



## EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *BOMBEX CEIBA* GUM

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### ABSTRACT

Pain and inflammation are complex physiological responses involved in many acute and chronic disorders, and their treatment is often limited by the adverse effects associated with conventional analgesic and anti-inflammatory drugs. Although several plant-based remedies are used in traditional medicine for managing these conditions, many lack adequate scientific validation. *Bombax ceiba* gum, belonging to the family Bombacaceae, is traditionally claimed to possess anti-inflammatory and analgesic properties, but experimental evidence is scarce. The present study aimed to evaluate the analgesic and anti-inflammatory potential of *Bombax ceiba* gum using standard experimental animal models. Analgesic activity was assessed through central and peripheral pain models, while anti-inflammatory activity was determined using carrageenan-induced paw edema. The extract showed a significant, dose-dependent reduction in pain and inflammation compared to controls. Greater inhibition during the late phase of inflammation suggests interference with prostaglandin-mediated pathways. The observed effects may be due to bioactive constituents such as flavonoids and phenolic compounds. These findings scientifically validate the traditional use of *Bombax ceiba* gum and support its potential as a natural therapeutic agent.

### INTRODUCTION:

Living tissue uses inflammation as a defense mechanism against damage or damaging stimuli. Classical symptoms like pain, redness, heat, and swelling are what define it. Capillary dilatation, which increases blood flow to the afflicted tissue, is the cause of redness. Vasodilation, which permits improved blood circulation and greater metabolic activity of inflammatory cells like neutrophils and macrophages, also

contributes to the rise in temperature. Increased capillary permeability brought on by vasodilation causes fluids and plasma proteins to seep into the interstitial spaces, causing swelling. Numerous harmful substances, such as pathogenic microbes, antibodies, chemical irritants, or physical trauma, can cause inflammation [4]. Many of the clinical signs and symptoms of disease are caused by the host's inflammatory response, which also plays a

crucial role in stopping and resolving infectious processes. The complement system, kinin system, and coagulation pathways are only a few of the intricate host defense mechanisms involved in this reaction. Persistent inflammation and gradual tissue damage are frequently the outcome of failing to eradicate or contain invasive germs [42]. There are two types of inflammation: acute and chronic. While chronic inflammation is defined by a protracted and dysregulated inflammatory response that can lead to tissue damage and functional impairment, acute inflammation is the body's initial reaction to damaging stimuli. The production of prostaglandins, prostacyclins, and thromboxanes—all significant mediators of inflammation, pain, and platelet aggregation—is largely dependent on cyclooxygenase (COX) enzymes [43]. After blood cells lyse, mediators including prostaglandins and bradykinin are released, which is the main cause of pain related to inflammation.

. Nociceptive pain, which results from damage to the skin, muscles, joints, bones, or visceral organs. Neuropathic pain, which results from illness or injury to the somatosensory nerve system. Inflammatory pain, which arises from inflammatory mediators sensitizing and activating nociceptive pathways at tissue damage sites. Additionally, there are two types of pain: acute and chronic. While chronic pain is becoming more often acknowledged as an illness in and of itself, acute pain is a sign of tissue damage. Four basic mechanisms are involved in the production of pain after tissue damage: transduction, in which nociceptors are activated by noxious stimuli; transmission, which is the movement of pain signals from the peripheral site to the central nervous system; modulation (plasticity), which modifies nociceptive signaling at the spinal and supraspinal levels; and perception, which is the subjective interpretation of pain that incorporates cognitive and emotional elements. Pro-inflammatory cytokines including interleukin-1 $\alpha$ , interleukin-1 $\beta$ , interleukin-6, and tumor necrosis factor- $\alpha$ , as well as chemokines, reactive oxygen species, vasoactive amines, lipids, ATP, and acidic

metabolites, all play important roles in inflammation and pain. Resident mast cells, endothelial cells, and invading leukocytes all release these mediators. The pathophysiology of a number of illnesses, such as arthritis, cancer, and cardiovascular diseases, includes pain and inflammation as essential elements. Pain and inflammatory diseases have historically been treated using a variety of natural ingredients [2]. At the moment, both steroidal and non-steroidal anti-inflammatory medications (NSAIDs and SAIDs) are extensively used to treat inflammatory conditions. These substances mainly work by blocking the COX-1 and COX-2 enzymes, which lowers the production of prostaglandins. However, long-term use of these medications is linked to serious side effects, especially renal and gastrointestinal issues [44]. With about 80% of the population depending on traditional medicine, herbal therapy is widely used and an essential component of healthcare systems in many nations, despite the fact that it is not entirely standardized. Rheumatoid arthritis and other chronic inflammatory illnesses continue to be significant global health issues. Even though synthetic anti-inflammatory medications are widely used, their long-term harm cannot be disregarded. Chronic treatment frequently results in adverse consequences such peptic ulcers and gastrointestinal bleeding. As a result, there is an increasing need to create anti-inflammatory drugs that are safer, more effective, and have fewer adverse effects.

