reactive oxygen species that stimulate inflammatory responses. Folk medicine and anecdotal reports suggest that whole *Chlorophytum borivilianum* tuber powder aids in prevention and treatment of arthritis.

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