RESEARCH PAPER

In-Vivo Anti-Inflammatory Potential of Anogeissus sericea

Ravi Gupta[1*], Abhishek Nagar[2]
[1]Scholar, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan (Corresponding Author)
Email: ravi19960723@gmail.com
[2]Professor, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan
Email: abhishek.nagar@cpur.edu.in

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ABSTRACT
Inflammation is a multifaceted biological reaction initiated by tissue damage, infection, or immunological dysfunction, involving several cellular and molecular mediators, including cytokines, prostaglandins, leukotrienes, and transcription factors such as NF-κB. This study aimed to assess the anti-inflammatory activity of Anogeissus sericea Leaves and Anogeissus sericea Bark. Acute toxicity experiments were performed on albino Wistar rats in accordance with OECD recommendations, demonstrating the safety of both extracts at doses up to 2000 mg/kg, with no recorded mortality. The anti-inflammatory effect was measured using the carrageenan-induced hind paw edema model, while the analgesic effect was examined by Eddy's hot plate method. The findings indicated a significant ($p \leq 0.05$) decrease in paw edema in the groups administered ASL and ASB Anogeissus sericea extract relative to the carrageenan-induced control group. The anti-inflammatory impact was determined to be dose-dependent, with elevated doses exhibiting increased suppression of edema. Significantly, Anogeissus sericea Leaves formulations demonstrated enhanced efficacy relative to Anogeissus sericea bark. The data indicate that Anogeissus sericea Leaves and Anogeissus sericea Bark extracts exhibit significant anti-inflammatory effects, possibly through the regulation of inflammatory mediators. These findings endorse the prospective application of these plant-derived formulations as efficacious substitutes for mitigating inflammation with no adverse effects.

Keywords: Anti-inflammatory, Anogeissus sericea, Carrageenan induced paw edema
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INTRODUCTION

The term "inflammation" originates from the Latin word "inflammo," which translates to "I set alight," "I ignite," or "to set on fire." In Greek, inflammation is referred to as a heated phenomenon. The Greek term "phlegmon" has been employed to describe inflammatory lesions within **(Mayer et al., 2013)**.

Inflammation is characterized as a localized defensive reaction of living mammalian or vascularized tissues to injury or infection caused by any factor. This is a typical reaction to disrupted homeostasis resulting from illness, injury, and trauma. In the Unani framework, Waram (inflammation) is a comprehensive term that denotes any atypical swelling resulting from the buildup of blood, pus, water, or gas. Waram is a swelling resulting from the absorption of aberrant substances in any organ. Inflammation often arises when pathogenic microorganisms, including bacteria, viruses, or fungi, infiltrate the body, inhibit specific tissues, and/or circulate throughout the bloodstream **(Loane et al., 2016.)**. Inflammation may also occur in reaction to phenomena such as tissue injury, cellular necrosis, malignancy, ischemia, and degeneration **(Fernandes et al., 2015)**. Both the innate and adaptive immune responses are primarily implicated in the development of inflammation **(Rock et al., 2011)**, **(Waisman et al., 2015).**The innate immune system is the primary defense mechanism against invading microbes and cancer cells, involving the action of different cells such as macrophages, mast cells, and dendritic cells. The adaptive immune system entails the function of specialized cells, namely B and T cells, which are tasked with eliminating invading pathogens and cancer cells through the production of specific receptors and antibodies.A variety of inflammatory mediators are synthesized and released during various types of inflammatory responses. Inflammatory compounds are often categorized into two primary groups: pro-inflammatory and anti-inflammatory mediators. Nonetheless, several mediators, like interleukin (IL)-12, exhibit both pro-inflammatory and anti-inflammatory characteristics **(Vignali et al., 2012.)**. Cytokines (e.g., interferons, interleukins, and tumor necrosis factor α), chemokines (e.g., monocyte chemoattractant protein 1), eicosanoids (e.g., prostaglandins and leukotrienes), and the influential inflammation-regulating transcription factor nuclear factor κ B are among the inflammatory mediators and cellular pathways that have been thoroughly investigated in relation to human pathological conditions.