Alcoholic and petroleum ether extracts of several plant gums have been shown in earlier research to have anti-inflammatory properties. However, there is still no systematic pharmacological data to support the traditional usage of *Bombax ceiba* (family: Bombacaceae) as an anti-inflammatory and analgesic. Thus, the goal of this study is to assess *Bombax ceiba* gum's analgesic and anti-inflammatory properties.[63]

## **METHODS AND MATERIALS**

**Chemicals and Test Drugs:** We purchased *Bombax ceiba* gum from Jain & Co. Pvt. Ltd. in Ballari, Karnataka. We bought tramadol and diclofenac sodium from the

neighborhood market. SD Fine Chemicals, Merck India Ltd., Mumbai, and Sigma-Aldrich provided all additional analytical-grade chemicals and reagents.

**Experimental Animals:** We purchased 150–200 g adult male Wistar albino rats in good health from Sri Venkateshwara Enterprises in Bangalore. The animals were kept in polypropylene cages with controlled temperatures ( $25 \pm 1$  °C), relative humidity ( $55 \pm 5\%$ ), and a 12-hour light/dark cycle. The regular mouse pellet diet and unlimited water were given to the animals. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) criteria were followed in all experimental methods. The Institutional Animal Ethics Committee gave its approval to the study protocol (IAEC Approval No. CPCSEA/1677/PO/Re/2012/IAEC-April 2017/40).

**Phytochemical Screening of *Bombax ceiba* Gum:** Using standard qualitative assays as outlined by Kokate et al. (2006), a preliminary phytochemical screening of the gum was conducted to find proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, and terpenoids. Preparing Test Drugs Gum mucilage was prepared at a concentration of 10 mg/ml by powdering air-dried *Bombax ceiba* gum and triturating it with distilled water. Before being used in the experiment, fresh mucilage was made every day.

#### Qualitative Phytochemical Tests

Tests for Proteins

**Millon's test:** Adding Millon's reagent resulted in a white precipitate that, when heated, turned red, signifying the presence of proteins.

**Ninhydrin test:** The presence of proteins and amino acids was verified by the development of a violet hue upon boiling with ninhydrin reagent.

#### Tests for Carbohydrates

**Fehling's test:** Red precipitate formation signified the presence of reducing carbohydrates.

**Benedict's test:** Carbohydrates were confirmed by a reddish-brown precipitate.

**Molisch's test:** Carbohydrates were suggested by the appearance of a violet ring

at the interface.

**Iodine test:** Polysaccharides were confirmed by the development of a purple or dark blue tint.

**Test for Phenols and Tannins:** When ferric chloride was added, phenols and tannins were visible as a blue-green or black hue.

#### Tests for Flavonoids

**Shinoda test:** Formation of pink or scarlet color indicated flavonoids.

**Alkaline reagent test:** Intense yellow color that disappeared on acidification confirmed flavonoids.

#### Test for Saponins

Persistent foam formation upon vigorous shaking indicated saponins.

#### Tests for Glycosides

**Liebermann's test:** Green coloration indicated steroidal glycosides.

**Salkowski's test:** Reddish-brown coloration confirmed steroidal aglycone.

**Keller–Kiliani test:** Formation of a brown ring indicated cardiac glycosides.

#### Test for Steroids:

Steroids were validated by the green coloring in the acetic acid–sulfuric acid test and the red coloration in the chloroform layer.

#### Test for Terpenoids:

Grey coloration after sulfuric acid treatment confirmed terpenoids.

#### Determination of Total Phenolic Content

The Folin–Ciocalteu spectrophotometric technique was used to calculate the total phenolic content (TPC) [5]. After combining gum mucilage (1 mg/ml) with sodium bicarbonate solution and Folin–Ciocalteu reagent, the mixture was incubated for 45 minutes at 45 °C. At 765 nm, absorbance was measured. The results were reported as mg gallic acid equivalents (GAE)/g of extract, with gallic acid serving as the standard. Every measurement was done three times.

