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"Antinociceptive and Anti-Inflammatory Activity of Tabebeuia Aurea Leaf Extracts"

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ABSTRACT

Tabebeuia aurea (Bignoniaceae) is used in the treatment of bronchitis, grippes (viral diseases), anti-inflammatory agent and stem bark is used for treatment of cancer. The present study has is to evaluate antinociceptive and anti-inflammatory activities of alcohol and aqueous extracts of *Tabebeuia aurea* leaves. Both extracts showed dose dependent activity. The antinociceptive activity was investigated using hot plate, acetic acid induced writhing and formalin induced paw licking methods. Anti-inflammatory activity was evaluated using carrageenan induced paw oedema method. Alcohol extract (500 mg/kg) showed highest 76.92% inhibition of inflammation after 24 hrs. Both the extracts produced increased in latency time compared to vehicle but alcohol extract showed highest activity after 150 min in hot plate method (4.63 ± 0.08 sec) and inhibit nocipeptive response in both phase. Extracts also produced significant inhibition of writhing. The experimental data demonstrates that the alcohol leaf extract of *Tabebeuia aurea* have excellent antinociceptive and anti-inflammatory activity.

Key-words: *Tabebeuia aurea* ; antinociceptive; anti-inflammatory; hot plate method, Acetic acid induced writhing, Formalin induced paw licking, carrageenan induced paw oedema.

INTRODUCTION:

Tabebuia aurea (*Bignoniaceae)* is one of the most spectacular of flowering trees. commonly called as 'silver trumpet tree'[1] with Silvery gray leaves, bark is corky, Leaves are palmate, opposite, to 11 inches long and 4 inches wide with 5-7 oblong-elliptic to oblong lanceolate leaflets, flowers are funnel form, flaring mouth, bright yellow to 3 ½ inches long and 1 inch, fruit is capsule, oblong.[2] The survey showed the presence of β-sitosterol, lapachol and veratric acid, methyl cinnamate, ethyl p-hydroxycinnamate, betulinic acid, 3,4´,5-trihydroxy-7-metoxyflavone(flavanoid) and p-anisic acid.[3] *Tabebuia* used in traditional medicine as astringents and against skin disease. It was used in the treatment of grippes (viral diseases) and bronchitis[4] and used in folk medicine as anti-inflammatory agent and also used against influenza. The Stem bark was effective against cancer.

Tabebuia was claimed to be useful in treatment of inflammation , there are no reports regarding antinociceptive and analgesic activity. Hence the current study, the anti-inflammatory and antinociceptive activity of *Tabebuia aurea* was studied in different animal models. The study is an approach to evaluate the traditional use of leaves of *Tabebuia aurea*.

MATERIALS AND METHODS:

Collection of plant material:

The leaves of *Tabebuia aurea* (Bignoniaceae) were collected and authenticated from Dr. Prathiba Devi, Head of the Department of Botany, Osmania University, Hyderabad. The Voucher specimen has been deposited in herbarium for future reference. The plant material was dried and powdered. Powdered material weighing 500g was extracted by soxhlet using alcohol and water and extracts were concentrated using rotary flash evaporator to get semi solid mass. The alcohol and aqueous extract yield is 12.09% and 25.68%.

Animals :

Albino mice and Wistar rats weighing 18-24g and 150-200g respectively, were maintained in the animal house, they had access to standard pellet and water ad libitum. Animals were divided into six groups of six each.

Preliminary phytochemical screening:

Alcoholic and aqueous extracts of *Tabebuia aurea* were investigated for qualitative chemical examination.

DETERMINATION OF ACUTE ORAL TOXICITY (LD_{50})

The acute LD_{50} value for *Tabebuia aurea* leaves extracts was found to be safe up to 2000 mg/kg p.o.

Antinociceptive activity:

Hot plate method: The animals were placed on Eddy's hot plate maintained at a temperature of 55 ± 0.5°C. A cut-off period of 15 s was observed to avoid damage to the paw. Reaction time and the type of response were noted using a stopwatch. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. Pentazocine was used as standard (10 mg/kg) which was administered i.p. The alcohol and aqueous extracts of *Tabebuia aurea* (250 and 500 mg/kg) were administered orally.[5] The response was observed at 0, 30, 60, 120 and 150 min.

