 doi:10.20944/preprints201907.0204.v1

Assessment of analgesic and anti-inflammatory potential of ethanolic extract of *Gossypium arboreum* leaves in experimental animals

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 doi:10.20944/preprints201907.0204.v1

Abstract: *Background and objectives*: *Gossypium arboreum* commonly known as cotton plant, this variety of cotton plant available throughout India. Cotton plant was used traditionally for the treatment of infection, diarrhea and other inflammatory conditions. The aim of present study is to evaluate analgesic and anti-inflammatory activity of *Gossypium arboreum* leaves extract on experimental animals. *Materials and Methods*: The ethanolic extract of *Gossypium arboreum* leaves (EEGA) was subjected to assess *its* antioxidant potential using DPPH radical scavenging assay; further anti-inflammatory and analgesic activity was assessed by using carrageenan-induced rat paw edema and tail flick test respectively in experimental animals. *Results*: It was observed that free radicals were scavenged by the EEGA in a concentration dependent manner. The Ethanolic extract showed maximum 72% scavenging activities at 200 µg/ml concentration. The ethanol extract exhibited significant analgesic activity in the tail-flick model ($P<0.01$) by increasing the reaction time of the mice to 8.9 sec at 180min after treatment in comparison to control (3.4 sec). The EEGA (100, 200 and 400 mg/kg, p.o.) showed dose-dependent, inhibition of carrageenan-induced rat paw edema from 30 min onwards ($P<0.01$), *Conclusions*: Present study revealed that the ethanolic extract of *Gossypium arboreum* displayed prominent analgesic and anti-inflammatory activity in experimental animals owing to its antioxidant property.

Keywords: *Gossypium arboreum*; antioxidant; analgesic; anti-inflammatory.

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1. Introduction

In numerous diseases and disorders oxidative stress and inflammation play a vital role [1,2]. Free radicals particularly, the reactive oxygen species (ROS) creates oxidative stress within cells resulting in inflammatory and infectious condition. Phagocytic cells including polymorphonuclear leukocytes (neutrophils, eosinophils) and mononuclear cells (macrophage and lymphocytes) produce excessive amount of reactive oxygen species which play a significant role in the host defense mechanism [3,4].

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. The generation of oxygen free radicals is known to be involved in the development of the inflammatory response. In addition to their actions as noxious mediators generated by inflammatory cells, these molecules play also a crucial role for progression of inflammation in various organs [5]. Inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. The standard signs of inflammation are expressed by increased blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids and cellular [6].

Although non-steroidal anti-inflammatory drugs (NSAID) and opioids are widely used in the treatment of inflammatory diseases, these drugs have numerous side effects which include gastritis, peptic ulceration; gastrointestinal bleeding and tolerance may develops due to chronic administration of NSAIDs [7]. Therefore, it is necessary to identify analgesic and anti-inflammatory agent with higher potency and minor side effects.

Presently use of natural products or herbal drugs for the treatment of various diseases and disorders increased tremendously [8]. Various herbal drugs being also used to treat inflammation and oxidative stress related complications [9]. *Curcuma longa, Zingiber officinale, Boswellia serrata and Rosmarinus officinalis* these medicinal plants showed anti-inflammatory activity [10].

Gossypium arboreum commonly known as cotton plant, this variety of cotton plant available throughout India. The plant is a species of cotton native to India, Pakistan and other tropical and climatic zone of the world. *Cotton plant was* used traditionally for the treatment of infection,

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diarrhea and other inflammatory conditions [11]. *Gossypium arboreum* leaf extracts are already reported for its antidiabetic, antihypertensive, antibacterial and antifungal activity [12-14].

Infusion of the leaf of *Gossypium arboreum* used to reduce respiratory complications like cough and cold as it reduce smooth muscle contractions [15,16]. The important active phytochemicals present in *Gossypium arboreum* are glycosides, monoterpenes, phenolic acids, triterpenoid, carbohydrates, flavonoids, alkaloids, fatty acids, and essential oils, some of these phytochemicals are reported for its antioxidant potential [11,17].