#### In Vitro Antioxidant Activity [33]

DPPH Radical Scavenging Assay Principle:

The assay relies on the decrease of DPPH radicals from deep violet to yellow when they react with antioxidants that donate hydrogen.

#### Method:

Gum mucilage concentrations ranging from 100 to 1600 µg/ml were combined with

DPPH solution (0.1 mM) and left in the dark for 30 minutes. At 517 nm, absorbance was measured. The standard was rutin. The following formula was used to determine percentage inhibition

$$\{\text{DPPH scavenging activity (\%)} = (\text{A}_{\text{control}} - \text{A}_{\text{test}}) / \text{A}_{\text{control}} \times 100\}$$

### Nitric Oxide Radical Scavenging Assay

#### Principle:

Nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite, which is measured using Griess reagent.

#### Procedure:

Gum mucilage at different concentrations (10–160 µg/ml) was treated with sodium nitroprusside (10 mM) for 150 minutes at 25 °C. After adding the Griess reagent, absorbance was measured at 546 nm. Accordingly, percentage inhibition was determined (Nur et al., 2013).

### ABTS Radical Scavenging Assay

After reacting ABTS with potassium persulfate and incubating for 16 hours, the ABTS<sup>•+</sup> radical was produced. At 734 nm, the solution was diluted to achieve an absorbance of 0.700 ± 0.020. Absorbance was measured after adding gum extract at various concentrations. The standard was BHT. IC<sub>50</sub> values were used to express the results.

### In Vitro Anti-Inflammatory Activity [33] HRBC Membrane Stabilization Method

The human red blood cell (HRBC) membrane stabilization experiment was carried out in accordance with Chatterjee and Das's (1996) instructions. The standard was 50 µg/ml of diclofenac potassium. Membrane stability was evaluated by measuring absorbance at 560 nm.

### Evaluation of Analgesic Activity[4]

**Eddy's Hot Plate Test:** Animals were divided into four groups (n=5). Tramadol (50 mg/kg, i.p.) served as standard. Reaction time was measured at 55 °C with a cut-off time of 15 seconds (Eddy and Leimbach, 1953).

**Tail Flick Test:** The tail flick latency was recorded by immersing the tail tip in water maintained at 58 °C with a cut-off time of 10 seconds (Kulkarni, 1999).

### In Vivo Analgesic Activity[4][7]

**Allyl Isothiocyanate-Induced Nocifensive Behavior:** AITC (1%) was injected into the plantar surface of the hind paw. Paw lifting duration was recorded for 5 minutes post-injection (Samer et al., 2008).

### In Vivo Anti-Inflammatory Activity[8]

**Carrageenan-Induced Paw Edema:** A 1% carrageenan solution was used to cause paw edema. A plethysmometer was used to measure paw volume at predetermined intervals. The conventional formula was used to compute the percentage inhibition of edema (Winter et al., 1962).

**Statistical Analysis:** The mean ± SD was used to express the results. Using GraphPad Prism version 6, one-way ANOVA and Dunnett's test were used to assess statistical significance. Statistical significance was defined as a p-value of less than 0.05.

## RESULTS

### 1. Preliminary Phytochemical screening of Bombax ceiba gum

The preliminary phytochemical screening of the *Bombax ceiba* gum mucilage showed the presence of glycosides, phenols, flavonoids, steroids and terpenoids.

**1.2 Quantification of Poly Phenols:** The Folin-Ciocalteu method was used to calculate the total polyphenolic content of Bombax ceiba gum using the calibration curves of gallic acid ( $y = 0.007x$ ,  $R^2 = 0.978$ ). It was discovered that Bombax ceiba has a total polyphenolic concentration of 42.86 mg GAE/gm dry extract. The gallic acid standard curve is shown below.

### 1.3 In Vitro Anti Oxidant Activity of Bombax ceiba Gum

#### 1.3.1 DPPH radical scavenging assay:

In the DPPH free radical scavenging assay, Bombax ceiba and Rutin have shown IC<sub>50</sub> values of 87.87 ± 7.20 and 127.21 ± 21.4 µg/ml respectively. This indicates that Bombax ceiba has the ability to scavenge the stable free radical DPPH, though efficacy is less than Rutin. The % inhibition was given in the (Fig-7).

