

**NOVEL EVALUATION OF IN VIVO ANTI-INFLAMMATORY  
ACTIVITIES OF PERGULARIA DAEMIA FORSSK**

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Article Received on  
15 November 2018,

Revised on 05 Dec. 2018,  
Accepted on 26 Dec. 2018

DOI: 10.20959/wjpr20191-13794

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**ABSTRACT**

The pharmacological and chemical constituents of plants from the plant *Pergularia daemia* Forssk which is widely used in folk medicine. The purpose of this study was to evaluate and compare the anti-inflammatory activity of the aqueous stem and leaves extract of *Pergularia daemia* Forssk. in experimental acute and chronic inflammatory animal models. Methanol extract of *Pergularia daemia* Forssk was prepared and tested for anti-inflammatory activity in albino rats weighing 150-280 grams. The animals were randomly divided into 3 groups of 6 each; one group served as control and other two groups received indomethacin and *Pergularia daemia* orally 1 hour prior to

experimentation. The *in vivo* anti-inflammatory activity was studied using the acute (Carrageenan induced paw edema) and chronic (Cotton pellet induced granuloma) animal models. Anti-inflammatory activity was expressed as Percent inhibition (PI). Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by Scheffe's post hoc test.  $P < 0.05$  was considered statistically significant. The PI with indomethacin and *Pergularia daemia* Forssk in carrageenan induced paw edema were 51.8% and 33.98% and in cotton pellet induced granuloma were 35.53% and 10.9% respectively. Indomethacin showed highly significant anti-inflammatory activity in both the models. However, *Pergularia daemia* Forssk showed highly significant activity in acute model and but a trend of anti-inflammatory activity in chronic model studied. As *Pergularia daemia* Forssk showed significant anti-inflammatory activity in the models studied, it can be a promising anti-inflammatory agent.

**KEYWORDS:** *Pergularia daemia* Forssk, anti-inflammatory, carrageenan, granuloma, edema.

## INTRODUCTION

Inflammation is a defense reaction caused by tissue damage or injury, characterized by redness, heat, swelling and pain'. The primary objective of inflammation is to localize and eradicate the irritant and repair the surrounding tissue. Inflammation aids disposal of microbes, toxins or foreign material at the site of injury, prevents their spread to other organs, and prepares the site for tissue repair. Thus it helps restore tissue homeostasis. Non-steroidal anti-inflammatory drugs (NSAIDs) make up one of the largest groups of drugs used for pain and inflammation. Currently available anti-inflammatory agents are associated with unwanted side effects and have their own limitations. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to the inhibition of the protective cyclooxygenase enzyme in gastric mucosa. The added advantages of indigenous medicinal treatment would include its complementary nature to the conventional treatment making latter safer, well tolerated and economical remedy for acute and chronic inflammatory conditions.



**Fig. 1:** *Pergularia daemia* Forssk stem, leaves and fruits.

*Pergularia daemia* Forssk Synonyms *Pergularia extensa* N.E.Br, *Daemia extensa* R.Br. Family (Asclepiadaceae). It find throughout the Dhaka, Jessore, Kushtia, Khulna, Magura, Rajshahi in village groves of Bangladesh, up to 900m. Leaves are bitter, anthelmintic and depurative. The juice of the leaves is used as an expectorant in catarrhal affections of the lungs. It is given internally in asthma and is applied locally to rheumatic swellings. In combination with lime or ginger it is given internally in amenorrhoea and dysmenorrhoea. Fruits are acrid and digestive and are useful in vitiated conditions of kapha and dyspepsia.

The plant is astringent, acrid, emetic and expectorant, and anthelmintic, antipyretic and laxative. It is useful in urethrorrhoea, strangury, metropathy, inflammations, vitiated conditions of vata and kapha, cough, asthma, intermittent fevers. A perennial twinning herb with milky juice when brusied and grows extensively throughout Bangladesh.



**Fig. 2: *Pergularia daemia* Forssk stem and leaves.**

From the previous studies, methanol extract of *Pergularia daemia* Forssk leaves showed significant anti-inflammatory activity at a dose of 100 mg/kg. However, studies on aqueous extract of root and its evaluation in different anti-inflammatory models are sparse. Hence, here is an attempt to evaluate the anti-inflammatory activity of aqueous extract of *Pergularia daemia* stem and leaves in albino rats and to compare the activity between the acute and chronic inflammatory animal models.

