World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 6, Suppl 2, 2017 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article Full Proceeding Paper

EVALUATION OF ANTI OXIDANT AND ANTI INFLAMMATORY ACTIVITY OF METHANOLIC FLOWER EXTRACT OF *DELONIX REGIA*

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Received on: 05-10-2017; Revised and Accepted on: 08-11-2017

ABSTRACT

Inflammation is a series of pathological changes associated with local vascular reaction and cellular response, the living tissue, an injury insufficient to kill the tissue. Delonix regia (family: Legminosae, sub family: fabaceae) commonly called as gulmohor in Hindi. Gulmohar flower is actinomorphic or somewhat irregular; it is reported to contain rich content of β flavonol, phenolic acid, carotenoid and anthocyanin. The goal of present study is evaluate the anti-oxidant and anti-inflammatory activity of methanolic extract of flowers of Delonix regia. The DPPH method was used to evaluate the anti-oxidant activity. The percentage of radical scavenging property of the 400 mg dose of methanolic flower extract and standard in DPPH radical scavenging assay were 73.53±0.41% and 89.70± 0.98% at 100µg/ml respectively. Therefore, the anti-inflammatory activity of Delonix regia were studied in Swiss albino mice by Carrageenan and formalin induced paw edema method. The administration of mice with 200 and 400mg/kg b.wt. of Delonix regia flower of methanolic and aqueous extracts reduced inflammation that concluded Delonix regia possesses potent anti-oxidant and anti-inflammatory activities. The maximum anti-inflammatory activities were observed in high dose 400mg/kg b.wt. of methanolic flower extract of Delonix regia. Our study indicates that methanolic flower extract of Delonix regia possesses anti-inflammatory activity and it may be useful as an anti-inflammatory agent in the inflammation related disorders.

KEYWORDS: *Anti-inflammatory, Anti-oxidant, Delonix regia, Carrageenan, Paw Edema.*

INTRODUCTION

Gulmohar flower is actinomorphic or somewhat irregular, slightly fragrant and up to 5-13 cm across. Calyx is 5 lobed, glabrous. Sepals are thick, reddish with yellow border within and green outside. There are 5 petals. Petals are orbicular, broadly spoon shaped, rounded, broader, 5-6.5 cm long and 2-3 cm wide. 4 petals are orange-red, almost scarlet and 1 is whitish inside with red spots, longer and narrower than the others. Number of stamens range from 9 to 10. Stamens are completely free, separate and monadelphous. Filaments are hairy, villous and red or pink in color. The extract of *D. Regia* consists of mixture of various components, sach as flavonol, phenolic acid, carotenoid and anthocyanin from its flowers[1]. The methanolic flower extract of *D. Regia* consist of mixture of various components, sach as flavonol, phenolic acid, carotenoid and anthocyanin from its flowers. The major medicinal properties of *D. Regia* include anti-oxidant, anti-inflammatory, anti-pyretic, anti-bacterial, carminative and anti-diabetic.

MATERIALS AND METHODS

Animals:

Twelve week-old healthy swiss albino mice (18–22 g) of either sex procured from inbreed facility of Aurobindo College of Pharmacy, Gangadevipally, Warangal were used for this study. They are maintained under standard conditions (temperature 22 ± 2°C, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water and libidum. The institutional animal ethics committee (IAEC) approved the protocol to carry out the experiment. The study was conducted in accordance with the ethical rules on animal experimentation, approved by Institutional Animal Ethics Committee, (Registration number- 1761/P0/Ere/S/14/ CPCSEA).

Plant material:

Plant flowers of *Delonix regia* were collected fresh in bulk from village Annaram 75 Km away from Warangal Specimens were collected and identified by Prof. Ajmeera Ragan, Incharge of the herbarium at Department of Botany, Kakatiya University, and Warangal. The leaves were collected separately from plant dried under shade was then powdered using mechanical grinder. Compounds with medicinal properties, reported in preliminary screening and compounds which were not screened in preliminary tests are subjected to qualitative analysis.