#### 1.3.2 Effect of Bombex cieba on NO Scavenging assay

In the NO Scavenging assay, Bombax ceiba and rutin have shown IC<sub>50</sub> values of 142 ± 19.26 µg/ml and 113.7 ± 15.92 µg/ml

respectively. This indicates that *Bombax ceiba* has the ability to capture the nitrile free radical formed in the assay. The % inhibition were given in the (Fig-8).

### 1.3.3 Effect of *Bombax ceiba* gum on

#### ABTS Scavenging assay

In the ABTS Scavenging assay, *Bombax ceiba* and Rutin have shown IC<sub>50</sub> values of  $166.2 \pm 5.70$   $\mu\text{g/ml}$  and  $835 \pm 24.86$   $\mu\text{g/ml}$  respectively. This indicates that *Bombax ceiba* has the ability to prevent the conversion of ABTS its radical cation. The % inhibition were given in the (Fig-9).

### 1.4 Effect of *Bombax Ceiba* gum on HRBC membrane stabilizing assay

In the HRBC membrane stabilizing assay, *Bombax ceiba* and diclofinac potassium have shown IC<sub>50</sub> values of  $126 \pm 12.74$  and  $88.40 \pm 7.20$   $\mu\text{g/ml}$  respectively. This indicates that *Bombax ceiba* protected the human erythrocyte membrane against lysis induced by heat. The % inhibition was given in the (Fig-10).

### 1.5 In Vivo Analgesic Activity

#### 1.5.1 Effect of *Bombax ceiba* gum in Eddy's hot plate test

The jumping latencies have significantly ( $p < 0.05$ ), increased in the animals treated with *Bombax ceiba* (400mg/kg, p.o) and tramadol (50mg/kg, i.p) when compared to the disease control group and the effect was noted to be dose dependent. There is no significant difference at low dose (200mg/kg, p.o) when compared to the disease control group. The results were given in (Table-11; Fig-11).

#### 1.5.2 Effect of *Bombax ceiba* gum in Tail flick test

The tail with drawl latencies have significantly ( $p < 0.05$ ), increased in the animals treated with *Bombax ceiba* (400mg/kg, p.o) and tramadol (50mg/kg, i.p) when compared to the disease control group and the effect was noted to be dose dependent. There is no significant difference at low dose (200mg/kg, p.o) when compared to the disease control group. The results were given in (Table-12; Fig-12).

#### 1.5.3 Effect of *Bombax ceiba* gum in Allyl isothiocyanate induced analgesia

The number of paw lickings have significantly ( $p < 0.05$ ), decreased in the animals treated with *Bombax ceiba*

(400mg/kg, p.o) and diclophenac sodium (50mg/kg, i.p) when compared to the disease control group and the effect was noted to be dose dependent. There is no significant decrease at low dose (200mg/kg, p.o) when compared to the disease control group. The results were given in (Table-13; Fig-13).

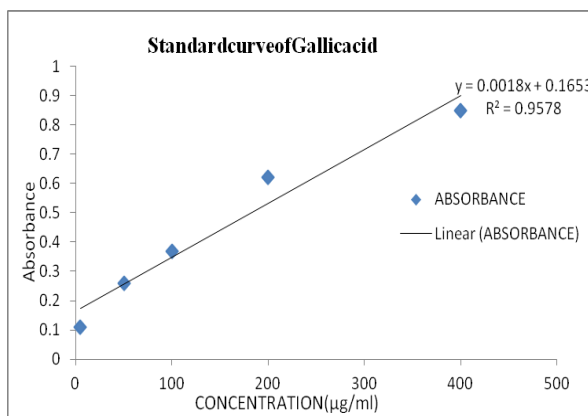
### 1.6 Effect of *Bombax ceiba* gum in Carrageenan-induced paw edema model:

When compared to the disease control group, the animals treated with *Bombax ceiba* (400 mg/kg, p.o.) and diclophenac sodium (50 mg/kg, i.p.) showed a substantial ( $p < 0.05$ ) decrease in paw size, with a large displacement of the volume in the plethysmometer and a dose- dependent impact. When compared to the disease control group with low displacement volume, there is no discernible reduction in paw size at low dose (200 mg/kg, p.o.). Table 14 and Figure 14 presented the findings.