Formalin induced paw licking model: One hour after oral administration of test compounds (250 and 500 mg/kg alcohol and aqueous extracts of *Tabebuia aurea*), 20 µl of 1% formalin was injected into the paw of each animal. Duration of paw licking was monitored 0-5min (first phase) and 15-30min (second phase) after formalin injection. Pentazocine was used as a standard (10mg/kg) which was administered i.p.[6,7]

Acetic acid induced writhing test: Albino mice were administered with different treatments orally one hour before acetic acid injection. Control group received only vehicle, and animals under standard group received Diclofenac sodium (10 mg/kg, p.o.). One hour after drug administration, 1% v/v acetic acid (0.1ml/10 g, i.p.) was injected. Five minutes after the intraperitoneal injection of acetic acid, number of writhing were counted for the period of 20 minutes.[8,9]

Anti-inflammatory activity

Carrageenan-induced rat paw edema: Acute inflammation was produced by injecting 0.1ml of (1%) carrageenan (in a normal saline solution) into plantar surface of rat hind paw. The alcohol and aqueous extracts (250 and 500 mg/kg, orally), Diclofenac sodium (10 mg/kg, orally) as a reference agent were administered 60min before carrageenan injection. The paw edema volume was recorded using a plethysmometer at a different time intervals.[8]

Statistical anlysis:

Data were analysed by one-way analysis of variance (ANOVA) and one-way repeat measure ANOVA, wherever applicable followed by Dunnett's *post hoc* test. The difference of $P < 0.05$ was considered as significant in all cases.

RESULTS

Preliminary phytochemical analysis: Our qualitative preliminary phytochemical tests showed the presence of alkaloids, glycosides, saponins, phenols, tannins, proteins, carbohydrates, phytosterols in alcoholic extract of *Tabebuia aurea,* and aqueous extract of the plant showed similar constituents positive except phytosterols, while fixed oils & fats are absent in both the extracts.

Antinociceptive activity: The extracts of *Tabebuia aurea* has shown significant dose dependent antinociceptive activity, however the alcohol extract (500 mg/kg) produced better activity than the aqueous extract. The alcohol extract (500 mg/kg) showed highest activity after 150 min in hot plate method as latency time increases to 4.63 ± 0.08 sec after 150 min compare to 1.08 ± 0.08 of control (Table 1).

Alcohol extract (250 and 500 mg/kg) produced inhibition of 28.48% and 37.43% pain response in first phase and inhibition of 53.41% and 74.56% paw licking response in second phase. Aqueous extract produce comparatively less effect than the alcohol extract and produced inhibition of 23.98% and 29.73% pain response in first phase and inhibition of 40.33% and 59.46% pain response in second phase at a dose of 250 mg and 500 mg/kg respectively (Table 2).

Alcohol extract produced 36.5% and 53.5% inhibition of writhing response in low and high dose respectively. Aqueous extract has produced less inhibition than the alcohol extract (Table 3).

Anti-inflammatory activity: The test extracts at doses of 250 and 500 mg/kg as well as diclofenac sodium (10 mg/kg), showed significant inhibition of edema in dose dependent manner 3 h after carrageenan-induced inflammation, when compared with the control. Both alcohol extract and aqueous extract of *Tabebeuia aurea* produced dose dependent inhibition of paw edema, The percentage inhibition of edema was 63.3%, 76.92%, 57.05% and 64.74% against alcohol extract (250 mg/kg and 500 mg/kg) and aqueous extract (250 mg/kg and 500 mg/kg) respectively after 24h (Table 4).

