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Evaluation of *Delonix regia* Linn. flowers for antiarthritic and antioxidant activity in female wistar rats

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Abstract

The alcoholic extract of the flowers of Delonix regia Linn. (Family: Caesalpinaceae) was evaluated for antiarthritic activity in adult female Wistar rats. Two doses of 200mg/kg and 400mg/kg of alcoholic extract of Delonix regia were administered intraperitoneally to the Freund`s incomplete adjuvant induced arthritis in rats for 28 days. During the experimental period, paw edema volume (primary lesion) was observed. Liver homogenate was utilized for the assessment of oxidative stress. Our results suggest that both the doses of alcoholic extract of Delonix regia significantly reduced the paw edema volume as compared to diseased control animals. Treatment with Delonix regia also showed significant increase in antioxidant enzyme levels like Catalase (CAT), Glutathione peroxidase (GPx), Glutathione –S- Transferase (GST), reduced glutathione (GSH), Vit – C with decreased protein level and the values were almost analogous to that of normal values.

Keywords: Arthritis, Antiarthritic activity, Antioxidant enzyme levels, *Delonix regia*, Freund`s incomplete adjuvant.

INTRODUCTION

Arthritis is a characteristic chronic disease of the joints, cartilages and bones. The clinical feature is heterogenous with a wide variation in the age of 20-40 years. Treatment has been based on the use of sequential use of Non-Steroidal Anti-inflammatory Drugs ($NSAID_S$) progressing to disease modifying agents such as Gold, Sulfasalazine and Methotrexate. The fatalities are usually due to the complications of arthritis such as systemic amyloidosis and vasculitis or to iatrogenic effects of therapy-Gastro intestinal bleeding [1]. Antioxidants have been known to prevent the

formation of oxygen free radicals. The reactive oxygen species (ROS) in the body affects various enzyme systems (Superoxide dismutase, Glutathione peroxidase, Catalase and the reducing agents such as Glutathione, Ascorbate) and causes oxidative damage which contributes to conditions such as cancer, ischemia, aging, adult respiratory distress syndrome, rheumatoid arthritis etc[2,3].

Delonix regia (DR) Family: Caesalpinaceae known as Royal Poinciana is a flamboyant tree with flowers some say the world's most colorful free. The flowers are striking, they have four spoon shaped spreading scarlet or orange-red petals and one upright slightly which is marked with yellow and white. The aqueous and alcoholic extracts of flowers were active against round worm infestation. Though we know much about the antioxidant activity of species such as *Ananas, Camellia, Glycine, Arachis, Beta vulgarius* etc., there is paucity of information regarding the effect of flowers of *Delonix regia* against oxidative stress in arthritis [4, 5].

MATERIALS AND METHODS

Collection of plant material and extraction: The flowers of *Delonix regia* (family:Caesalpinaceae) were collected, shade dried, pulverized using a cutter mill and stored in an air tight, light resistant container for further use. The pulverized plant material was subjected to cold maceration using alcohol. For pharmacological experimentation, a weighed amount of dried extract was freshly suspended in 1% of carboxy methyl cellulose (CMC).

Animals: Adult Wistar Strain rats of female sex weighing 120-150g were procured from the Veterinary Hospital, Madhavaram animal house, Chennai, India. They were maintained under controlled conditions of temperature (25°±2°C) and light and dark cycle of 10 and 14 hours respectively for 1 week before and throughout the experiment. Animals were fed with standard rodent pellet diet (Hindustan Lever) and tap water *ad libitum*. The animals were randomly divided into five groups of eight rats each, the groups being balanced for body weight. The animals were fasted for 24hrs before experimentation but allowed free access to tap water throughout The project proposal was approved by the Institutional Animal Ethical Committee (IAEC/32/2008).

Induction of arthritics and drug treatment: For inducing arthritics, all the rats were injected with 0.1ml of Freund's incomplete adjuvant (FIA) in right hind paw [6, 7]. Group 1 was fed with normal saline (10ml/kg p.o.). Freund's incomplete adjuvant was given to groups 2, 3, 4 and 5 to induce arthritis. Groups 3, 4 and 5 were administered with 200mg/kg, 400mg/kg, p.o. of extract and 5mg/kg of standard Diclofenac respectively for a period of 28 days.