**Table-6: Phytochemical screening of *Bombax ceiba* gum**

Sl. No	Phytoconstituents	Interference
1	Carbohydrates	+Ve
2	Proteins	- Ve
3	Phenols	+Ve
4	Glycosides	+Ve
5	Saponins	+Ve
6	Flavonoids	+Ve
7	Terpenoids	+ Ve
8	Steroids	+ Ve
9	Alkaloids	+ Ve

+ Ve = Presence, -Ve = Absence

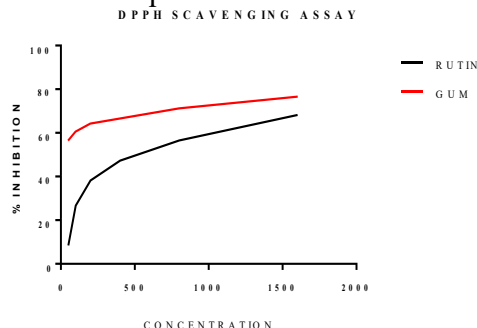


**Fig-6: STANDARD CURVE OF GALLIC ACID**

**Table-7: Effect of Bombax Ceiba gum on DPPH scavenging assay**

S. No	Concentration (µg/ml)	Rutin (%Inhibition)	Bombax ceiba gum (%Inhibition)
1	50	8.57	56.6
2	100	26.8	60.7
3	200	38.3	64.4
4	400	47.4	66.6
5	800	56.6	71.3
6	1600	68.3	76.6
		<b>IC<sub>50</sub> = 127.21 ± 21.4</b>	<b>IC<sub>50</sub> = 87.87 ± 7.20</b>

All the values were expressed as Mean ± SD of triplicates. IC<sub>50</sub> values were calculated from the linear equation

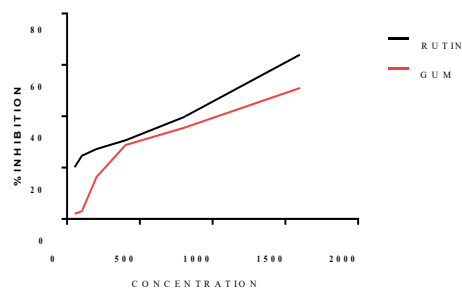
**Fig-7 Effect of Bombax Ceiba gum on DPPH Scavenging assay**

All the values were expressed as mean ± SD of triplicates.

**Table-8: Effect of Bombax Ceiba gum on NO Scavenging assay**

S.No	Concentration (µg/ml)	Rutin (%inhibition)	Bombax ceiba gum (%inhibition)
1	50	20.2	2.06
2	100	24.7	3.09
3	200	27.3	16.4
4	400	30.7	28.8
5	800	39.7	35.5
6	1600	64.04	51.03
		<b>IC<sub>50</sub> = 113.7 ± 15.92</b>	<b>IC<sub>50</sub> = 142 ± 19.26</b>

All the values were expressed as mean ± SD of triplicates. IC<sub>50</sub> values were calculated from the linear equation

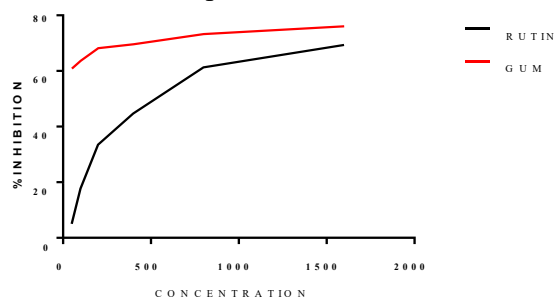
**Fig-8: Effect of Bombax Ceiba gum on NO Scavenging assay**

All the values were expressed as mean ± SD of triplicates.

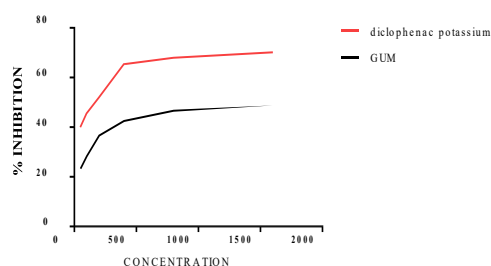
**Table-9: Effect of Bombax ceiba gum on ABTS Scavenging**

Sl. No	Concentration (µg/ml)	Rutin (%inhibition)	Bombax ceiba gum (%inhibition)
1	50	4.95	60.9
2	100	17.6	63.7
3	200	33.4	68.2
4	400	44.6	69.6
5	800	61.2	73.3
6	1600	69.3	76.1
		<b>IC<sub>50</sub> = 835 ± 24.86</b>	<b>IC<sub>50</sub> = 166.2 ± 5.70</b>

All the values were expressed as mean ± SD of triplicates. IC<sub>50</sub> values were calculated from the linear equation.