Table 1: Effect of alcohol and aqueous leaf extract of *Tabebeuia aurea* on Hot plate model

		Initial	30min	60min	120min	150min
Control	10	1.02±0.09	0.94±0.05	1.16±0.07	1.27±0.01	1.08±0.08
Pentazocine	10	1.14±0.04	2.42±0.19***	4.06±0.03***	5.36±0.08***	5.80±0.06***
Alcohol extract	250	0.97±0.01	1.08±0.05	1.94±0.01***	2.62±0.06***	3.26±0.03***
Alcohol extract	500	0.93±0.04	1.17±0.01*	2.69±0.04***	3.74±0.07***	4.63±0.08***
Aqueous extract	250	1.01±0.02	1.09±0.05	1.18±0.01	2.10±0.01***	2.35±0.06***
Aqueous extract	500	0.95±0.07	1.15±0.02*	2.06±0.03***	3.17±0.07***	3.98±0.03***

Values are mean ± SE, n=6, ***P < 0.001, **P < 0.01 and *P < 0.05 using one-way ANOVA followed by Dunnet's test.

Table.2 Effect of alcohol and aqueous leaf extract of *Tabebeuia aurea* on Formalin induced paw licking model

Treatment	Dose mg/kg	%inhibition of Paw licking in early phase 0-5min	%inhibition of Paw licking in late phase 15-30min
Control	10	-	-
Pentazocine	10	47**	80.22***
Alcohol extract	250	28.48*	53.41***
Alcohol extract	500	37.43**	74.56***
Aqueous extract	250	23.98	40.33***
Aqueous extract	500	29.73*	59.46***

Values are mean ± SE, n=6, ***P < 0.001, **P < 0.01 and *P < 0.05 using one-way ANOVA followed by Dunnett's test.

Table 3: Effect of alcohol and aqueous leaf extract of *Tabebeuia aurea* on acetic acid induced writhing model

Treatment	Dose (mg/kg)	Number of writhing	% Inhibition
Control		70.5	
Diclofenac	3	25.8	63.4***
Alcoholic extract	250	44.7	36.5**
Alcohlic extract	500	32.9	53.3**
Aqueous extract	250	50.2	28.7*
Aqueous extract	500	39.3	44.2***

Values are mean ± SE, n=6, ***P < 0.001, **P < 0.01 and *P < 0.05 using one-way ANOVA followed by Dunnett's test.

Table 4: Effect of alcohol and aqueous leaf extract of *Tabebeuia aurea* on Carrageenan induced rat paw edema

Groups	Do)se(mg/kg)	% Inhibition of paw edema inflammation	
		3hr	24hr

Control	-	0.00	0.00
Diclofenac sodium	10	35.60***	79.48***
Alcoholic extract	200	23.48	63.34***
Alcoholic extract	500	30.06***	76.92***
Aqueous extract	200	11.36	57.05***
Aqueous extract	500	21.20	64.74***

Values are mean ± SEM, n=6, ***P < 0.001, **P < 0.01 and *P < 0.05 using one-way ANOVA followed by Dunnett's test.

DISCUSSION

Both the extracts showed activity in a dose dependent manner. The alcohol extracts showed potent antinociceptive and anti-inflammatory activity compare to aqueous extract. The alcoholic and aqueous extracts at low and high doses (250 and 500 mg/kg) increase the reaction time in dose dependent manner to the thermal stimulus. The highest antinociception of thermal stimulus was exhibited at higher dose of alcohol extract than aqueous extract. This could be the possible explanation for its central analgesic activity observed in hot plate test. The alcoholic and aqueous extracts exhibited a significant antinociception in both early and late phase of the formalin test. The alcohol and aqueous extract exhibited significant, dose-dependent decrease in the number of abdominal constrictions. However alcohol extract has shown good activity compare to aqueous extract.

The phytochemical analysis of both extracts showed the presence of carbohydrates, alkaloids, glycosides, tannins, saponins, phytosterols, phenolic compounds, proteins, amino acids, flavonoids, gums and mucilage. The anti-inflammatory effect of *Tabebeuia aurea* may be due to the presence of flavonoids. It has been reported that flavonoids possess anti-inflammatory and analgesic activity. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception. Hence, the presence of flavonoids may be contributory to the anti-inflammatory and analgesic activities of *Tabebeuia aurea*.

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