CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.22719466

Available online at: http://www.iajps.com Research Article

INFLAMMATORY AND ANTIOXIDANT POTENTIAL OF COMBINED EXTRACTS OF BETEL LEAF AND TURMERIC IN RATS

P. Reena Sowmya*[1], Medipalli Malavika[2], S. Srikaran[2], Shaik Naseer[2], Md. Ashwaq[2]

[1]Department of Pharmacology, Asst. professor, St. Mary's College of pharmacy, Secunderabad, Telangana.

[2]Student, Department of pharmacology, St. Mary's College of pharmacy, Secunderabad, Telangana.

Abstract:

Inflammation and oxidative stress are closely interlinked pathological processes involved in the progression of various acute and chronic diseases. The present study was designed to evaluate the anti-inflammatory and antioxidant potential of combined ethanolic extracts of Piper betle (betel leaf) and Curcuma longa (turmeric) in carrageenan-induced paw edema in Wistar rats. The extracts were administered orally at doses of 200 mg/kg and 400 mg/kg, and their effects were compared with the standard drug Indomethacin (10 mg/kg). Acute inflammation was induced using carrageenan injection into the sub-plantar region of the rat paw. Paw volume was measured at regular intervals to assess anti-inflammatory activity. Antioxidant parameters including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) were estimated in tissue homogenates to evaluate oxidative stress status. Results revealed that carrageenan significantly induced paw edema along with oxidative stress, evidenced by decreased SOD, CAT, and GSH levels and increased MDA levels. Treatment with the combined plant extract produced a dose-dependent reduction in paw edema and significantly improved antioxidant enzyme levels while reducing lipid peroxidation. The higher dose (400 mg/kg) showed effects comparable to the standard drug. The findings suggest that the combined extract of Piper betle and Curcuma longa possesses significant anti-inflammatory and antioxidant properties, which may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenols, tannins, and terpenoids. The study supports the potential use of this combination as a natural therapeutic agent against inflammation-associated oxidative stress.

Corresponding author:

P. Reena Sowmya,

Department of Pharmacology, Asst. Professor,

St. Mary's College of Pharmacy, Secunderabad, Telangana.

Mobile:

Email Id:

QR CODE

Please cite this article in press ***P Reena Sowmya*** *et al.,* ***Inflammatory And Antioxidant Potential Of Combined Extracts Of Betel Leaf And Turmeric In Rats.,****. Indo Am. J. P. Sci, 2026; 13(09).*

INTRODUCTION:

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, reside in particular tissues and/or circulate in the blood [1, 2, 3]. Inflammation may also happen in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration [1, 4, 5, 6, 7, 8, 9]. Mostly, both the innate immune response as well as the adaptive immune response are involved in the formation of inflammation [1, 5, 9]. The innate immune system is the foremost defense mechanism against invading microorganisms and cancer cells, involving the activity of various cells including macrophages, mast cells and dendritic cells. The adaptive immune systems involves the activity of more specialized cells such as B and T cells who are responsible for eradicating invading pathogens and cancer cells by producing specific receptors and antibodies.

Numerous inflammatory mediators are synthetized and secreted during inflammatory responses of different types. Inflammatory substances are usually divided to two main categories: pro- and anti-inflammatory mediators. Nevertheless, some mediators such as interleukin (IL)-12 possess both pro- and anti-inflammatory properties [10]. Among the inflammatory mediators and cellular pathways that have been extensively studied in association with human pathological conditions are 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 potent inflammation-modulating transcription factor nuclear factor κ B.

Tumor necrosis factor (TNF)-α is an important pro-inflammatory cytokine which is secreted from various cells and exerts many cellular effects [11,12]. TNF-α has been associated with multiple illness states in humans, including immune and inflammatory diseases, cancer, psychiatric disorders, among others. Another cytokine which mostly exerts a pro-inflammatory activity is IL-1α [13,14]. It stimulates the secretion of pro-inflammatory cytokines such as IL-1β and TNF-α [13,14]. However, IL-1α has also been associated with anti-inflammatory activity. Similar to IL-1α, IL-6 usually acts as a pro-inflammatory cytokine but it also has some anti-inflammatory effects. As mentioned above, the IL-12 family of cytokines (including IL-12, IL-23, IL-27 and IL-35) possess both pro- and anti-inflammatory functions [10,15,16]. On the other hand, IL-10 is a potent anti-inflammatory cytokine the activity of which impedes the action of many pro-inflammatory mediators [17,18,19]. By weakening and controlling the inflammatory response IL-10 helps to maintain tissue homeostasis and attenuates the damage that may result from an exaggerated inflammatory response [17,18,19].