Tumor necrosis factor (TNF)-α is a significant pro-inflammatory cytokine released by many cells, influencing numerous cellular processes. TNF-α has been linked to various pathological conditions in humans, including immunological and inflammatory illnesses, malignancies, and mental disorders, among others. IL-1α is another cytokine that mostly exhibits pro-inflammatory action **(Zelová et al., 2013)**. It induces the release of pro-inflammatory cytokines, including IL-1β and TNF-α **(Rider et al., 2013)**. Nonetheless, IL-1α has also been linked to anti-inflammatory activities. Like IL-1α, IL-6 typically functions as a pro-inflammatory cytokine, although it also exhibits certain anti-inflammatory properties. The IL-12 cytokine family, comprising IL-12, IL-23, IL-27, and IL-35, exhibits both pro-inflammatory and anti-inflammatory properties. Conversely, IL-10 is a powerful anti-inflammatory cytokine that inhibits the function of numerous pro-inflammatory mediators. IL-10 aids in preserving tissue homeostasis and mitigating damage caused by an excessive inflammatory response by diminishing and regulating the inflammatory reaction **(Kwilasz et al., 2015)**.

Prostaglandin (PG) E2 is likely the most extensively researched prostaglandin in relation to human physiological and pathological states **(Goetzl et al., 1995)**. It performs multiple physiological functions, including the management of appropriate body temperature, maintenance of stomach mucosal integrity, modulation of renal blood flow, and support of the female reproductive system's function. Conversely, modifications in PGE2 activity are linked to pathological illnesses like inflammatory diseases, dysregulation of body temperature, and colorectal cancer, among others. The production pathway of prostaglandins commences with the liberation of arachidonic acid from cell membrane phospholipids by phospholipase A2 (PLA2). Arachidonic acid is then transformed to prostaglandins (PGs) by the enzyme cyclooxygenase (COX). Of the three identified COX isoforms (COX-1, COX-2, and COX-3), the inducible enzyme COX-2 is acknowledged as the most active during inflammatory events. Leukotrienes (LTs), including LTB4, have been associated with several human pathological conditions such as inflammation, asthma, and depression. Leukotrienes (LTs) are synthesized by the enzyme 5-lipoxygenase (5-LOX) **(Moncada et**

al., 2006). A further enzyme closely linked to inflammatory situations is nitric oxide synthase (NOS), which generates nitric oxide (NO) **(Oeckinghaus et al., 2011)**. Like COX-2, inducible NOS (iNOS) is the most pro-inflammatory isoform of nitric oxide synthase.

The transcription factor nuclear factor κB (NFκB) is a key regulator of immunological and inflammatory responses and plays a significant role in the pathophysiology of cancer. In mammals, the NFκB complex consists of many components (e.g., p50 and p65) that govern both physiological and pathological processes. Under resting (unstimulated) conditions, NFκB is located in the cytoplasm. Upon activation by diverse infectious, inflammatory, or mitogenic stimuli, NFκB proteins translocate to the nucleus and initiate the transcription of genes linked with inflammation **(Ling et al., 2016.)**.

Phytochemicals from medicinal plants serve as the foundation for treatments of diverse health ailments and represent prospective sources for novel medication research and development **(Bachheti et al., 2019)**. Medicinal plant products continue to be the most accessible and cost-effective medications for primary health care in under developed countries **(Gumisiriza et al., n.d.)**. The phytochemistry of plants primarily facilitates the use of plant products as potential remedies for various illnesses. Due to their negligible side effects, plant-based medicines serve as the principal treatment for various human and livestock disorders and maintain a high level of community acceptance **(Kerdar et al., n.d.)**. Consequently, the utilization of plant-derived natural compounds as alternative therapies for various health conditions is fast escalating **(Gumisiriza et al., n.d.)**.

