



(RESEARCH ARTICLE)



## *Invitro* and *Invivo* anti-inflammatory activity of *Tabebuia pallida* leaves

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### Abstract

As any change in the body like injury or destruction and inflammation is characterized by the changes in the body evaluation like heat, discomfort, swelling, redness and some physiological changes in the processes. The inflammatory process is a complicated series of relationship among substances and some elements that included in the connective tissue. An inflammatory response is the primary cause for wide range of disorders like allergies, metabolic syndromes, cancer and syndromic disorders etc., Immune related disorder plays as financial burden on individuals, in such conditions some drugs and medicines are used like steroids, nonsteroids and immunosuppressants which are some common medications for controlling the inflammatory response in the body. Most of these medications have side effects on the body like allergies and gastric ulcers. In such conditions they find the natural goods like plants having historically an excellent medication activity for inflammatory illnesses. But there is no specific information on the inflammatory activity reports used in literature. Scientists need to find new process with low cost, straightforward to invitro methodologies and to access the natural anti-inflammatory activity and focus on the anti-inflammatory methodology which can evaluate the natural anti-inflammatory substances. These are cost effective and easy to operate with minor alternatives. The activity is performed on egg albumin to know about its anti-inflammatory properties.

**Keywords:** Anti-inflammatory, Egg albumin; *Invitro* methodology; Wistar rats; Diclophenac-sodium

### 1. Introduction

Inflammation is a reaction to the living tissues or the local part of body that is injured. Inflammation also follows various changes in the tissues, in blood and in the collective tissues for the purpose of elimination of the irritant and to repair the damaged tissues or organs.[1] We say that the importance of the plants plays an important role in maintaining the human health. In the ancient. The plans were used in the treatment of the diseases and cure of the diseases. The *Tabebuia* genus consists of different species among which the plant *Tabebuia Pallida* is one of them. The plant is likely to grow in lightly tropical and full sunny areas and usually planted as an ornamental tree in India and other Asian countries. The plant grows up to 15 to 20 feet height and grows in dry soils which has a little need of water.[2,3] The scientific study of the literature does not show any anti-inflammatory activity in terms of hind paw and Protein denaturation. So, we followed the anti-inflammatory activity procedure to evaluate the anti-inflammatory activity of *Tabebuia Pallida*.

#### 1.1. Requirement of chemicals

Diclofenac sodium, Buffer solution.

#### 1.2. Plant material

To investigate the plant *Tabebuia Pallida* the leaves of the plant are collected in the month of February, near English union school, Jyothinagar, Karimnagar. The plant was authenticated by certified botanist (ENM-100130). The leaves

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were dried in a shadow place for one week and made into powder by using electrical grinder. After obtaining the fine powder it is sieved by sieve no. 40 and the powder is stored in the air airtight container for further use.

### 1.3. Preparation of extract

The extract was prepared by using Methanol as a solvent. *Tabebuia Pallida* leaves were followed by the soxhlation process under suitable temperature. About 50 grams of powder was used in the thimble and the soxhlation was carried out. At the end of the process the extract was collected in the RB flask, that is after a time period of 6 hours. The extract was collected and dried in desiccator.

Maceration process was also carried out by using the same solvent (Methanol) and same amount of drug that has been used in soxhlation process which is kept for 1 week.

### 1.4. Animals

The study included healthy Wistar rats of both sexes, roughly the same age, and weighing between 150 and 180 g, which were procured from Mahaveer Enterprises in Hyderabad. They received the prescribed amount of water and a regular chow meal. The animals were kept in regular environmental conditions (12 h light/12 h dark cycle; 25 ± 3°C, 35-60% relative humidity) in polypropylene cages. The investigation was carried out with approval from the Institutional Animal Ethics Committee (IAEC), and the animals were handled strictly in accordance with CPCSEA norms.

### 1.5. Acute Toxicity and Gross Behavioral Study

After an overnight fast, the rats (n=6) were split up into groups and given oral dosages of Methanol extracts are suspended in water at progressively higher rates (250, 500, 750, and 1000 mg/kg body weight). Following extract administration, the animals were examined for the first two hours to assess any noticeable behavioural changes, then every 30 minutes for the following four hours, and finally every 24 hours for the following seventy-two hours to determine the percentage of death [4,5, 6].

### 1.6. Assessment of In-Vivo & In-Vitro AntiInflammatory Activity:

In the current study, the anti-inflammatory properties of several *Tabebuia Pallida* leaf extracts were evaluated using the in-vitro egg albumin denaturation process of proteins [2,7, 8] and the (chemical) carrageenan-induced rat paw edoema technique with Diclofenac sodium as standard [9,10].