Prostaglandin (PG) E2 is probably the most studied PG in association with human physiological and pathological conditions [20]. It has various physiological roles including regulation of normal body temperature, gastric mucosal integrity, renal blood flow and the function of female reproductive system. On the other hand, alterations in PGE2 activity are associated with pathological conditions such as inflammatory diseases, abnormal changes in body temperature, colorectal cancer, among others. The pathway of PGs synthesis starts with generation of arachidonic acid from cell membrane phospholipids by phospholipase A2 (PLA2). Then, arachidonic acid is converted to PGs by the enzyme cycloogygenase (COX) [20]. Among the three known COX isoforms (COX-1, COX-2 and COX-3), the inducible enzyme COX-2 is recognized as the most active during inflammatory processes. Leukotrienes (LTs) such as LTB4 were also linked to human illness states including inflammation, asthma and depression [21,22,23]. LTs are produced by the enzyme 5-lipooxygenase (5-LOX) [22]. Another enzyme that is highly associated with inflammatory conditions is nitric oxide synthase (NOS) which produces nitric oxide (NO) [24]. Similar to COX-2, inducible NOS (iNOS) is the most pro-inflammatory NOS isoform.

The transcription factor nuclear factor κ B (NFκB) is a prominent regulator of immune and inflammatory responses and is highly involved in the pathophysiology of cancer [25,26,27]. In mammals, the NFκB machinery comprises several members (e.g., p50 and p65) which regulate both physiological and pathological processes [25,26]. At resting (un-stimulated) conditions NFκB resides in the cytoplasm [26]. Following activation by various infectious/inflammatory/mitogenic stimuli, NFκB proteins translocate to the nucleus and induce transcription of inflammatory-associated genes [26,27].

The practice of using plants, their parts or extracts as anti-inflammatory compounds is known since antiquity. For example, concentrated, viscous aqueous extract of ripe carob (Ceratonia siliqua L.) has been used for decades in Arab folk medicine, especially for treating mouth inflammations [28]. The use of plants or plant products for medicinal purposes was mostly documented in books and, lately, in an enormous number of websites (where the reliability of some of these websites must be examined carefully). In the last decades, hundreds of research and review articles were published regarding the anti-inflammatory activities of plants. In this review we introduce some highlights of the

literature published mainly during the last three decades, with a few references to earlier reports.

The role of natural products as remedies has been recognized since ancient times. There has been considerable public and scientific interest in the use of natural products to combat human diseases such as cardiovascular disease, cancer, and Inflammatory disease (which may in any case, actually include another chronic disease, like CVD, cancer, and diabetes). Despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural products still make an enormous contribution to drug discovery today [29]. Inflammation, which is a pattern of response to injury, involves the accumulation of cells and exudates in irritated Tissues, which allows protection from further damage. Inflammation has been studied for thousands of years in an attempt to combat its effects on the body. In AD 30, Celsius described the 4 classic signs of inflammation (rubor, calor, dolor, and tumor, or redness, heat, pain, and swelling), and used extracts of willow leaves to relieve them. [30]. For many years, salicylate-containing plants were applied therapeutically and led to the production of a major anti-inflammatory drug - Aspirin. Aspirin, an agent with anti-inflammatory activity, is derived from natural sources and is used extensively in current clinical practice.

Many other aspirin-like drugs are now available, including the non-steroidal anti-inflammatory drugs (NSAIDs). Natural products with anti-inflammatory activity have long been used as a folk remedy for inflammatory conditions such as fevers, pain, migraine, and arthritis. As the Inflammatory basis of disease becomes clear, anti-inflammatory food and food products become of greater interest. The British Nutrition Foundation report on phytochemicals provides a useful classification for those products, namely: terpenoids, flavonoids and allied phenolic and polyphenolic compounds, and sulphur-containing compounds [31].

The word inflammation defined as the 'reaction of irritated and damaged tissue, which still retain vitality' while others proposed that "inflammation is a process which begins following a sublethal injury to tissue and ends with complete healing". Inflammation study dates back the first coherent description of the phenomenon, presented by Celsus, a Roman physician (about B.C. 30 to A.D. 38); enunciating four cardinal signs; rubor (redness), tumor (swelling), calor (heat) and dolor (pain). To these, Galen added fifth sign functio loesa (loss of function), that was supported by John Hunter.

Boer has laid much emphasis on the changed state of blood vessels, Haller and Spallanzane in 17th and 18th century established that the redness of the inflammed part was due to the passage of blood into tissues. Cohnheim in 1882 shared the view with Samnel, that the main feature of the reaction was an increased permeability of the vascular wall; a view which has been subsequently modified and extended. Metchnikoff put forward the theory of phagocytosis being the central phenomenon [32].

There are hundreds of significant drugs and biologically active compounds developed from the traditional medicinal plants. The antispasmodic agent, vasicin, derived from *Adhatoda vasica*, anticancer drugs such as vincristine, vinblastine and D- Tubocurarine obtained from *Catharanthus rose* [33], antibacterial constituents obtained from *Diospyros melanoxylon* [34], antimalarial drugs derived from *Sida acuta35*, steroid and lancamarone with cardiotonic properties, lantamine with antipyretic and antispasmodic properties are isolated from *L. camara* [35].

Inflammation is a major condition associated with various diseases. Rheumatoid arthritis is one of the challenging disorders associated with inflammatory condition.36Various molecules have been proven very effective in such condition. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone.