## **MATERIAL AND METHODS**

### **Collection of the plant parts**

The fresh stem and leaves of the plant were selected for the study. The stem and leaves of these plants were collected during month of April 2017 from the area of Khulna, Bangladesh and were identified by the experts of Bangladesh National Herbarium, Dhaka, where voucher specimen were retained.

### **Drying, Pulverization and Preservation of plant parts**

The stem and leaves were first washed with water to remove the adhering dirt and then cut into small pieces, sun dried for 05-07days. After complete drying, the entire portions were

pulverized into coarse powder with the help of grinding machine and were stored in an air tight container for use.

### Preparation of Extract

The stem and leaves coarse powder was authenticated and procured. Aqueous extract was prepared in methanol and soaked overnight and boiled next day until the volume reduced to 1/4<sup>th</sup>, cooled and drained. The extract was prepared freshly on the day of experiment.

### Phytochemical test

The freshly prepared aqueous extract of *A. Marmelos* was subjected to phytochemical screening tests for various constituents. A pilot study of extract with different doses (5mg/kg, 50 mg/kg, 100mg/kg and 200mg/kg) was done to estimate the dose for study. The anti-inflammatory activity was significant at dose of 100mg/kg and hence the same dose was used in this study.

### Chemicals

Indomethacin (Sigma), Carrageenan (Sigma) and all other chemicals were of analytical grade.

### Animals

Adult albino rats of either sex weighing between 150 to 250 grams were randomly selected from central animal facility. Animals were housed in groups of 3, at an ambient temperature of 25±1°C with ad libitum access to food and water. The study protocol was approved by Institutional Animal Ethics Committee.



**Fig. 3: Adult Albino rats.**

## Methods

Animals were randomly divided into 3 groups of 6 rats each; I group: Control (1ml of Vehicle, 2% Gum acacia suspension); II group: Standard drug (Indomethacin 100mg/kg); III group: Test drug (plant extract 100 mg/kg). Following models were used to screen the anti-inflammatory activity of *Pergularia daemia*.

### Carrageenan Induced Rat Paw Edema Animal Model

In this method, rats were divided in 3 groups of six animals each. The animals were pretreated with drugs orally 1 hr before the experiment. 0.05 ml of 1% carrageenan was injected aseptically into the subplantar surface of right hind paw of each rat. Paw edema was measured by Mercury Plethysmograph (UGO Basile, Italy) at '0'hour and at the end of '4' hours. The difference between the zero and 4 hours gives the actual edema. Percentage inhibition (protection) against edema formation was taken as an index of acute anti-inflammatory activity.

It was calculated by

The percent inhibition of edema =  $100 \times (1 - V_t / V_c)$

Where,  $V_c$  = mean paw edema volume in the control group.

$V_t$  = mean paw edema volume in the drug treated group.

### Cotton Pellet Induced Granuloma Animal Model

This is an established animal model to screen the chronic anti-inflammatory activity of the drugs. Four sterile cotton pellets weighing 10mg each were implanted subcutaneously in both the axilla and groin of each rat. Rats were fed with the respective drug daily for 7 days along with free access to water and food ad libitum. Later the animal were sacrificed on the 8<sup>th</sup> day and the cotton pellets with granulation tissue were removed, cleaned of the extraneous tissue and dried in a hot air oven to a constant weight and the dry granuloma weight was determined. The dry weight of the granuloma (i.e. the amount of actual granulation tissue formed) was calculated by noting the difference in the dry weight of the cotton pellets recorded before and after implantation. The animal weight was also recorded on day 1 as initial weight and on day 8 as the final animal weight. The percentage change of granuloma weight relative to vehicle control group was taken as an index of chronic anti-inflammatory activity.

It was calculated by

$$\text{Percent anti-granuloma activity} = 100 \times (1 - W_t / W_c).$$

Where,  $W_t$  = mean dry weight of granuloma in drug treated group.

$W_c$  = mean dry weight of granuloma in drug untreated control group.

