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Anti-Arthritic and Immune Modifying Potential of *Delonix Elata* Bark Extracts.

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ABSTRACT

Arthritis is a chronic, systemic inflammatory disorder that may potentially affect many tissues and organs, but principally attacks flexible musculo-skeletal joints in human body. *Delonix elata* bark extracts were studied for its therapeutic benefit against arthritis and its immunomodulatory effects in animal models. A 70% methanolic extract exhibited significant protection of arthritic inflammation on 6th and 10th day. This extract also produced comparable immune modification in cell mediated and humoral immunity. SRBC (please use full form here) induced DTH (full form), Heamoaggulutination and Phagocytosis models 70% methanolic extract of *Delonix eleta* produced significant change in such models when compared to control and standard treatments.
Key words : Arthritis, Immune, *Delonix elata,* Methanol, Extract.

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INTRODUCTION

Allopathic anti-inflammatory drugs are the mainstay of treatment for a variety of immune disorders, including rheumatoid arthritis (RA).[1,2] The non-steroidal anti-inflammatory drugs (NSAIDs) and biologics like (TNF)-α *a*ntibody and the decoy TNF- α receptor represent a prominent group of such drugs. However, the usage of these drugs is associated with severe adverse effects, including gastrointestinal bleeding and cardiovascular complications [3]. Due to the side effects and the high cost of conventionally used anti-inflammatory drugs, patients with arthritis are increasingly using complementary and alternative medicine (CAM) modalities of treatment[4,5] Over 36% Americans used CAM products annually for different disorders and the trend is on the rise[6,7]. Traditional Chinese medicine, Ayurvedic medicine, Kampo, and Homeopathy are among the major contributors to the natural products consumed by patient population.

A diverse group of diseases is characterized by inflammation that can be triggered not only by foreign microbial antigens but also by self-antigens. The response to self-antigens results in autoimmune inflammation. Therefore, like the infectious diseases, the autoimmune diseases are also associated with inflammation. Considering that autoimmune diseases result from a deregulated immune system[8], it is imperative to examine the therapeutic claims of CAM products for their immunological basis of curative properties against autoimmune disorders as well as other conditions involving inflammation[9,10].

MATERIALS AND METHODS

Plant Material

The barks of the plant *Delonix elata* were obtained from the outskirts of Tiruchengode, Namakkal Dt. Tamilnadu, India. It was identified by Prof. Sreenath (Taxonomist) Bangalore University, Bangalore. A voucher specimen has been prepared and deposited at the Department of Pharmacognosy, PES College of Pharmacy, Bangalore. The collected barks were dried in shade, crushed to coarse powder and used for the studies.

Preparation of Extracts

The dried plant barks were subjected to continuous hot extraction successively with petroleum ether, chloroform and 70% methanol for 8 hours. The extract was filtered, concentrated and the solvent was removed by rotary flash evaporator. The extract was dried over a dessicator. The dried residue thus obtained, was used for this study. The extracts were subjected to preliminary qualitative tests to identify the various phytoconstituents present in the barks. It was observed that petroleum ether extract showed presence of Oils, Phytosterols and Steroidal compounds. Chloroform extract showed positive test for Lipids, Steroidal compounds and trace of alkaloids. Alcoholic extract showed positive test for Saponins, Flavonoids, Tannins, Carbohydrates and trace of alkaloids.

Animals

Albino Swiss mice (18-25g) and Swiss Albino rats (220-250g) were procured from Bioneeds, Nelamangala, Tumkur, India. The animals were housed under standard conditions of temperature (25 ± 1^{o}C), relative humidity (55±10%), and 12/12 hour light / dark cycles and fed with standard pellet diet (Pranav Agro Industries Ltd, Bangalore) and portal water *ad libitum* under strict hygienic conditions. The animal studies were conducted after obtaining clearance from Institutional Animal Ethics Committee and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Pharmacological Studies

Delayed-type Hypersensitivity (DTH) Response [11]

Six animals per group of four groups of mice weighing 23 to 25 gm were selected for the study. All the animals were immunized by i.p. administration of 0.1 ml of 0.5×10^{9} SRBCs/ mice on day -14. Group 1 treated as control group 2, 3 and 4 received 250 mg of pet ether, chloroform and 70% methanolic extract of *Delonix eleta* bark which were prepared by suspending them in tween 80.