**Fig-9: Effect of Bombax Ceiba gum on ABTS Scavenging assay**

All the values were expressed as mean ± SD of triplicates.





**Fig-10: Effect of Bombax Ceiba gum on HRBC membrane stabilizing assay**

All the values were expressed as mean  $\pm$  SD of triplicates.

**Table-10: Effect of Bombax Ceiba gum on HRBC membrane stabilizing assay**

S. No	Concentration ( $\mu$ g/ml)	Diclofenac potassium (%inhibition of hemolysis)	Bombax ceiba gum (%inhibition of hemolysis)
1	50	40.12	56.6
2	100	45.61	60.7
3	200	52.12	64.4
4	400	65.53	66.6
5	800	68.12	71.3
6	1600	70.28	76.6
		<b>IC<sub>50</sub> = 88.40 <math>\pm</math> 7.20</b>	<b>IC<sub>50</sub> = 126 <math>\pm</math> 12.74</b>

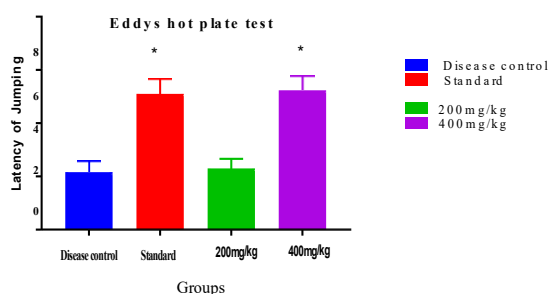
All the values were expressed as mean  $\pm$  SD of triplicates. IC<sub>50</sub> values were calculated from the linear equation

**Table-11: Effect of Bombax ceiba gum in Eddy's hot plate test**

Sl. No	Groups	Jumping latency (in sec)
1	Disease control (Thermal stimulus)	2.16 $\pm$ 0.37
2	Tramadol (50mg/kg, i.p)	5.10 $\pm$ 0.55
3	Gum low dose (200mg/kg)	2.30 $\pm$ 0.36
4	Gum high dose (400mg/kg)	5.23 $\pm$ 0.50

Values were expressed as Mean  $\pm$  SD (n=6);  
Analysed by one way ANOVA

\*=p<0.05, considered statistically significant when compared to the Disease control

**Fig-11: Effect of Bombax ceiba gum in Eddy's hot plate test**

Values were expressed as Mean  $\pm$  SD (n=6);

Analysed by one way ANOVA

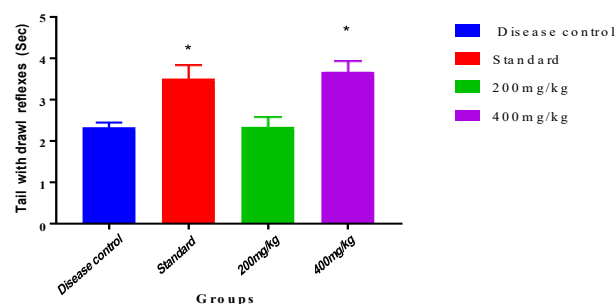
\*=p<0.05, considered statistically significant when compared to the Disease control

**Table-12: Effect of Bombax ceiba gum in Tail flick test**

Sl. no	Groups	Tail with drawl reflex (in sec)
1	Disease control	2.23 $\pm$ 0.11
2	Tramadol (50mg/kg, i.p)	3.50 $\pm$ 0.30
3	Gum low dose (200mg/kg, p.o)	2.33 $\pm$ 0.23
4	Gum high dose (400mg/kg, p.o)	3.66 $\pm$ 0.25

Values were expressed as Mean  $\pm$  SD (n=6);  
Analysed by one way ANOVA

\*=p<0.05, considered statistically significant when compared to the Disease control

**Fig-12 : Effect of Bombax ceiba gum in Tail flick test**

Values were expressed as Mean  $\pm$  SD (n=6);  
Analysed by one way ANOVA

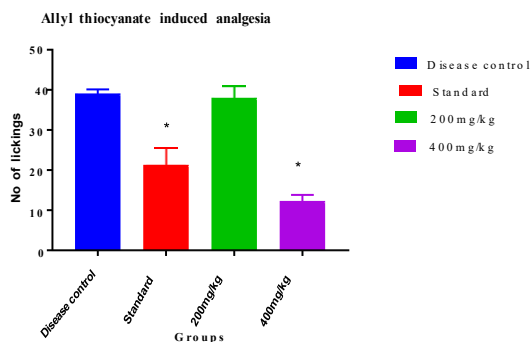
\*=p<0.05, considered statistically significant when compared to the Disease control.