So, based on the literature review, reported pharmacological activities and chemical constituents found in cotton plant the present study is aimed to investigate analgesic and anti-inflammatory potential of ethanolic extract of leaves of *Gossypium arboretum*.

2. Materials and Methods

2.1 Extraction

Leaves of *Gossypium arboreum* (Family Malvaceae) was collected in the month of January 2019 from the local region of Nanded district of Maharashtra state, and it was identified and authenticated from Taxonomy Research in Botany, N.E.S Science College, Nanded. Leaves of *Gossypium arboreum*, were washed 2-3 times with tap water and then shade dried for 7 days then coarsely powdered using grinder. 100 g of dried powder was extracted with ethanol by using Soxhlet apparatus. The % yield of ethanolic extract of leaves of *Gossypium arboreum* (EEGA) was found to be *4.7% w/w*.

2.2 Animals

Albino Wistar rats (180-220 g) and Swiss albino mice (20-30 g) of either sexes were used for the study. The animals were obtained from the animal house of Sudhakarrao Naik Institute of Pharmacy, Pusad, Maharashtra, India. The animals were housed in standard laboratory condition of dark,light cycle (12,12 h) and temperature (25 ± 2°C). Animals were given standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (**Reg. No. 729/PO/Re/S/11/CPCSEA**).

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2.3 Dose Selection

The dose was selected as per literature survey, the dose 100mg/kg, 200mg/kg and 400 mg/kg was selected for this study.

2.4 Experimental protocols

2.4.1 *In-vitro* antioxidant

2.4.1.1 DPPH radical scavenging activity [18].

The free radical scavenging activity of *Gossypium arboreum* was measured by DPPH radical scavenging assay, where in the bleaching rate of the stable free radical, (1, 1-Diphenyl-2-picrylhydrazyl) DPPH is monitored at a characteristic wavelength in the presence of the ethanolic extract of *Gossypium arboreum*. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, its absorbance decreases. Briefly, 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of *Gossypium arboreum* solution in water at various concentrations (50, 100, 150 and 200 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm.

% inhibition of extract was calculated against control by using following formula

$$\%\ \text{scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\ \text{X } 100$$

2.4.2 Analgesic activity

2.4.2.1 Tail flick method [19].

Swiss albino mice were divided into five groups with six animals in each group. Group I (control) received normal saline (1mL/mouse p.o.). Group II (Standard) received 20 mg/kg,p.o. of diclofenac sodium. Group III, IV and V received 100, 200 and 400 mg/kg. p.o. of EEGA respectively.

About 2-3cm of the tail of animal were dipped into a water bath containing warm water maintained at a temperature of 50±1°C and, the reaction time was recorded before 0 min and after 15, 30, 45, and 60 min of the treatments. The time taken for the mice to flick its tail or withdraw it from the warm water known as the reaction time was recorded; the cut off time was set at 10 s**.** The maximum possible analgesia (MPA) was calculated as follows,

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$$\text{MPA} = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15\ \text{sec} - \text{reaction time for saline}} \times 100$$

2.4.3 *In-vivo* anti-inflammatory activity

2.4.3.1 Carrageenan induced rat paw edema [20].

The animals were randomly divided into five groups of six animals in each. Group I (control) received normal saline (1 mL/rat p.o.). Group II (Standard) received 20 mg/kg, p.o. of diclofenac sodium. Group III, IV and V received 100, 200 and 400 mg/kg of EEGA respectively. After an hour of drug/extract treatment, 0.1 mL of 1% w/v suspension of Carrageenan in normal saline was injected into the sub-plantar region of the right hind paw of each rat; the left hind paw of the animal considered as control. The paw volume was measured at 0 min and after 15, 30, 60, 90, 120 and 180 min, of carrageenan administration by using plethysmometer and % inhibition was calculated.

$$\%\ \text{inhibition} = \frac{(\text{Vt} - \text{Vo})\text{control} - (\text{Vt} - \text{Vo})\text{treated}}{(\text{Vt} - \text{Vo})\text{control}}\ \text{X}\ 100$$

2.5 Statistical analysis

The data obtained were statistically analyzed using ANOVA followed by Dunnett's test to detect any significant difference among different means, with level of significance set at $p< 0.05$. The results were expressed as mean ± S.E.M.