The inventory list from WHO indicates that over 20,000 species of medicinal plants have been cataloged to date **(Vaou et al., n.d.)**. Certain plant-derived natural compounds are utilized as nutraceuticals **Mbendana et al. (n.d.)**. The understanding of medicinal plants provides prospective secondary metabolites with pharmaceutical applications, with approximately 50% of contemporary medications derived from these substances. Phytochemicals such as phenolic compounds, saponins, proanthocyanidins, nitrogenous compounds, alkaloids, and terpenoids have been documented for their potential pharmacological activities.

2. Experimental:

2.1 Material

Carrageenan was acquired from CDH C, Formaldehyde was acquired from CDH. The Plethysmometer was purchased from IMCORP. The data obtained from various groups were statistically analyzed with GraphPad in Stat version 3.10, using two tailed paired t-test. Values at $p \leq 0.05$ were considered significant.

2.2 Method

All the protocols were in accordance with the guidelines of the Committee for the purpose of control and supervision of experiments on animals, Ministry of Environment and Forests, Government of India. Animals were kept under standard laboratory conditions, at temperature 25 ± 2°C and relative humidity 55 ± 5%. The animals were housed in proper cleaned cages, 6 per cage, with free access to standard laboratory diet and water.

❒ **Acute Toxicity Study**

Acute toxicity studies were carried out according to OECD (The Organization for Economic Co-operation and Development) guidelines **(OECD, 2002)**.

Animals: Male albino Wistar rats are weighing 180-220 g were used for the acute toxicity study.

Administration of doses: ASL as well as ASB dissolved in water were administered in a single dose using an intubation cannula (#18). Food, but not water, was withheld for 4 hours before dosing. After the dose was administered, food was withheld for another 4 hours.

Number of Animals and dose levels: Five animals were used for each group. Four dose levels were selected at 5, 50, 300, and 2000 mg/kg body weight of animals. Then the animals were observed for the first 4 hours after dosing and then every day upto 14 days for any mortality in animals.

Experimental protocol for an acute toxicity study

Each Group Contains 5 animals

Group I: Animals treated with ASL at dose of 5mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).

Group II: Animals treated with ASB at dose of 5mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).

Group III: Animals treated with ASL at dose of 50mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).

Group IV: Animals treated with ASB at dose of 50mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).

Group V: Animals treated with ASL at dose of 300mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).
Group VI: Animals treated with ASB at dose of 300mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).
Group VII: Animals treated with ASL at dose of 2000mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).
Group VIII: Animals treated with ASB at dose of 2000mg/kg body weight orally, in a single dose, on the first day and observed for 14 days (1ml/100g).

Anti-inflammatory study

An anti-inflammatory study was evaluated by the carrageenan-induced hind paw edema method using a plethysmometer in healthy male albino Wistar rats (180-200g). The animals were divided into ten groups, each comprising 6 animals, as shown in table 1.

Table 1: Groups of animals used in an anti-inflammatory study.

Sr.no.	Group no.	Treatment
1.	Group I	Control
2.	Group II	Carrageenan induced animals
3.	Group III	Standard formulation (OMNIGEL contains Linseed oil (3% w/w) + Diclofenac sodium (1% w/w)
4.	Group IV	Placebo formulation
5.	Group V	ASL Extract (0.000025% w/w)
6.	Group VI	ASL Extract (0.00005% w/w)
7.	Group VII	ASL Extract (0.0001% w/w)
8.	Group VIII	ASB Extract (0.000025% w/w)
9.	Group IX	ASB Extract (0.00005% w/w)
10.	Group X	ASB Extract (0.0001% w/w)

Formulations were applied to groups (except group I and II) on hind paw gently before 30 minutes of carrageenan injection. Acute inflammation was produced by injecting 0.1 ml of 1% (w/v) carrageenan suspension in the sub-plantar region of the left hind paw 30 minutes after treatment. Paw volume measurements was made at intervals of 1, 2, 3, 6,12, 24 hours by mercury displacement method using a plethysmometer. The %inhibition of paw edema in formulation treated and carrageenan-induced animals was compared with the control group and calculated according to formulae·

$$\%\textbf{Edema} = \frac{\textbf{Final volume of paw} - \textbf{Initial volume of paw}}{\textbf{Initial volume of paw}} \times 100$$

$$\%\textbf{Inhibition} = \frac{\%\textbf{Edema (Control)} - \%\textbf{Edema (Formulation)}}{\%\textbf{Edema (Control)}} \times 100$$

3. Result and discussion

Both ASL and ASB were found to be non-toxic in Acute toxicity studies ASL and ASB are classified as category 5 substances according to the Globally Harmonized Classification System for chemical substances and mixtures.