**Table-13: Effect of Bombax ceiba gum in Allyl isothiocyanate induced analgesia**

Sl. No	Groups	No of lickings
1	Disease control	39.00 $\pm$ 1.00
2	Diclophenac sodium (50mg/kg, i.p)	21.33 $\pm$ 4.16
3	Gum low dose (200mg/kg)	38.00 $\pm$ 2.64
4	Gum high dose (400mg/kg)	12.33 $\pm$ 1.52

Values were expressed as Mean  $\pm$  SD (n=6);  
Analysed by one way ANOVA

\*=p<0.05, considered statistically significant when compared to the Disease control



**Fig -13: Effect of Bombax ceiba gum in Allyl isothiocyanate induced analgesia**

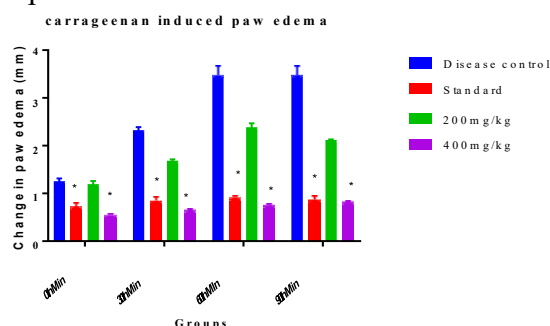
Values were expressed as Mean  $\pm$  SD (n=6); Analysed by one way ANOVA

\*=p<0.05, considered statistically when compared to the Disease control

**Table-14: Effect of Bombax ceiba gum in Carrageenan-induced paw edema model**

Values were expressed as Mean  $\pm$  SD (n=6) Analysed by one way ANOVA

\*=p<0.05, considered statistically when compared to the Disease control.



**Fig-14: Effect of Bombax ceiba gum in Carrageenan-induced paw edema model**

Values were expressed as Mean  $\pm$  SD (n=6); Analysed by one way ANOVA

\*=p<0.05, considered statistically significant when compared to the Disease control.

## DISCUSSION

Although non-steroidal anti-inflammatory medicines (NSAIDs) are frequently given to treat pain and inflammatory diseases, their long-term use is restricted because of major side effects such kidney damage, gastrointestinal ulcers, and an increased risk of hemorrhagic stroke. The hunt for safer and more efficient substitutes, especially those derived from natural sources, has accelerated because to these safety concerns. Particularly in underdeveloped nations where a sizable section of the populace relies on traditional medical systems, medicinal plants continue to be essential to primary healthcare. In this regard, the goal of the

current study was to objectively assess the analgesic, anti-inflammatory, and antioxidant capabilities of Bombax ceiba gum, a plant-derived substance that has been used traditionally but has no pharmacological documentation. The pathophysiology of inflammatory illnesses, pain, and a number of chronic diseases is significantly influenced by oxidative stress. Reactive oxygen and nitrogen species are examples of free radicals that can cause cellular damage through DNA damage, protein oxidation, and lipid peroxidation. By scavenging free radicals and stopping chain reactions, antioxidants mitigate these effects. Bombax ceiba gum showed strong free radical scavenging activity in the DPPH, nitric oxide, and ABTS tests used in this

Change in paw edema (mm)				
Groups	0th min	30th min	60th min	90th min
Disease control	1.03 $\pm$ 0.05	1.79 $\pm$ 0.06	2.56 $\pm$ 0.18	2.67 $\pm$ 0.17
(Diclophenac sodium 50mg/kg)	0.73 $\pm$ 0.06 *	0.84 $\pm$ 0.06*	0.9 $\pm$ 0.02*	0.85 $\pm$ 0.06*
Low dose 200mg/kg	1.19 $\pm$ 0.06	1.68 $\pm$ 0.03	2.38 $\pm$ 0.08	2.1 $\pm$ 0.01
High dose (400mg/kg)	0.54 $\pm$ 0.01*	0.65 $\pm$ 0.00*	0.75 $\pm$ 0.01*	0.82 $\pm$ 0.00*

investigation. Strong antioxidant capability is suggested by the dose-dependent decrease in radical activity seen in all of these tests. The gum's ability to neutralize both hydrophilic and lipophilic radicals was demonstrated by its ABTS scavenging activity, which was found to be equivalent to DPPH scavenging. This antioxidant characteristic might be essential for reducing oxidative stress linked to inflammation.