3. Results

3.1 *In-vitro* antioxidant activity

Several concentrations (50, 100, 150 and 200 µg/ml) of the EEGA were tested for their antioxidant activity in DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity. It was observed that free radicals were scavenged by the EEGA in a concentration dependent manner. The EEGA showed maximum radical scavenging activities (72%) at 200 µg/ml concentration, which is comparable to that of standard ascorbic acid (89%).

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Table 1.Effect of ethanolic extract of *Gossypium arboreum* on DPPH radical scavenging assay

Concentration (µg/mL)	DPPH radical scavenging activity			
	EEGA		Ascorbic acid	
	Absorbance	% Scavenging activity	Absorbance	% Scavenging activity
50	0.35±0.01	28	0.22±0.01	54
100	0.20±0.05	58	0.15±0.05	69
150	0.18±0.05	63	0.09±0.06	81
200	0.13±0.04	72	0.05±0.08	89
IC_{50}	105.83		68.37	

All values are expressed as mean ± SEM. three parallel measurements. EEGA- Ethanolic extract of *Gossypium arboreum* leaves.

3.2 Analgesic activity

Analgesic activity of EEGA was performed by using tail flick method. In the tail flick test, EEGA at the dose of 100 mg/kg reveled significant ($p<0.05$) alteration in the reaction time at 30 and 60 min of EEGA administration. EEGA at the dose of 200 and 400 mg/kg, p.o. depicted prominent analgesic activity ($p<0.01$) from 30 min onwards. An increase in the dose of EEGA there was significant improvement ($p<0.01$) in analgesic activity and that was comparable to that of diclofenac treated animals.

EEGA at the dose of 400 mg/kg showed maximum possible analgesia i.e. 48% after 180 min of EEGA treatment whereas, diclofenac 20 mg/kg p.o. treated animals showed a maximum possible analgesia i.e. 53% after its administration.

Table 2. Analgesic effect of ethanol extract of *Gossypium arboreum* by tail-flick method in mice.

Groups	Reaction time in seconds (Maximum possible analgesia %)					
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Control	3.25±0.06	3.38±0.06	3.13±0.05	3.2±0.05	3.3±0.09	3.4±0.08
Diclofenac	3.7±0.08	5.4±0.09** (17)	6.7±0.09** (30)	7.48±0.05** (36)	8.9±0.10** (47)	9.7±0.02** (53)
EEGA 100	3.33±0.06	4.18±0.09* (06)	4.92±0.09* (15)	5.45±0.10** (19)	6.26±0.12** (25)	7.10±0.08** (32)
EEGA 200	3.5±0.09	4.63±0.01* (10)	5.01±0.05** (16)	6.17±0.15** (25)	7.2±0.06** (33)	7.9±0.05** (38)

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EEGA 400	3.25±0.03	4.43±0.01* (04)	4.36±0.05** (10)	6.59±0.03** (28)	7.8±0.04** (38)	8.9±0.05** (48)

All values are expressed as mean ± SEM. (n=6), in each group; (One way ANOVA followed by Dunnett's test),* (P<0.05), ** (P<0.01), when compared to the control group. EEGA- Ethanolic extract of *Gossypium arboreum* leaves.