Preparation of extract:

Leaves of *Delonix regia* (400gm) were extracted by 70% methanol by soxhlet apparatus (weight of extract 4.37gm). It was then concentrated and dried in hot air oven. Dried extract was fractionated using different solvents in increasing order of polarity i.e. hexane, ethyl acetate and chloroform and methanol. Upon these chloroform fraction was not used for the experiment due to very low percentage yield. All the extracts were dried and stored in desiccator until further use. For experimental method the dried extracts were suspended in 1% gum tragacanth in normal saline and used for anti-inflammatory activity.

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Percentage yield:

400 gm of powdered *Delonix regia* was taken and after evaporation it yields 2.64 gm of *Delonix regia* methanolic flower extract. So, percent yield is {(2.64/400) ×100} = 0.66%.

Drugs and dosage:

The methanolic and aqueous extracts of flower of *Delonix regia* were administered orally at doses of 200 mg/kg and 400 mg/kg in the form of suspension prepared in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC). Carrageenan, Diclofenac sodium, and formaldehyde (4%), Gum tragacanth, methanol, DPPH, Ascorbic acid.

Acute toxicity study:

Healthy adult Swiss albino mice of either sex weighing between 20 and 25 g were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development[2]. Groups of six mice each were administered orally graded doses ranging from 0.1 to 2 g/kg. The mice were observed continuously for 2 h for behavioral, neurological, and autonomic profiles for any lethality or death for the next 48 h.

Determination of Anti-oxidant activity of *Delonix regia* methanolic flower extract:

Working procedures:

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the scavenging potential of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical by modified method[3]. Ascorbic acid was used as standard anti-oxidant. Seven different concentrations (10, 20, 40, 80 and 100 µg/ml respectively) of *Delonix regia* methanolic flower extract and ascorbic acid were used in this study. 100 mg of *Delonix regia* methanolic flower extract was dissolved in 20 ml of pure (95%) methanol to give final concentration of 5 mg/ml. Then further dilutions from this stock were also prepared in methanol for each concentration of extract tested. Ascorbic acid solution was prepared in the same way and same for each concentration, the optical density of both the sample and standard was recorded against the blank and % inhibitionof DPPH free radical was calculated usingthe formula given below All experiments were performed in triplicate.

Percent (%) inhibition of DPPH = A – B/ A X 100

Where A = optical density of the blank and B =optical density of the sample.

Then, log C for each concentration of plant extract and ascorbic acid was calculated and plotted in the graph against corresponding % of DPPH scavenging activity. Then, IC50 (Inhibitory concentration at which 50% DPPH are neutralized) value for both plant extract and ascorbic acid was calculated by regression analysis and using statistical software "Biostat 2009". The diluted working solutions of the test extracts and ascorbic acid were prepared in methanol. 0.002% of DPPH was prepared in methanol. For each concentration, 1 ml of DPPH solution was mixed with 1 ml of sample solution. For standard, 1 ml of DPPH solution was mixed with 1 ml of ascorbic acid. For, negative control or blank, one ml of DPPH solution was mixed with 1ml of methanol to see whether the solvent used has any anti-oxidant activity or not. These solution mixtures were kept in dark for 30 min and shaken vigorously, and optical density was measured at 517 nm using Cecil-Elect UV Spectrophotometer.

Drug treatment:

Swiss Albino mice were divided into six groups of 6 animals in each.

Group I served as Control (1% (w/v) CMC in double distilled water, p.o).

Group II was administered standard drug Diclofenac sodium ((75mg/kg I.V.).

Group III-IV served as test groups and treated with aqueous extracts of *Delonix regia* (200 and 400 mg/kg, p.o in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC) respectively).

Group VI served as test groups and treated with methanolic extracts of *Delonix regia* (200 and 400 mg/kg, p.o in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC) respectively).

The prepared extracts were administered once daily for 7 consecutive days for anti-inflammatory activity.

Carrageenan-induced paw edema:

The mice were injected with 0.1 ml of carrageen (1% w/v in water) into the sub-plantar area of right hind paw. The drugs were given orally one hour prior to carrageen injection and treatment continued for 7 consecutive days. The volume of mice paw was measured at 7th day using plethysmometer during treatment period[4]. The results were expressed as the mean hind paw swelling as compared with the initial hind paw thickness.