When lysosomal membranes become unstable due to inflammation, proteolytic enzymes are released, which worsen tissue damage. One important way that anti-inflammatory drugs provide their protective benefits is thought to be the stabilization of these membranes. Since the lysosomal membrane and the human red blood cell (HRBC) membrane are similar, the HRBC membrane stabilization approach is a dependable in vitro model for evaluating



anti-inflammatory effectiveness. Bombax ceiba gum significantly and dose-dependently stabilized HRBC membranes in the current investigation, demonstrating its capacity to stop membrane lysis in hypotonic environments. Phenolic and flavonoid chemicals found during phytochemical screening may be responsible for this action because they are known to improve membrane integrity and prevent the release of lysosomal enzymes. Inflammatory pain is mediated by both cerebral and peripheral pathways. Thermal models like the hot plate and tail flick tests were used to assess Bombax ceiba gum's analgesic efficacy. The tail flick test primarily represents spinal reflexes, while the hot plate test reflects supraspinal responses. These models are well-established for evaluating centrally mediated analgesic activity. Pain response latency was considerably increased when Bombax ceiba gum was administered at doses of 200 and 400 mg/kg in both models. These findings suggest that the gum exerts analgesic effects at both spinal and supraspinal levels, possibly through modulation of central nociceptive pathways. The allyl isothiocyanate (AITC)-induced nocifensive behavior paradigm was employed to investigate peripheral nociceptive processes in more detail. Acute pain reactions result from AITC's activation of C-fiber nociceptors' transient receptor potential ankyrin 1 (TRPA1) channels. Bombax ceiba gum dramatically decreased AITC-induced paw lifting behavior in the current investigation, suggesting suppression of TRPA1-mediated nociception. This implies that the gum's antinociceptive action involves both cerebral and peripheral processes. Bombax ceiba gum's broad analgesic efficacy is demonstrated by its capacity to inhibit both chemically and thermally generated pain responses. The in vivo anti-inflammatory efficacy was evaluated using the carrageenan-induced paw edema paradigm. This model's biphasic inflammatory response has made it generally accepted. Histamine and serotonin mediate the first phase, whereas prostaglandins, bradykinin, and lysosomal enzymes mediate the second. Clinically effective anti-

inflammatory drugs exhibit inhibition of the second phase, which is especially suggestive of cyclooxygenase inhibition. Bombax ceiba gum considerably reduced paw edema in the later stages of the current study, indicating disruption of prostaglandin production and other inflammatory mediators. This finding validates the plant's historic application for inflammatory ailments. Bioactive phytoconstituents found in Bombax ceiba, such as flavonoid glycosides, coumarins, and phenolic compounds, may be responsible for the pharmacological effects seen in this investigation. Pro-inflammatory cytokines, cyclooxygenase production, MAPK and NF- $\kappa$ B signaling pathways, and oxidative stress are all known to be suppressed by flavonoids such as vicienin, isovitexin, and vitexin. All of these processes work together to provide analgesic and anti-inflammatory effects. The phytochemical profile of the gum indicates a synergistic impact of numerous substances, even if the precise elements responsible for the reported activity were not isolated in the current investigation.

## CONCLUSION

The results of this study offer pharmacological evidence in favor of the traditional analgesic and anti-inflammatory properties of Bombax ceiba gum. Its efficacy in both peripheral and central modes of action is demonstrated by the observed decrease in pain and inflammation across experimental models. The anti-inflammatory activity raises the possibility of inhibiting inflammatory mediators including prostaglandins and cytokines, especially in the later stages of inflammation. Suppression of nociceptive signaling and modification of inflammatory pain pathways may be responsible for the analgesic effect. These actions are probably caused by the gum extract's bioactive phytoconstituents. All things considered, Bombax ceiba gum exhibits promise as a natural medicinal agent with potential advantages in the treatment of pain and inflammatory disorders, supporting more thorough phytochemical and mechanistic.

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