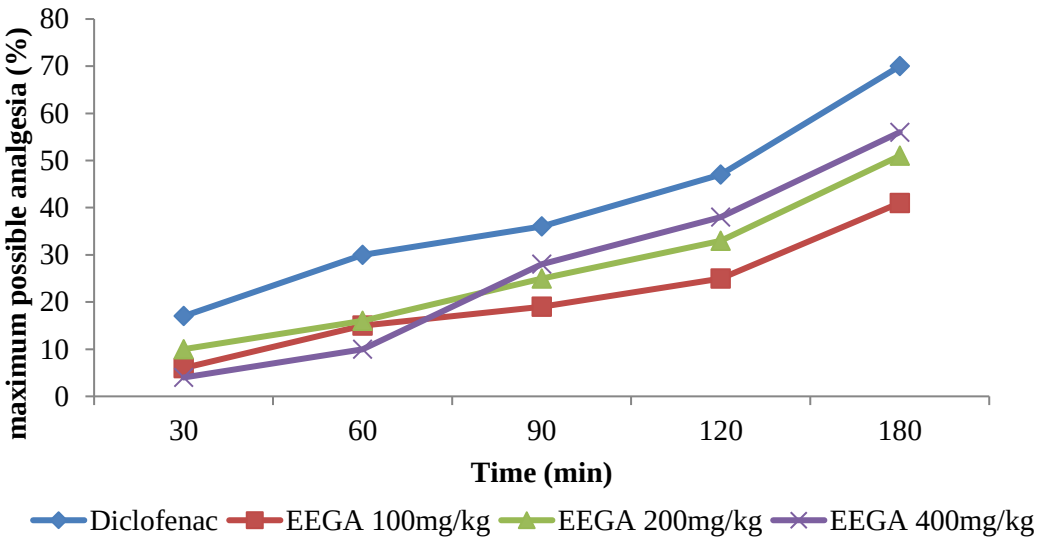

Figure 1. Effect of ethanolic extract of *Gossypium arboreum* leaves on Maximum possible analgesia (%)

3.3 Anti-inflammatory activity

Anti-inflammatory activity of EEGA was performed by using carrageenan induced paw edema. In the carrageenan induced paw edema test, EEGA at the dose of 100 mg/kg reveled significant ($p<0.05$) alteration in the paw volume at 15, 30 and 60 min of EEGA administration. EEGA at the dose of 200 and 400 mg/kg, p.o. depicted prominent anti-inflammatory activity ($p<0.05$) from 30 min onwards of treatment. An increase in the dose of EEGA there is significant improvement ($p<0.01$) in anti-inflammatory activity and that was comparable to that of diclofenac treated animals.

EEGA at the dose of 400 mg/kg showed maximum percentage inhibition i.e. 61% after 180 min of EEGA treatment whereas, diclofenac 20 mg/kg p.o. treated animals showed a maximum percentage inhibition i.e. 74% after its administration.

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Table 3. Effect of ethanolic extract of *Gossypium arboreum* leaves in Carrageenan induced rat paw edema

Groups	Mean paw volume (% Inhibition)						
	0min	**15min**	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Control	0.51±0.07	0.65±0.06	0.8±0.05	1.02±0.01	1.22±0.03	1.09±0.02	0.9±0.01
Diclofenac	0.5±0.07	0.59±0.01** (30)	0.69±0.02** (37)	0.77±0.06** (47)	0.74±0.01** (66)	0.71±0.09** (63)	0.6±0.08** (74)
EEGA 100	0.51±0.01	0.63±0.06* (14)	0.74±0.01* (20)	0.86±0.08* (31)	0.97±0.01** (35)	0.80.±0.01** (40)	0.73±0.09** (43)
EEGA 200	0.5±0.01	0.61±0.09* (21)	0.71±0.01** (27)	0.82±0.01** (37)	0.9±0.07** (43)	0.77±0.01** (53)	0.74±0.09** (38)
EEGA 400	0.49±0.01	0.59±0.09* (28)	0.7±0.01** (27)	0.81±0.01** (37)	0.88±0.04** (45)	0.75±0.06** (55)	0.64±0.01** (61)

All values are expressed as mean ± SEM. (n=6), in each group; (One way ANOVA followed by Dunnett's test),* ($P<0.05$), ** ($P<0.01$), as compared to the control group. EEGA- Ethanolic extract of *Gossypium arboreum* leaves.