All the groups were challenged by sub plantar administration of 0.1ml of 0.025×10^{9} SRBCS/ml into right hind foot paw on day 0. All the three extracts were administered orally from day – 14 until day 0. DTH response was measured at 24th and 48th hour after SRBCs challenge on days 0 and expressed as mean percent increase in paw volume by using Plethysmometer. The results were analysed by Dunnett's *t*-test (*n* = 6).

Effects of *Delonix eleta* bark extracts on antigenically challenged mice

Delayed-type hypersensitivity (DTH) response Mean percent increase in paw oedema		
Treatment	24h	48h
Control	29.12 ± 1.29	18.12 ± 0.98
Pet.Ether extracted residue (250mg)	33.15 ± 2.45**	21.85 ± 1.85*
Chloroform extracted residue (250mg)	32.20 ± 1.24*	19.50 ± 1.45
70% Methanol extracted residue (250mg)	36.50 ± 1.80**	22.24 ± 2.27**

n = 6 per group. Values are Mean+SEM. *P< 0.05, ** P < 0.001

Macrophage Phagocytosis by Carbon Clearance Assay [12]

Mice of either sex were divided into five groups of six each. Group 1 served as control. Group 2 received cyclophosphamide (50 mg /kg, i.p) on 4th, 5th and 6th day. The test

extracts of *Delonix elata* pet ether, chloroform and 70% methanol extracts 250mg/kg, p.o were administered to group 3, 4 and 5 respectively 7 days prior to the injection of carbon particles. On 7th day all the animals received an intravenous injection of Indian ink suspensions**.** As soon as mouse eyes turned black 50 µl of blood samples were collected on the slide from each animal by retro orbital bleeding by using glass capillaries at an interval of 2 min (*t1*) and 10 min (*t2*) after the injection of ink suspension lysed with 4 ml of 0.1 % sodium carbonate solution. Absorbances of these samples were measured at 675 nm using spectrophotometer. Then the liver and spleen of individual mice were culled and weighed. Rate of carbon clearance (K) and Phagocytic index (α) are calculated by the formula:

$$(K) = log\ A1 - log\ A2 \,/\, (t1 - t2) \text{ and } (\alpha) = K1/3 \text{ X body weight / Liver wt. + spleen wt.}$$

Rate of carbon clearance and phagocytic index of treated group animals was compared with the control group animals**.** The results are presented as mean ± SEM from 6 animals. Statistical analysis between the groups was analysed by using One-Way ANOVA followed by Dunnett's test. $P<0.05$ was considered to be statistically significant.

Effects of *Delonix eleta* bark extracts on carbon clearance

Phagocytic activity		
Treatment	Rate of carbon clearance (K) (10^{-02})	Phagocytic index (α) (10^{-04})
Control	0.081±0.013	0.167±0.065
Cyclophasphamide (50mg)	0.049±0.007*	0.035±0.012*
Pet. Ether extracted residue (250mg)	1.679±0.012*	1.316±0.271*
Chloroform extracted residue (250mg)	0.841±0.007	0.615±0.012
70% Methanol extracted residue (250mg)	2.091±0.007*	1.630±0.012*

n = 6 per group. Values are Mean ± SEM. *P < 0.01

Hemagglutination Antibody Titre[13]

Humoral immune response was performed using SRBC as antigen. The mice weighing 22-25g were divided into 5 groups of six animals each. The vehicle (2% w/v CMC) was administered to each mouse of group I (control). Group II (cyclophosphamide-treated group) received the vehicle for a period of 7 days, petroleum ether, chloroform and 70% methanol extracts 250 mg/kg were administered to mice of group III, IV and V respectively for 7 days. The animals of groups II &III were injected with cyclophosphamide (30 mg/kg, i.p.) on the 4^{th}, 5^{th}, and 6^{th} day, 1 h after the administration of dose treatment.