### Statistical Analysis

Results were expressed as mean  $\pm$  Standard deviation (SD). Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by Scheffe's post hoc test.  $P < 0.05$  was considered statistically significant. All the statistical methods were carried out through the SPSS for Windows (version 13).

## RESULTS AND DISCUSSION

Preliminary phytochemical screening of aqueous extract of *Pergularia daemia*, stem and leaves revealed the presence of alkaloids and glycosides.

### Carrageenan-Induced Rat Paw Edema

The aqueous extract of *Pergularia daemia* (100mg/kg) and indomethacin (100mg/kg) both significantly inhibited carrageenan induced rat paw edema ( $P < 0.001$ ). The maximum inhibition of paw edema was observed in both indomethacin and *Pergularia daemia* at the end of four hours when compared to the control group. Anti-inflammatory activity is expressed as Percent Inhibition (PI). The PI with Bilwa and indomethacin were 33.98% and 51.8% respectively [Table 1].

**Table 1: Carrageenan-Induced Rat Paw Edema data.**

Groups	Number of rats	Mean paw edema (cm) $\pm$ SD	Percent inhibition
Control (1ml of vehicle)	6	14.86 $\pm$ 4.024	00
Indomethacin (100 mg/kg)	6	7.15 $\pm$ 2.286	51.8%
Plant extract	6	9.81 $\pm$ 1.517	33.98%

The indomethacin (100mg/kg) showed significant ( $P < 0.001$ ) anti-inflammatory activity in granuloma induced by cotton pellets. Bilwa (100g/kg) did not show significant anti-inflammatory activity in reducing granuloma formation, compared to control group but showed a trend of anti-inflammatory activity ( $P > 0.05$ ). The PI with *Pergularia daemia* and indomethacin were 10.9% and 35.53% respectively [Table 2].

**Table 2: Cotton Pellet Induced Granuloma data.**

Groups	Number of rats	Mean paw edema (cm)±SD	Percent inhibition
Control (1ml of vehicle)	6	65.66 ± 4.844	00
Indomethacin (100 mg/kg)	6	42.33 ± 11.910	35.53%
Plant extract	6	58.5 ± 5.357	10.9%

The body weight of all animals was recorded on day of implantation and on the day of removing the cotton pellets. The weight loss was observed more in indomethacin treated animals than the control group. The body weight in *Pergularia daemia* treated animals showed negligible loss of weight and the results obtained, however were statistically non-significant ( $P > 0.05$ ).

## DISCUSSION

Inflammation is the integral part of the body's defence mechanism. Acute inflammation is characterized by vasodilatation, exudation of plasma, release of various inflammatory mediators, cytokines, growth factors and emigration of leukocytes. While the features of chronic inflammation includes infiltration of mononuclear cells, proliferation of fibroblasts, blood vessels and increased connective tissue formation. Tissue infection is a prototype of inflammatory response. Anti-inflammatory drugs inhibit different stages of inflammation.

*Pergularia daemia* is one of the most frequently used drug in the traditional and folklore systems of medicine. The decoction of the *Pergularia daemia*, stem and leaves are used to cure palpitations, abdominal pain, fever, urinary troubles, hypochondriasis and melancholia.<sup>[19,20]</sup> Preliminary phytochemical screening of aqueous extract of *Pergularia daemia* root revealed the presence of alkaloids and glycosides. Studies have shown the evidence of the presence of various chemical constituents in the *Pergularia daemia*. Coumarins like marmin, marmesinin, umbelliferone, skimmianine alkaloid and triterpenes like b-sitosterol and lupeol were identified. Marmin, a coumarin isolated from the *Pergularia daemia* (1g/kg p.o.) showed anti-inflammatory effect against carrageenan induced inflammation in rats. Marmin, marmesin, umbelliferone and skimmianine are identified from the stem and leaves which contribute to the anti-inflammatory property of *Pergularia daemia*. Lupeol, a pentacyclic triterpenes showed reduction in paw swelling by 39% compared to 35% by indomethacin. Oxidative stress and neutrophil infiltration are common for inflammatory diseases and it is now accepted that gastric ulcer is mainly caused by oxidative stress. Intra gastric administration of marmin at a dose of 25mg/kg body weight

exhibited antiulcer activity in experimental ulcer models. In another study the in vitro antibacterial potential of chloroform extract of the *Pergularia daemia* of A. marmelos (Correa) Linn was determined by agar dilution and disc diffusion techniques. The in vitro activity was found to be comparable to that of ciprofloxacin. Further stem and leaves extract treated animals showed significant inhibitory activity against castor oil induced diarrhea. Therefore the additional property of antimicrobial along with anti-inflammatory activity of *Pergularia daemia* may have synergistic effects on the inflammation induced by the invading pathogens.