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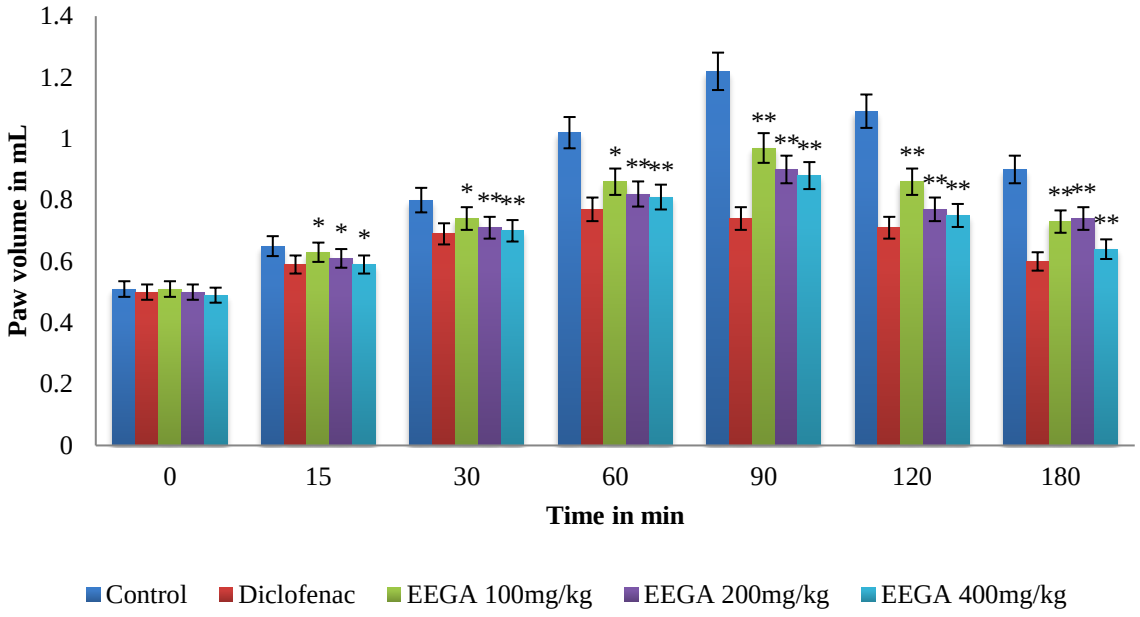

Figure. 2. Effect of ethanolic extract of *Gossypium arboreum* leaves in Carrageenan induced rat paw edema.

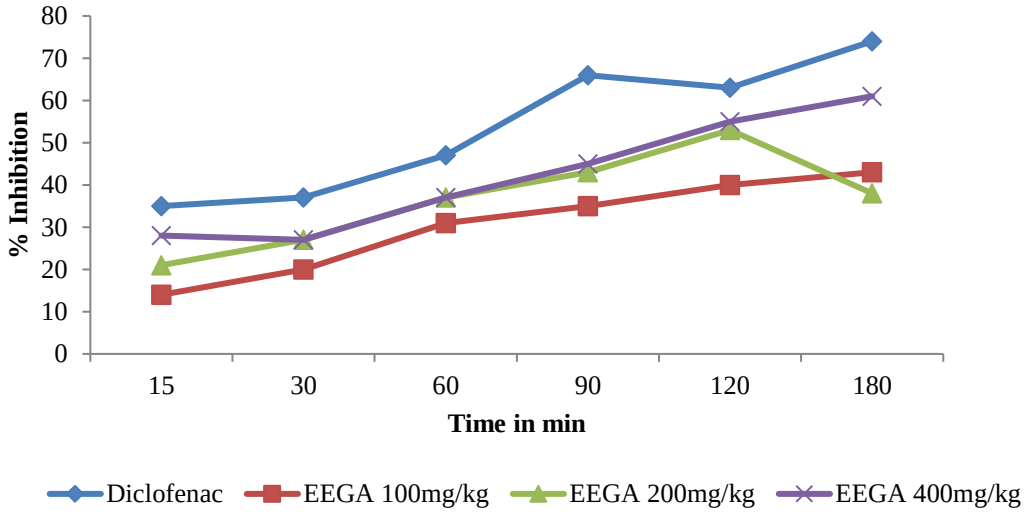

Figure 3. % Inhibition of ethanolic extract of *Gossypium arboreum* leaves in Carrageenan induced rat paw edema.