Paw swelling assessment: To follow the course of the disease, swelling of the adjuvant-injected hind paw was determined plethysmographically. Paw volume was measured three times a week and the percentage deviation from the control group was calculated. The incidence of arthritis development is presented as a percentage of animals per group with at least one joint swollen in a non-injected paw.

Estimation of biochemical parameters: The rats were kept for fasting for one day before sacrificing. The animals were sacrificed by cervical dislocation followed by decapitation. Blood

was collected by cutting Jugular vein liver was dissected out, blotted of blood, rinsed in phosphate buffered saline (pH-7.4) and immediately preceded for bio-chemical estimations. The anti oxidant enzyme levels like catalase (CAT)[6], glutathione peroxidase (GPx)[7] and glutathione -s- transferase (GSH)[8], vitamin-C and protein estimation were carried out in liver homogenate of the experimental animals.

Statistical analysis: The experimental data were expressed as the Mean ± SEM and the statistical significance was assessed by the one way analysis of variance (ANOVA) followed by Bonferroni test.

RESULTS

Rheumatoid arthritis is a chronic inflammatory disease affecting about 1% of the population in developed countries [9]. *DR* has shown the persuasive protective effect against arthritis induced by FIA. The mean change in paw swelling was about 1.90±0.02 in the FIA induced control group on 28th day. *DR* significantly ($P < 0.001$) reduced the mean change in paw swelling at 28th day evaluation and was found to be 1.48±0.01 and 1.42±0.00 in a dose dependent manner at 200 and 400 mg/kg b.wt respectively. However, the standard drug Diclofenac sodium exhibited significant (1.32±0.02, $P < 0.001$) protection as compared with the control group (Table 1).

The results of biochemical changes in FIA induced rat paw edema are shown in Table 2. There was significant elevation in protein level with reduction in the levels of antioxidants like Catalase (CAT), Glutathione peroxidase (GPx), Glutathione –S- Transferase (GST), reduced glutathione (GSH), Vit – C (Table-1). Both the doses of alcoholic extract of *Delonix regia* Linn. reduced the elevated levels of serum protein and stabilized the deficient protein levels significantly ($P<0.001$) and there was a significant ($P<0.001$) increase in antioxidant on comparison with the saline treated control group. The 200mg/kg of *Delonix regia* Linn. did not show much activity and was statistically insignificant. The activity exhibited by the 400mg/kg of *Delonix regia* Linn. was comparable with the standard drug Diclofenac.

DISCUSSION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, systematic approaches are made to find out the efficacy of plants against arthritis so as to exploit them as herbal antiarthritic agents [10, 11].

The rat adjuvant arthritis is the most frequently used chronic inflammatory model used in the screening of NSAIDs, steroids and immunosuppressive drugs. In adjuvant arthritis, bacterial peptidoglycan and muramyl dipeptide are responsible for its induction. It occurs through cell mediated autoimmunity by structural mimicry between *Mycobacterium* and cartilage proteoglycans in rats [12,13].

Our body has an excellent system to prevent and neutralize the free radicals induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as CAT, GPx and GSH etc. when the balance between ROS production and antioxidant defenses is lost there by

oxidative stress results in a series of events that deregulate the cellular functions leading to various pathological conditions [14].

In present arthritic study of rats, it is a clear, manifestation of excessive formation of free radicals in adjuvant induced arthritic animals. The significant decline in these constituents in alcoholic extract of DR-200mg/kg treated animals indicates antioxidant effect of alcoholic extract of DR.
GSH is a major non-protein thiol in living organisms, which plays a central role in co-coordinating the body's antioxidant defense processes.

CAT, GPX constitutes a mutually supportive team of defense against ROS. CAT is a haeomoprotein, localized in the peroxisomes or the microperoxisomes. These enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting cells from oxidative damage of H_2O_2 and OH [15].

In our study decline of these enzyme levels in adjuvant induced animals and attainment of near normal values in alcoholic extract of DR 400mg/kg treated rats reveals that oxidative stress is elicited by arthritis induction and the intoxication has been nullified due to the effect of alcoholic extract of DR.

GST plays an important role by eliminating toxic compounds by conjugation with glutathione. In arthritis induced animals these levels restored to normal activity in alcoholic extract of DR treated animals.

overall increase in life span of freund's incomplete adjuvant induced arthritic rats treated with alcoholic extract of DR-200mg/kg was quite significant and in agreement with data.