### 1.7. Anti-inflammatory assays

#### 1.7.1. Invitro anti-inflammatory activity of the *tabebuia pallida* leaves on the egg albumin denaturation process of proteins

For the preparation of the egg albumin solution the fresh egg flakes or the egg albumin powder or fresh hens' egg that is accessible in the store is taken and used to make the 1% egg albumin solution. About 1 gram of egg flakes are taken and transferred to a 100 ml volumetric flask containing distilled water to dissolve the egg flakes. The formed clear solution is the required 1% egg albumin solution.

#### 1.7.2. Assay

The Anti-Inflammatory activity of crude extracts can be determined by the in vitro for denaturation of the egg albumin protein.

The procedure of the Anti-inflammatory activity with the egg albumin was done with the samples that were prepared as mentioned below:

#### 1.7.3. Standard

0.2 ml of 1 percent egg albumin solution, 2ml of diclofenac sodium (1mg/ml) at different concentrations and 2.8 ml of phosphate buffered saline (pH 7.4) and make up the volume to 5ml.

#### 1.7.4. Control

In 2 ml triple distilled water add 0.2 ml of 1% egg albumin solution and 2.8 ml of phosphate buffered saline and volume make upto 5ml.

### 1.8. Preparation of phosphate buffer of pH 7.4

Take 25ml of 0.2M potassium dihydrogen phosphate in 200ml conical flask and add 19.55ml of 0.2M NaOH then make the volume to 200ml with distilled water.

### 1.9. Preparation of 0.2M potassium dihydrogen phosphate

Dissolve 2.721g of potassium dihydrogen phosphate in 100ml volumetric flask and make the volume to 100ml using distilled water.

### 1.10. Preparation of 0.2M NaOH

Dissolve 0.8g of sodium hydroxide in 100ml volumetric flask using distilled water.

#### 1.10.1. Test samples

Maceration

- TEST 1: 0.2 ml of 1% egg albumin solution + 2ml of 1mg/ml methanolic extract of *Tabebuia Pallida* + 2.8 ml of phosphate buffered saline.
- TEST 2: 0.2 ml of 1% egg albumin solution + 2ml of 3mg/ml methanolic extract of *Tabebuia Pallida* + 2.8 ml of phosphate buffered saline.
- TEST 3: 0.2 ml of 1% egg albumin solution + 2ml of 5mg/ml methanolic extract of *Tabebuia Pallida* + 2.8 ml of phosphate buffered saline.

#### 1.10.2. Soxhlation

- TEST 4: 0.2 ml of 1% egg albumin solution + 2ml of 1mg/ml methanolic extract of *Tabebuia Pallida* + 2.8 ml of phosphate buffered saline.
- TEST 5: 0.2 ml of 1% egg albumin solution + 2ml of 3mg/ml methanolic extract of *Tabebuia Pallida* + 2.8 ml of phosphate buffered saline.
- TEST 6: 0.2ml of 1% egg albumin solution + 2ml of 5mg/ml methanolic extract of *Tabebuia Pallida* + 2.8 ml of phosphate buffered saline.

Then the mixtures of all samples are incubated for  $37 \pm 2$  °C for 30 mins and then it is kept on the water bath for 15 minutes at  $70 \pm 2$  °C. After that the samples are kept for cooling then the sample absorbance was measured under the UV or visible spectrophotometer at the suitable nanometers 660 nm by using the triple distilled water as the blank.

The percentage equation of the absorbance of the sample was determined by the 1% inhibition of protein denaturation.

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The extract of the plant and positive control concentration for the 50% inhibition (IC50) was determined by giving the percentage inhibition concerning against the concentration.(11,12,13)

### 1.11. *In vivo* anti-inflammatory activity

In the laboratory, 150–180 grammes of juvenile adult Wistar rats were employed to test the *Tabebuia Pallida* leaves' *In-vivo* anti-inflammatory properties. The rats spent the night on an empty stomach prior to the exercise. The rats were split up into four groups, with six animals in each group. Water was used to prepare the control and standard samples. The animals were given the standard, control, and test extracts, and the concentrations of these samples were measured as indicated in Table 1. Two hind paws that were somewhat beyond the tibiotalar junction were used for identification. The paws were dipped in the mercury column to the designated level during the test to guarantee a steady paw volume. Following an hour, the test and reference materials were given, and a 26 G needle was used to subcutaneously inject 0.1 ml of 1% carrageenan suspension (in normal saline) into the rat's dorsal subplanter surface of the hind paw. Each rat's initial paw volume was recorded prior to the drug's administration, and the paw volumes are measured at the end of 0.5, 1, 2, 3, and 4 hours using a plethysmometer.