Formaldehyde-induced paw edema:

The volume of the hind paw of the animals was measured initially using plethysmometer. After taking the initial reading, 0.1 ml of formaldehyde (2% v/v in water) was injected into sub-plantar aponeurosis of the left hind foot. The drugs were given orally one hour prior to formaldehyde injection and treatment continued for 7 consecutive days. The paw volume was measured at 1, 2, 3, 4, 5, 6 and 7 day after injection [5, 6].

Statistical analysis:

The observations are represented as Mean ± S.E.M. The data were processed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoch test. *P < 0.05 was considered significant.

RESULTS AND DISCUSSION

1. Anti-oxidant activity:

DPPH radical scavenging assay:

DPPH test provides simplified version to detect the antioxidant properties of various molecules present in the extracts. A DPPH solution is decolourized when the odd electron becomes paired off in the presence of a free radical scavenger. The colour becomes light yellow from deep violet. The results of the assay are given in the table1. The percentage of radical scavenging property of the highest dose of methanolic extract and standard in this assay were 73.53±0.41% and 89.70± 0.98% at 160µg/ml respectively.

Table No. 1: Comparison of anti-oxidant activity of *Delonix regia* and Ascorbic acid

DPPH assay concentration (µg/ml)	Inhibition (%) of *Delonix regia*	Inhibition (%) of ascorbic acid
10	20.43±0.27	25.35±0.30
20	27.70±0.12	32.20±0.21
40	36.29±0.09	45.42±0.17
80	58.63±0.03	69.20±0.73
100	73.53±0.41*	89.70± 0.98**

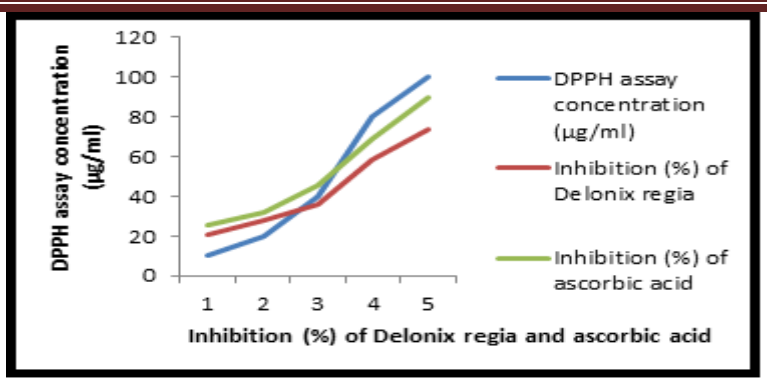

Fig. 1: Anti-oxidant activity of *Delonix regia* and Ascorbic acid

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating activity. As DPPH is considered as the lipophilic radical, it readily accept electron from the antioxidant compound and converts its colour from violet to yellow which is detected at 517nm. In this study, free radical scavenging activities of *D. regia* flower methanolic extract and standard ascorbic acid were determined by using DPPH method. The result obtained in the study indicates that the high dose (400 mg) extract exhibited good radical scavenging activity but was to a moderate significant when compared to standard ascorbic acid.

2. Assessment of anti-inflammatory activity:

a). Carrageenan induced rat hind paw oedema method:

Diclofenac showed more inhibition of edema. Methanolic fraction 400 mg/kg showed more significant mean paw volume at 5th hr when compared with control and aqueous extract groups of carrageenan induced edema. Out of two methanolic fractions from *D. regia* flower, 400 mg/kg of methanol fraction was more significant than the other fraction and moderately significant * p<0.05when compared with standard.

Table No. 2: Effect of methanolic extract of *D. regia* flower by using carrageenan induced paw edema method

Groups Dose (mg/kg)		Time before Carrageenan administration	Time after Carrageenan administration			
			paw edema volume (mm)			% Inhibition
			0 hr	3hr	5 hr	
Group-I	Vehicle	0.64±0.03	2.30± 0.20	6.50± 0.17	6.44± 0.21+9	0
Group-II	Diclofenac sodium75 mg	1.25±0.78	1.96± 0.21	2.56± 0.28	1.92± 0.860**	96
Group-III	200 mg AEDR	2.08±0.67	3.01± 0.16	4.86± 0.51	4.02± 0.670	68
Group-IV	400 mg AEDR	2.58±0.25	3.12± 0.17	3.39± 0.62	3.91± 0.529	75
Group-V	200 mg MEDR	2.84±0.91	3.09± 0.43	2.72± 0.72	3.0± 0.81	79
Group-VI	400 mg MEDR	2.32±1.08	3.54± 0.25	2.82± 0.97	2.80± 0.03*	87

Results are presented as mean ± SEM, (n=5), *: p<0.05, **: p<0.001 dunnet test as compared to control.