Carrageenan is regarded as an established phlogistic agent/oedemogen and edema induced by the subplantar injection of carrageenan in the rat hind paw is reported to have been inhibited by a number of steroidal and non-steroidal anti-inflammatory drugs. It has a biphasic effect. The first phase is due to release of histamine and serotonin (5-HT) (0-2 hr), plateau phase is maintained by a kinin like substance (3hr) and second accelerating phase of swelling is attributed to PG release (>4hr). In our study the aqueous extract of A. marmelos 100 mg/kg, p.o. significantly reduced edema induced by the carrageenan. The percent inhibition of paw edema by indomethacin was 51.8% while that of *Pergularia daemia* is 33.98%. Hence *Pergularia daemia* showed 69.32% anti-inflammatory activity as that of standard Indomethacin under the present experimental conditions. The results obtained in this study are in concurrence with the study done by Udupa *et al.*, where aqueous extract of *Pergularia daemia* was used in a dose of 5mg/100g body weight i.p once daily. The probable mechanism of the acute anti-inflammatory activity might be due to the inhibition of release of mediators like histamine, serotonin and prostaglandins. This activity probably will be due to the chemical constituents like marmin, lupeol etc.

Cotton wool granuloma is a method for testing the proliferative phase i.e granuloma formation, provoked by the subcutaneous implantation of compressed cotton pellets. After few days histologically gaint cells and undifferentiated connective tissue can be observed along with fluid infiltration. The amount of newly formed connective tissue can be measured after removal and weighing the dried pellets. The percent inhibition of granuloma formation by indomethacin was 35.53% and that of *Pergularia daemia* was 10.9%. Hence *Pergularia daemia* showed 37.24% ( $P > 0.05$ ) anti-granuloma activity as that of standard indomethacin ( $P < 0.05$ ) under the present experimental conditions. The additional finding, body weight loss observed in the indomethacin group than the *Pergularia daemia* treated animals, suggest that

indomethacin induced gastritis might be responsible for the reduced food intake and leading to loss of weight. Though the *Pergularia daemia* showed less chronic anti-inflammatory activity compared to indomethacin further assessment of *Pergularia daemia* with change in the dosage, solvent extracts and other chronic inflammatory models, will throw a more light on its chronic anti-inflammatory activity.

Studies on *Pergularia daemia* stem are fewer and studies on aqueous extract of stem on different anti-inflammatory models are sparse. Hence the present study has attempted to fill these lacunae of this invaluable drug. The standardization of the extracts, identification and isolation of active principles and pharmacological studies of these needs to be studied further. To conclude, a large number of studies on *Pergularia daemia* have showed antidiabetic, antiulcer, anti-inflammatory, analgesic, antipyretic, antidiarrhoeal, antihyperlipidaemic, antioxidant, anticancer, antimicrobial and radioprotective properties. In the present study aqueous extract of *Pergularia daemia* has showed promising results in acute model however these were less effective in the chronic model of experimental inflammation when compared to the standard indomethacin. These studies are valuable for identifying lead compounds for anti-inflammatory drugs, keeping in mind the side effects of NSAIDs and corticosteroids. Further human studies are needed to prove the safety and efficacy of long term administration of aqueous extract of *Pergularia daemia* as potential anti-inflammatory agent in routine clinical practice.

## CONCLUSION

Our total observation showed that *Pergularia daemia* Forsk is a medicinal plant which has anti-inflammatory activity. By using this activity we can play a role for treatment of inflammation.

## ACKNOWLEDGEMENT

I would like to express my heartiest gratitude to my respected teacher, Dr. Md. Siddiqui Islam, Chairman, Department of Pharmacy, Southeast University, for the continuous supervision, providing all facilities and congenial and comfortable atmosphere to perform the project work.

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