The paw volumes are deducted from the starting paw volumes at various intervals, and any resulting change in the rat's paw volume is then analysed. By averaging each group at various hours, the average value of edema was determined. For every group, the percentage of edema inhibition is computed in relation to the control group.

$$\text{Percentage Inhibition} = (A-B) \times 100/A$$

Where the A = Increase in paw volume in rats treated with control.

B = Increase in paw volume in rats treated with test.

## 2. Results and Discussion

After 72 hours, it was discovered that every animal in the acute toxicity investigation was still alive. This suggests that the extracts were deemed safe at the dose ranges under investigation. The LD50 of the extracts will be more than 1000 mg/kg body weight because every animal lived at a dosage of 1000 mg/kg body weight. During the study period, no significant alterations in behaviour were noted. After receiving all three extracts, the mice exhibited mild sedation Table 3.

### 2.1. Anti-Inflammatory Activity of Leaf Extracts of *Tabebuia Pallida*

*In-vitro* Anti-inflammatory activity for all the leaf extracts was performed in-vitro by protein denaturation at a dose of 1, 3 and 5 mg/ml (Figure 1). The results pertaining to this investigation were shown in the Table 4. The results obtained in this investigation indicate that there was significant anti-inflammatory activity for the Methanol (Soxhlation) extract. It showed about 78.06 percentage protection at a dose of 1mg/ml of diclofenac sodium (standard) and 1mg/ml, 3 mg/ml and 5mg/ml was 60.7, 63.6, 68.4 respectively percentage protection indicating that the activity was dose dependent.

Using the carrageenan-induced rat paw edema technique, all leaf extracts were tested for their *In-vivo* anti-inflammatory properties in vivo at doses of 250 and 500 mg/Kg body weight in rats. The investigation's findings were displayed in Tables 5 and 6 (Figure 2). The findings of this study demonstrate that all extracts (soxhlation) exhibited a considerable proportion of protection against edema development and did not exhibit dose-dependent anti-inflammatory action. Table 5 shows that the common medication Diclofenac sodium had 17.3, 26.4, 44, 61, 48% protection against carrageenan-induced inflammation at ½, 1, 2, 3, and 4 hours. Maximum activity of the methanolic extract was observed at 500 mg/kg at 3 hours.

**Table 1** Division of animals for anti-inflammatory activity of *Tabebuia Pallida*.

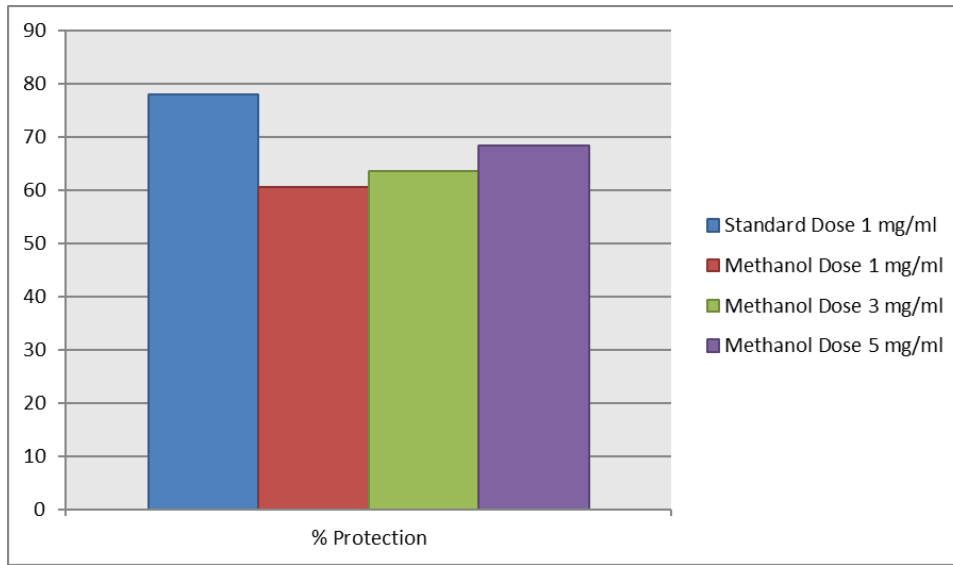
Group	Extract
Group I (Control)	Water
Group II (Standard)	Diclofenac sodium (1mg/kg)
Group III	Methanolic extract (250 mg/kg)
Group IV	Methanolic extract (500 mg/kg)