The anti-inflammatory effect of *Anogeissus sericea* extract was determined according to the procedure described in the section. A significant reduction ($p \leq 0.05$) in mean % edema was obtained in groups III and V to X compared to group II. Activity of *Anogeissus sericea* extract was found to be concentration-dependent. While anti-inflammatory activity of *Anogeissus sericea* extract of ASL was found to be superior to that of *Anogeissus sericea* extract of ASB. %inhibition in edema of ASL/ASB loaded *Anogeissus sericea* extract was significantly ($p \leq 0.05$) higher compared to control and placebo gel at the end of 24 hours. Therefore, *Anogeissus sericea* extract had significant anti-inflammatory activity. Comparison between the various formulations on mean % edema of rats is shown in Fig.1 while %inhibition of various formulations with time is compared is shown in Fig.2.

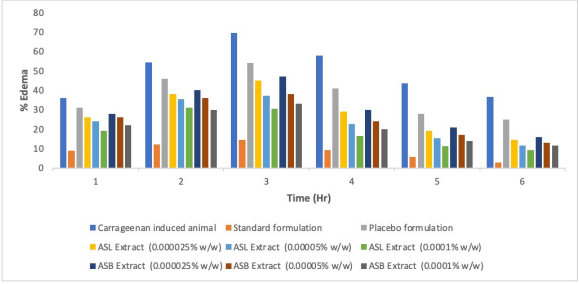

Fig.1: Effects of various formulations on mean % edema of rats (n=6) for 24 hours

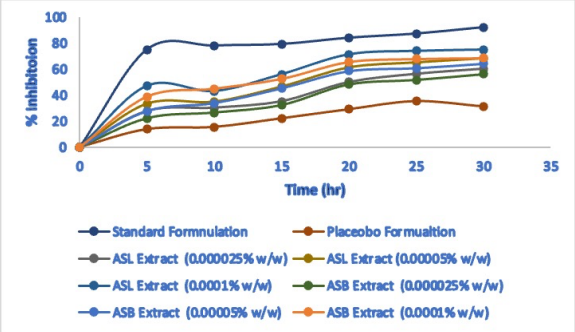

Fig.2: Effect of various formulations on % inhibition v/s time.

CONCLUSION

This work offers robust experimental data endorsing the anti-inflammatory and analgesic properties of *Anogeissus sericea* extract. Inflammation is a multifaceted physiological response governed by several routes and chemical mediators, and its successful treatment continues to provide a significant therapeutic challenge. This study's results unequivocally indicate that both ASL and ASB extracts considerably mitigate inflammation in experimental animal models, exhibiting dose-dependent and statistically significant benefits. ASL is frequently shown greater activity, signifying a higher concentration or potency of the active phytoconstituents responsible for its pharmacological actions. The carrageenan-induced paw edema model validated the anti-inflammatory effectiveness of the formulations, demonstrating a significant decrease in edema in the treated groups relative to the control. The analgesic investigation employing Eddy's hot plate method revealed an elevation in pain threshold, hence corroborating the therapeutic efficacy of these extracts. The observed activities may be ascribed to the suppression of essential inflammatory mediators, including prostaglandins, cytokines, and enzymes such as COX-2 and nitric oxide synthase.

The acute toxicity investigation confirmed the safety of both extracts at elevated doses, indicating a positive therapeutic index. This underscores the benefit of plant-based formulations as safer alternatives to traditional synthetic medications, which frequently entail severe consequences with prolonged usage.

In conclusion, ASL and ASB *Anogeissus sericea* extract are promising, safe, and effective plant-derived therapeutic agents for managing inflammation, with ASL demonstrating significantly larger potential.

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