4. Discussion

Certain types of inflammatory injuries are mediated by reactive oxygen species. The most likely sources of these oxidizing agents are the phagocytic leukocytes (e.g., neutrophils,

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monocytes, macrophages, and eosinophils) that invade the tissue [21, 22]. These reactive radicals and oxidants may harm cell and tissues directly via oxidative degradation of cellular components [23]. Reactive oxygen species may also initiate and amplify inflammation via the release of several mediators involved in the inflammatory response [24].

Chronic health problems such as asthma, diabetes, hypertension, cancer, kidney and liver failure and certain inflammation, showed involvement of free radicals induced damage [25]. Antioxidants prevent free radical induced tissue damage by stabilizing the unstable free radical by donating electron or may reduce its formation or enhance their decomposition [26]. Free radical induced damage can be reduced by use of certain antioxidant agents derived from synthetic or natural source. The rich sources of natural compounds with antioxidants property are present in traditional Indian diet and medicinal plants [27,28]. In present study, the antioxidant activity of *Gossypium arboreum* leaves was performed by using DDPH radical scavenging assay. DPPH produce a stable free radical, and in present assay the measurement of electron donating ability of EEGA and that can be analysed by colour change in the reaction mixture. The changes in colour (from deep violet to light yellow) were measured at 517 nm on a UV-visible light spectrophotometer [25]. EEGA exhibited a significant DPPH radical scavenging activity and that was comparable to ascorbic acid. Radical scavenging ability of EEGA may be due to presence of certain phytochemicals such as lipid, proteins, flavonoid and glycoside responsible for antioxidant effect [17].

Analgesics act on peripheral or central nervous system and selectively relieve pain without significantly altering the consciousness. Central effect is due to raising the threshold for pain and inhibit the generation of impulses at chemoreceptor site, when act peripherally [29]. In present study, tail-flick method was used for screening of analgesic activity, it is mediated by spinal reflex to a nociceptive stimulus and reaction time to the stimuli is measured. EEGA at the dose of 400 mg/kg showed significant analgesic effect by increasing the reaction time for tail withdrawal when compared to that of control group animals. EEGA possesses flavonoids that may contribute to analgesic effect [30, 31].

Carrageenan is the phlogistic agent; used to induce inflammation in the experimental animals to screen anti-inflammatory activity of compounds. When carrageenan injected locally into the rat paw, it produced inflammation, which was occur within 30 min [32, 33]. Carrageenan showed biphasic response, the inflammatory symptoms observed due to release of histamine, serotonin

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and similar substances during first phase (2nd hour) of administration; and in second phase (3rd hour) activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome responsible [34,35]. The EEGA inhibited the carrageenan induced rat paw edema, at both early and late phase. Results revealed the inhibitory effect of EEGA on edema formation is probably due to the inhibition of the synthesis and release of the inflammatory mediators such as histamine, serotonin and prostaglandins. Anti-inflammatory effect of EEGA may be due to presence of flavonide, saponins are responsible for reducing the release of inflammatory mediators [30, 36]. EEGA also reported for its membrane stabilizing activity which may contribute for its anti-inflammatory activity [37].

5. Conclusion

It can be concluded that the ethanolic extract of the leaves of *Gossypium arboreum* possess anti-inflammatory and analgesic activity thus validating the traditional claims. This knowledge could be tapped to formulate new agents to treat inflammatory ailments.

Author Contributions: Conceptualization, S.P. (Sharad Patange); Methodology, S.P. (Sharad Patange); Formal Analysis, P.Z. (Prashant Zagade); Investigation, S.P. (Sharad Patange) and V.N. (Vijay Navghare); Data Curation, R.M. (Rameshwar Mustapure); Writing—Original Draft Preparation, S.P. (Sharad Patange) and V.N. (Vijay Navghare);Writing—Review & Editing, R.M. (Rameshwar Mustapure) S.J. (Swarup Jadhav) A.K. (Akshay Kendre); Supervision, S.P. (Sharad Patange) and V.N. (Vijay Navghare); Project Administration, S.P. (Sharad Patange) and V.N. (Vijay Navghare).

Funding: This research received no external funding.

Acknowledgments: Sudhakarrao Naik Institute of Pharmacy, Pusad for providing animal house facility for completion of the present work.

Conflicts of Interest: The authors declare no conflict of interest.

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