The animals were immunized by injecting 0.1 ml of 20% of fresh SRBC suspension, intraperitonially on day 0. Blood samples were collected in micro centrifuge tubes by retro-orbital plexus on the 7^{th} day and serum was separated by centrifugation. Antibody levels

were determined by hemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. Two fold dilutions of pooled serum samples were made in 25 µl volumes of normal saline in micro titration plate and to that was added 25µl of 1% suspension of SRBC in saline. After mixing, the plates were incubated at room temperature for 1 h and examined for hemagglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre.

Humoral immune response by antibody titre by Extracts of *Delonix elata* Bark

Hemagglutination antibody titre	
Treatment	Day 7
Control	4.33±0.21
(Clyclophasphamide 30mg)	2.83±0.40**
Pet.Ether extracted residue (250mg)	4.66±0.21
Chloroform extracted residue (250mg)	5.33±0.21
70% methanol extracted residue (250mg)	8.16±0.30**

n = 6 per group. Values are Mean±SEM. ** P < 0.01

Formalin Induced Inflammatory Arthritis [14]

Wister albino rats weighing 220 to 250 gm were housed for two weeks. All animals were randomly divided into four groups of six animal each (n=6). Group I served as control and Group II as standard. Group III, IV and V were received 250 mg/kg of pet. ether, chloroform and 70% methanolic extract of *Delonix elata*. Rats were injected with 0.1 ml 2 %(v/v) of formaldehyde solution in the planter surface of the left foot, on the first and third day of the test. Drug treatment was started from the initial day i.e. from the day of formaldehyde injection (0 day) and continued till 10^{th} day. The rat paw volume was recorded on 3^{rd},6^{th} and 10^{th} day by using Plethysmometer.

Effect on Formaldehyde induced arthritis by Extracts of *Delonix elata* Bark.

Paw Volume (ml)				
Treatment	Day 0	Day 3	Day 6	Day 10
Control	1.04±0.11	1.57±0.31	1.98±0.33	1.92±0.20
(Diclofinac 10mg)	0.98±0.06	1.27±0.13*	1.33±0.09**	1.22±0.10**
Pet.Ether extracted residue (250mg)	0.97±0.08	1.55±0.18	1.72±0.31	1.60±0.18*
Chloroform extracted residue (250mg)	0.02±0.08	1.49±0.18	1.81±0.31	1.67±0.18
70% methanol extracted residue (250mg)	1.00±0.17	1.40±0.23	1.61±0.22*	1.34±0.17**

n = 6 per group. Values are Mean±SEM. *P< 0.05, ** P < 0.001.

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RESULTS AND DISCUSSION

In the present investigation, SRBC-induced delayed-type hypersensitivity was used to assess the effect of the extracts on cell-mediated immunity. The peak edema at 24 h was taken as a parameter for evaluating the reaction. *Delonix elata* 70% methanolic extract produced a significant decrease from DTH reactivity in mice.

Effect of extracts on humoral response was tested on sheep erythrocyte-specific haemagglutination antibody titre in mice. It was found that a significant change occurred in the production of circulating antibodies. In view of the pivotal role played by the macrophages in coordinating the processing and presentation of antigen to β-cells, the extracts were further evaluated for its effect on macrophage phagocytic activity. When the carbon particles are injected intravenously, the rate of clearance of carbon from blood by macrophage is governed by an exponential equation. This seems to be the general way in which inert particulate matter is cleared from the blood. From this study, the 70% methanolic extracts showed significant effect on macrophage phagocytosis. In formaldehyde induced arthritis also 70% methanol extract of *Delonix elata* Bark exhibited significant protection on day 6 and 10.

The present study establishes the cellular and humoral immunomodulatory property of the 70% methanolic extract of *Delonix elata* barks *in vivo*. Further studies are taken up to confirm the active constituents from the extract. Based on our results, the residue obtained from methanolic extract had Saponins, Flavonoids, Tannins, Carbohydrates and trace of alkaloids. Until further investigations are done, we can surmise that the beneficial effects are caused by one or combination of active chemical moieties present in the methanolic extract of *Delonix elata* bark.

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