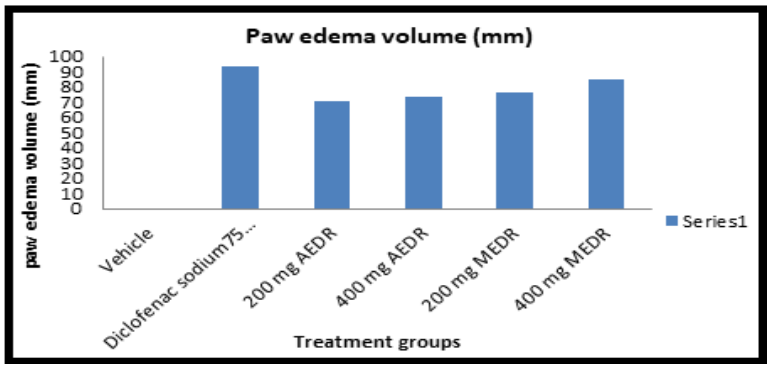

Fig. 2: Effect of methanolic flower extract of *D. regia* on Carrageenan induced paw edema

Table No. 3: Effect of methanolic extract of *D. regia* flower by using formalin induced paw edema method

Treatment groups	Dose	Paw edema (mm) at 3 (hr)after formalin administration	Paw edema (mm) at 5 (hr) after formalin administration	% Inhibition of paw edema
Group-I	**Vehicle**	0.96± 0.78	2.09.±0.31	0
Group-II	**Diclofenac sodium 75 mg**	1.21±0.52	0.87±0.86	94
Group-III	**200 mg AEDR**	1.07±0.59	1.32±0.74	71
Group-IV	**400 mg AEDR**	1.56±0.65	1.30±0.65	74
Group-V	**200 mg MEDR**	1.87±0.28	1.29±0.54	76
Group-VI	**400 mg MEDR**	1.49±0.93	0.94±0.37	85

Results are presented as mean ± SEM, (n=5), *: p<0.05, **: p<0.001 dunnet test as compared to control.

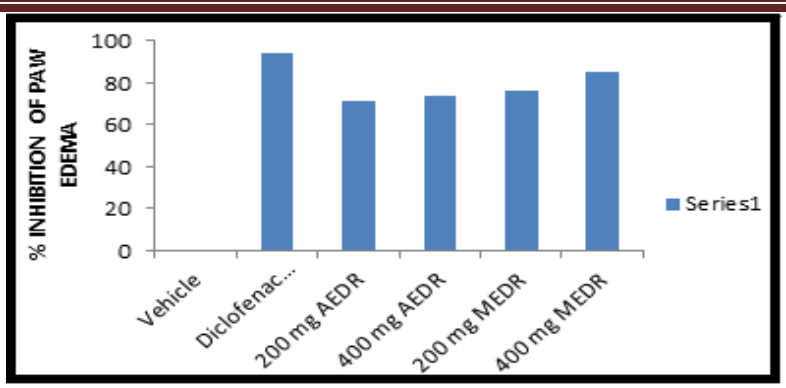

Fig. 3: Effect of methanolic flower extract of *D. regia* on formalin induced paw edema

CONCLUSION

The methanolic flower extract of *Delonix regia* showed more potent anti-oxidant and anti-inflammatory activities. It has more significant anti-inflammatory effect when compared with control as well as aqueous extracts. Whereas mild significant when compared with Diclofenac. The highest dose of methanolic flower extract of *Delonix regia* showed nearer value of % inhibition to the Diclofenac.

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How to cite this article:

Swathi Baswa et al. EVALUATION OF ANTI OXIDANT AND ANTI INFLAMMATORY ACTIVITY OF METHANOLIC FLOWER EXTRACT OF *DELONIX REGIA*. J Pharm Res 2017;6(Suppl 2):107-110.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil