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An Experimental Study On Phytochemical Screening And Determination Of Anti-Inflammatory Activity Of Terminalia Bellirica Leaves On Animal Model

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ABSTRACT

With globalization, consumer market patterns changed. However, dietary and lifestyle modifications are among their long-term effects. Millions of lives have been saved by medications developed and discovered thanks to research in the chemical and pharmaceutical sciences, but prolonged use of these medications has raised concerns about their safety and toxicological effects. Researchers looked at the plants that were previously utilized in Ayurvedic and Chinese medicine to confirm their traditional uses. Consequently, throughout the past few decades, communities' dependence on complementary and alternative therapies has begun to revive. One such plant, Terminalia bellerica, was known as the "king of medicinal plants" in Ayurveda because of its extensive use in herbal concoctions to treat a variety of health issues. The anti-inflammatory properties of Terminalia bellerica are investigated in this work. Following the collection and testing of the plant material for a number of criteria, an in vivo anti-inflammatory investigation was conducted. After Terminalia bellirica was extracted, the results indicate that while flavonoids, phenols, carbohydrates, and saponins were present, tannin and glycosides were not. Paw edema was

significantly inhibited by the Terminalia bellirica hydroalcoholic extract at doses of 200 and 300 mg/kg. After a 4-hour injection of carrageenan, the maximum percentage of edema inhibition for the Terminalia bellirica hydroalcoholic extract was recorded at 59.19% and 62.48% for oral doses of 200 and 300 mg/kg of the extract. According to the findings, Terminalia bellirica have notable anti-inflammatory properties.

KEY-WORDS: Inflammation, Carrageenan, *Terminalia bellerica*, Glycosides

INTRODUCTION

The word "inflammatory" comes from the Latin term "Inflammare," which meaning "burn." Any type of physical injury can lead to a variety of chemical changes in the affected region. There was formerly a belief that inflammation was a singular illness brought on by abnormalities in bodily fluids. The general consensus holds that inflammation is a normal reaction to an illness or other disturbance. Heat, redness, swelling, discomfort, and loss of functioning are the classic indicators of inflammation. Many processes that may be categorized into three groups—the acute transitory phase, the delayed subacute phase, and the chronic proliferating phase—are often responsible for causing inflammation. Local edema develops in the early stages due to inflammatory exudates brought on by increased vascular permeability. The second phase is characterized by leukocyte and phagocyte migration through blood to vascular tissues, whereas the third phase is characterized by tissue fibrosis and disintegration. Endogenous mediators, including prostaglandins, histamine, serotonin, and bradykinin, are released in response to inflammation.[1]

The process of inflammation is not simple. The first step in the inflammatory cascade is the diagnosis of an illness or damage. Finding pathogen-associated molecular patterns (PAMPs), which are mostly aimed at broad themes of pathogen-expressed molecules necessary for pathogen survival, is one common way to do this. Damage-associated molecular patterns (DAMPs), another name for alarmins, are organic molecules that the innate immune system recognizes as indicators of damage or necrosis. Decreased inadvertent targeting of host cells and organs is one benefit of recognizing these signals. The innate immune system lacks the ability to differentiate between different pathogen strains and assess their toxicity, in contrast to adaptive immunity [2].

The most often prescribed drugs worldwide for the management of both acute and chronic pain associated to inflammation are non-steroidal anti-inflammatory drugs (NSAIDs). One common characteristic of the activities of the NSAID drug family is the reduction of COX activity in the synthesis of prostaglandins and thromboxanes. The main mechanism by which NSAIDs function is by blocking central and peripheral COX, which prevents arachidonic acid from converting to thromboxanes, prostacyclins, and prostaglandin E2. The method that NSAIDs work is largely different from the ways that the COX-1 and COX-2 enzymes act. Numerous cells, including those in the fetus and amniotic fluid, contain COX-1, which is involved in defense and regulation among other physiological processes. On the other hand, COX-2 is stimulated by proinflammatory cytokines and inflammation. Despite the medications' early effectiveness, significant negative effects on the heart, kidneys, and gastrointestinal system have been reported since the introduction of selective COX-2 inhibitors [3,4].

Since ancient times, herbal medicine has been used for medical purposes. Their abundance of therapeutic components, which might aid in the prevention of illnesses and afflictions, has led to their widespread appreciation on a global scale. China and India are rightfully referred to as the "Botanical Garden of the World" since they are the world's top producers of therapeutic plants. India is a special place in the globe since it is the birthplace of several internationally known traditional medical systems, including homeopathy, yoga, naturopathy, Siddha, and Unani. [5]

Terminalia bellerica, the plant known as bibhitaki, is a member of the Combretaceae family. This fruit, called vibheetaki (meaning "fearless" in Sanskrit), calms our fear of being ill. In India, Maharashtra , Madhya Pradesh, Uttar Pradesh, Punjab are the states where it is often noticed. Antispasmodic and bronchodilatory, antifungal, anti-salmonella, antimicrobial, antioxidant, anti-biofilm, anti-ulcer, anti-Alzheimer's, antihypertensive, anti-athrogenic, immune-modulating effect, healing of wounds, antifertility, anti-diarrheal, anti-cancer, and anti-plasmodial are just a few of the pharmacological effects it possesses. [6,7] Therefore, the purpose of this study is to examine Terminalia bellerica's anti-inflammatory properties.

The primary goals of this study are to screen for phytochemicals and evaluate the anti-inflammatory properties of Terminalia bellerica. The extraction procedure seeks to extract the bioactive compounds from

the plant material, while phytochemical screening searches for the presence of various secondary metabolites, including glycosides, alkaloids, flavonoids, tannins, and phenols, which are known to have a range of biological activities. [8] The plant extract's ability to reduce inflammation, which is a significant contributing factor to a number of chronic illnesses, is assessed using animal models.

MATERIALS & METHODS

Gathering of Botanical Specimens

Based on geographical availability, the leaves of plant, Terminalia bellirica, were harvested from a local location in Maharashtra and dried at room temperature after being cleansed with tab water. After the materials had dried, they were crushed and passed through a 20-mesh filter. Until they were required, the powdered medications were stored in sealed containers away from direct sunlight.

Extraction of plant material

The Soxhlet apparatus was filled with the proper amount of air-dried powdered plant material, beginning with petroleum ether and moving on to hydroalcohol (ethanol: water; 75:25) for the powdered leaves of Terminalia bellirica. Each time, the powdered material was air dried below 100°C before being removed and replaced with the next dissolvent substance. The water bath was used to evaporate the extracted solvent at 100°C. The extracted materials were kept refrigerated for further examination following the evaporation.

Table 1: Phytochemicals test[9]

Phytochemical	Test	Procedure
Alkaloids	Dragendroff's Test	The filtrates were subjected to a solution of potassium bismuth iodide, known as Dragendorf's reagent. The presence of alkaloids is shown by the formation of red precipitate.
Glycosides	Legal's Test	Sodium nitropruside was used to treat the extract with pyridine and sodium hydroxide. The presence of cardiac glycosides is indicated by the formation of a pink to blood red color.
Flavonoids	Alkaline Reagent Test	A few drops of sodium hydroxide solution were added to the extract. Flavonoids are indicated by the formation of a bright yellow color that becomes colorless when diluted acid is added.
Saponins	Froth Test	After diluting the extract with 20ml of distilled water, it was agitated for 15 minutes in a graduated cylinder. The presence of saponins is indicated by the formation of a 1 cm layer of foam.
Tannins	Gelatin Test	A 1% sodium chloride-containing gelatin solution was added to the extract. The presence of tannins is shown by the formation of white precipitate.
Phenols	Ferric Chloride Test	Three to four drops of ferric chloride solution were added to the extract. Phenols are present when blue black color begins to form.
Proteins and Amino acids	Xanthoproteic Test	A little amount of concentrated nitric acid was added to the extract. The development of a yellow hue signifies the existence of proteins.
Carbohydrates	Molisch's Test	In a test tube, filters were treated with two drops of an alcoholic α-naphthol solution. The presence of carbohydrates is shown by the formation of the violet ring at the junction.

Animal grouping

As per the protocol, six randomly selected groups (n=6) of mice received subplantar injections of 0.1 mL of λ-carrageenan (1% in NaCl 0.9%) in the right hind paw. Following that, they received an oral dosage (gavage) using the following solutions: Group I consisted of 10 mL/kg of sterile saline 0.9% NaCl; Group II was a

reference medicine consisting of 50 mg/kg of diclofenac sodium; Group III was an extract of Terminalia bellirica leaves at 200 mg/kg; and Group IV was an extract of the same at 300 mg/kg. One hour after the injection of carrageenan, an oral dosage of Terminalia bellirica extract and Diclofenac, both diluted in 0.9% NaCl, were delivered. To eliminate the methanol, the extract was first vacuum-evaporated at room temperature. The deposit was then dissolved in a 0.9% NaCl solution. [10]

RESULTS AND DISCUSSION

After extracting Terminalia bellirica, the results indicate that while flavonoids, phenols, carbohydrates, saponins were present, tannin and glycosides were not. Paw edema was significantly inhibited by the 200 mg/kg and 300 mg/kg hydroalcoholic extract of Terminalia bellirica after two hours of carrageenan treatment. This may be the result of the lower dosage having a more inhibitory impact on prostaglandin and bradykinin production. Following a 4-hour injection of carrageenan, the maximum percentage of edema inhibition for the Terminalia bellirica hydroalcoholic extract was seen at 200 and 300 mg/kg oral doses of the extract, with corresponding values of 59.19% and 62.48%.

Table 2: Preliminary phytochemical tests results of *Terminalia bellirica*

Phytochemical	**Test**	**Result**
Alkaloids	Dragendroff's Test	+ ve
Glycosides	Legal's Test	- ve
Flavonoids	Alkaline Reagent Test	+ ve
Saponins	Froth Test	+ ve
Tannins	Gelatin Test	- ve
Phenols	Ferric Chloride Test	+ ve
Proteins and Amino acids	Xanthoproteic Test	+ ve
Carbohydrates	Molisch's Test	+ ve

Table 3: Anti-inflammatory activity of Hydroalcoholic extract of *Terminalia bellirica* on Carrageenan-induced paws edema

Treatment group	**Dose (mg/kg)**	**The paw volume (ml), mean ± SEM**				
		Basal	**1 h**	**2 h**	**3 h**	**4 h**
Negative Control	Sterile saline 0.9% NaCl	0.282±0.01	0.281 ± 0.008	0.303 ± 0.014	0.304± 0.01	0.314 ± 0.008
Diclofenac sodium	50 mg/kg, p.o	0.268 ± 0.008	0.238 ± 0.004	0.208± .003	0.189±0.007	0.171±0.004
Terminalia bellirica (Extract)	200 mg/kg, p.o	0.294 ± 0.012	0.253 ± 0.008	0.229±0.008	0.200±0.004	0.186± 0.002
Terminalia bellirica (Extract)	300 mg/kg, p.o	0.278 ± 0.012	0.247 ± 0.005	0.209±0.004	0.194±0.005	0.178±0.005

Table 4: Maximum percentage of inhibition of edema for the Hydroalcoholic extract of *Terminalia bellirica* at 100 and 200 mg/kg

Treatment group	**Dose (mg/kg)**	**% inhibition**			
		1 h	**2 h**	**3 h**	**4 h**
Diclofenac sodium	50 mg/kg, p.o	22.98 %	46.12 %	55.75 %	66.23 %
Terminalia bellirica Extract	200 mg/kg, p.o	14.65 %	35.46 %	49.18 %	59.19 %
Terminalia bellirica Extract	300 mg/kg, p.o	17.43 %	48.12 %	53.77 %	62.48 %

CONCLUSION

To sum up, this study set out to examine the anti-inflammatory properties, phytochemical analysis, and extraction process of the Terminalia bellirica extract. The study yielded many noteworthy results that enhanced our comprehension of the plant extract's possible health benefits. The thorough extraction, phytochemical screening, and evaluation of anti-inflammatory activity processes improve our comprehension of Terminalia bellirica's potential medicinal uses. The study emphasizes the significance of conventional

medical herbs as sources of new bioactive chemicals and the necessity for more investigation to completely comprehend these plants' health advantages. The results of this investigation imply that ethanol-based leaf extracts of Terminalia bellirica has anti-inflammatory properties. Paw edoema inhibition has long been recognized as a trustworthy measure of an anti-inflammatory drug's effectiveness. According to the results of the current investigation, *Terminalia bellirica* hydroalcoholic extract exhibits significant anti-inflammatory efficacy.

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