**Table 2** Acute toxicity studies

Extracts	Group	Dose(mg/kg)	No of mice each group	After 4 hours	After 24 hours
Methanol	I	250	6	6	6
	II	500	6	6	6
	III	750	6	6	6
	IV	1000	6	6	6

**Table 3** *In-vitro* anti-inflammatory studies

Treatment	Dose mg/ml	%protection
Standard	1	78.06
Methanol	1	60.7
	3	63.6
	5	68.4



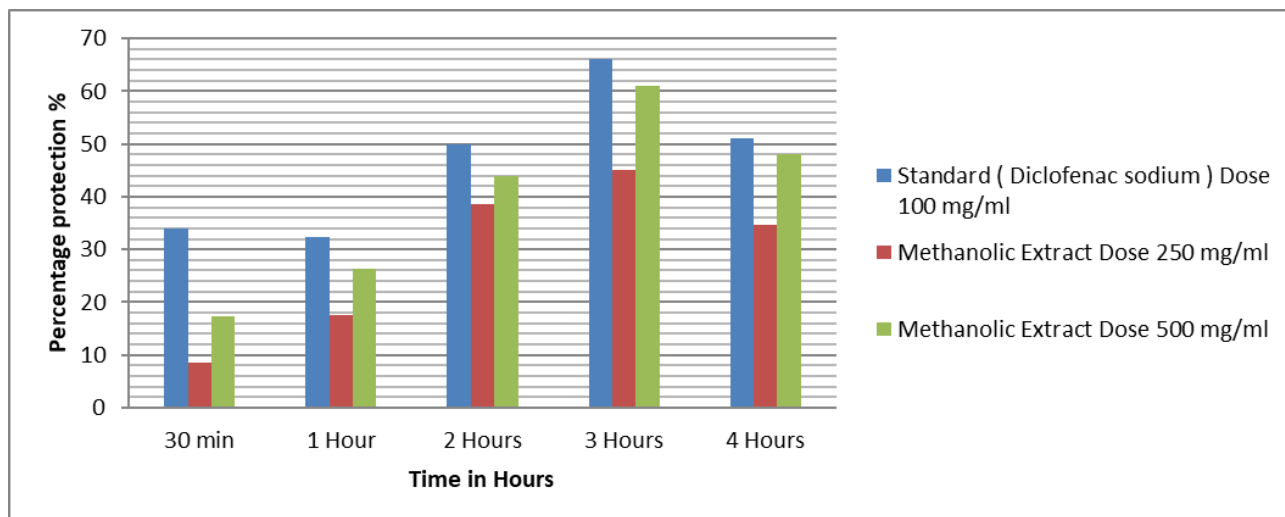
**Figure 1** *In-vitro* anti-inflammatory studies

**Table 4** Anti-inflammatory activity of leaf extracts of *Tabebuia Pallida*

Name of the drug	Dose (mg/kg)	Mean edema volume (ml) 30 min	1hr	2hr	3hr	4hr
Control	-	0.23 ± 0.02	0.34 ± 0.02	0.62 ± 0.05	0.75 ± 0.02	0.70 ± 0.03
Diclofenac Sodium	100	0.15 ± 0.03	0.23 ± 0.03	0.31 ± 0.03	0.27 ± 0.04	0.34 ± 0.03
Methanolic Extract	250	0.21 ± 0.03	0.28 ± 0.04	0.38 ± 0.03	0.49 ± 0.05	0.38 ± 0.06
Methanolic Extract	500	0.19 ± 0.01	0.25 ± 0.03	0.28 ± 0.03	0.29 ± 0.04	0.28 ± 0.04

**Table 5** Percentage Protection against edema formation.

Name of the drug	Dose (mg/kg)	Protection % 30 min	1hr	2hr	3hr	4hr
Standard (Diclofenac Sodium)	100	34	32.3	50	66	51
Methanolic Extract	250	8.6	17.6	38.7	45	34.6
Methanolic Extract	500	17.3	26.4	44	61	48



**Figure 2** Percentage Protection against edema formation

### 3. Conclusion

Based on the aforementioned findings, it can be said that *Tabebuia pallida* leaf methanolic extract has a notable *In vitro* anti-inflammatory effect. Significant *In-vivo* anti-inflammatory efficacy is achieved by reducing edema with the use of Methanolic extract, which is generated by the carrageenan. On the other hand, the activity was similar to the quantitative activity induced by a typical medication. The usage of crude extracts may be the cause of this. Therefore, it will be helpful to isolate the active principles in order to create novel bioactive components from these extracts that may have greater activity.

### Compliance with ethical standards

#### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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