The persistent reduction in GSH, GPx and GST observed in present investigation point out suppressed GSH dependent antioxidant system in adjuvant induced rats. The reduction in GSH was brought out by three mechanisms-Limited GSH synthesis, enhanced GSH utilization and enhanced ROS production. It directly interacts with free radicals and quenches them.

Vitamin-C is well known for its direct free radical quenching activity [16]. It can synthesize collagen and protect cells from free radicals and has been implicated in regulating body activities. The reduction in vit-C in arthritis induced animal might be due to decrease in conversion of dehydro ascorbic acid to ascorbic acid [16].

The present study reveals that oxidative stress in arthritis induced rats is by
1. Increased H_2O_2 concentration in animals as a result of enhanced spontaneous dismutation of O_2*. and concomitant decrease in activity of CAT and GPX, the H_2O_2 catalyzing enzymes.
2. Decrease in GSH content as a result of enhanced utilization, decrease synthesis and turn over and decreases the vitamin C.

Total protein was used to estimate diseased estimation and increased levels of protein were found in dehydration, multiple myeloma, cancer, chronic liver disease and decrease in malnutrition[17]. The protein level was found to increase significantly in adjuvant induced arthritis animals and reached near normal values in drug treated animals.

The life span was noted for 28 days. It was found that control and drug treated have greater survival time than Freund's incomplete adjuvant induced arthritis. As in arthritis state, there is formation of rheumatoid factor, immune complex and deposition which leads to joint injury. The rate of mortality increases in freund's incomplete adjuvant induced arthritis [17].

There is gradual increase in body weight of animals in the 28 days study which is more significant in arthritis induced animals. Due to high nutritional profile of DR, contains a wide spectrum of natural mixed phytopigments and glycosides which are favorable for animals and thus shows a proportional increase in body weight.

Table-1 Effect of *Delonix regia* on FIA induced arthritis in rats

S. No	Groups	Mean change in paw volume (ml)				
		Day 0	Day 7	Day 14	Day 21	Day 28
1	Control	1.01±0.12	1.01±0.25	1.01±0.09	1.01±0.21	1.01±0.18
2	FIA	1.07±0.02	1.43±0.02	1.76±0.02	1.88±0.02	1.90±0.02
3	AE-DR 200mg/kg	1.02±0.02	1.36±0.02	1.49±0.02	1.53±0.01	1.48±0.01
4	AE-DR 400mg/kg	1.02±0.03	1.29±0.02	1.41±0.02	1.46±0.01	1.42±0.00
5	Standard drug	1.02±0.02	1.23±0.03	1.34±0.02	1.39±0.03	1.31±0.02

**P<0.05 as compared to group-1.values are mean ±SEM of eight animals in each group.*
AE-DR Alcoholic extract of Delonix regia; FIA-Freund's Incomplete Adjuvant.

Table-2 Effect of *Delonix regia* on Protein and antioxidant enzyme levels

S. No	Drug Treatment	Protein in serum (g/dl)	GSH mM/100g tissue	Vit-C mM/100g tissue	CAT mM/100g tissue	GPx mM/100g tissue	GST mM/100g tissue
1	Control	6.99	5.04±0.15	3.54±0.14	77.03±4.10	76.29±1.90	9.46±0.52
2	FIA	8.89	3-72±0.10	2.80±0.20	50.06±1.45	52.28±2.91	5.54±0.29
3	AE-DR 200mg/kg	7.94	4.99±0.14	3.29±0.15	69.99±3.03	78.23±2.00	9.65±0.46
4	AE-DR 400mg/kg	6.53	4.85±0.11	2.34±0.17	69.99±3.03	77.03±2.45	8.83±0.32
5	Standard drug	8.00	4.68±0.11	3.35±0.19	71.77±2.01	76.51±2.80	9.83±0.42

**P<0.05 as compared to group-1.values are mean ±SEM of eight animals in each group.*
AE- Alcoholic extract of Delonix regia; FIA-Freund's Incomplete Adjuvant.

CONCLUSION

From the present investigation it is concluded that the alcoholic extract of *Delonix regia* 400mg/kg has antiarthritic activity in adjuvant induced arthritic rat when treated for a period of 28 days. However, further studies are required to provide a detailed phytochemical examination of the active extract of *Delonix regia* to identify the principle(s) responsible for the activity and to elucidate their exact mechanism of action.

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