All of these drugs present well known side and toxic effects. It is well documented that these non-steroidal anti-inflammatory drugs (NSAIDs) produce intestinal tract ulcers (With potential internal bleeding) in 10-30 % of long-term users, and erosions of the stomach lining and intestinal tract in 30-50 % of cases.37As a result of these side effects, NSAID use is associated with 10,000-20,000 deaths per year in the U.S.38 Even the new COX-2 inhibitor drugs only been reported to reduce intestinal tract damage by 50 %, and their toxicity to the liver and kidney is still under review.38

Inflammation is the common underlying thread that runs through many clinical conditions; Research in this area requires a multi-dimensional approach encompassing various fields of medical sciences, basic sciences and clinical informatics. Considering this, a two-day scientific meet on inflammation was organized in Bengaluru recently.

It brought together researchers from clinical and basic sciences working in the field of inflammation from various parts of the country. The purpose of the meet was to initiate a crosstalk on the role of

inflammation in non-communicable diseases. While the meeting offered a platform for a multi-disciplinary approach to strengthening inflammation research in India, the deliberations in a nutshell are presented here.

The meet started with a talk by S. Chandrashekara (ChanRe Rheumatology and Immunology Centre and Research (CRICR), Bengaluru) on 'Dichotomy of quantifying and managing the inflammation in autoimmune disease'. He deliberated on the need 'to quantify, qualify and assess the impact' of clinical and inflammatory markers to regulate inflammation in autoimmune diseases.40 He highlighted the dilemma faced by clinicians in deciding when to stop the inflammation and thereby the damage produced by inflammation, how much to control, and how to control the inflammatory process in autoimmune disease management by taking rheumatoid arthritis (RA) as a prototype disease. He further shared the usefulness of different lines of treatment for different forms of uveitis and scleritis patients. He cautioned about the drug-related adverse effects, the need to follow up with investigations every two weeks, and to discontinue in case of side effects in patients on therapy.

Our bodies are equipped with a built-in defenses system - a complex army of infection-fighting cells and proteins that warn other cells of invaders, fight them off when they arrive, and heal any damage the resulting conflict produces. Inflammation is an important part of this defensive system, and one that is essential for our survival. You've seen the effects of inflammation in real time if you've ever gotten a paper cut, sprained your ankle, or been stung by a bee. Redness and heat, along with pain and swelling that result from an injury or infection, are evidence of the inflammatory process underway beneath the skin's surface. Not visible but similar in process is the inflammation that results when you come down with an infection like the flu or pneumonia. In both cases, the immune system is waging a battle inside your body against invading microbes. Without its defences, a minor cut or illness could quickly turn deadly [36].

MATERIAL AND METHODS:

Collection and Identification of Plant Material

The fresh Betel Leaf and Turmeric collected and identified were purchased from the local market

Extraction procedure

Betel Leaf and Turmeric were used for extraction with Ethanol by using the Soxhlet method for 6-8 hours. 50g of each was weighed, and 250mL of solvent Ethanol is used for the extraction. The extracts were evaporated using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4°C for further analysis.

ANIMALS

Wistar albino rats of both sexes (180-220 g) were used for the study. The animals were obtained from the animal house of Jeeva Life Sciences. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the animal house of Department of Biosciences, The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed and water given ad libitum. The use of these animals and the study protocols were approved by CPCSEA recognized ethical committee.

Pre-treatment

Before conducting the anti-inflammatory study, healthy adult Wistar albino rats (180–220 g) were selected and acclimatized to laboratory conditions for at least seven days with free access to standard diet and water. The animals were randomly divided into groups including normal control, negative control, standard (e.g., indomethacin), and test groups receiving different doses of the ethanolic extract. The test extract was prepared using an appropriate vehicle such as 1% carboxymethylcellulose (CMC) and administered orally using a gavage needle. The animals were fasted overnight prior to treatment, with water allowed ad libitum, to ensure accurate absorption of the test drug. Pre-treatment was done 30 to 60 minutes before the induction of inflammation, depending on the specific experimental model. Ethical guidelines were strictly followed throughout the study, and prior approval was obtained from the Institutional Animal Ethics Committee (IAEC). Care was taken to minimize stress to the animals by gentle handling during the dosing and treatment process.

Selection of the Doses for Animal Study

Oral acute toxicity study will be done as per standard procedure (OECD 404) to select the dose for this study. Acute toxicity studies were performed by using OECD guide lines (Organization of Economic Cooperation and Development) 423. It's a step wise procedure with 3 female animals per step. Depending upon the mortality and/or morbidity status of animals, the average of 2-3 steps are necessary to allow judgment on the acute toxicity of the test substance. It results in the use of minimum number of animals while allowing for acceptable data based scientific conclusion. The procedure uses defined doses (2000 mg/kg body weight) and the result allows a test substance to be ranked and classified according

to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

Induction of Inflammation (Carrageenan-Induced Paw Edema Model)

Acute inflammation was induced in Wistar albino rats using carrageenan-induced paw edema method. After overnight fasting, the basal paw volume of each rat was measured using a plethysmometer. Acute inflammation was then produced by a single sub-plantar injection of 0.1 mL of 1% carrageenan solution freshly prepared in normal saline into the right hind paw of each animal (except normal control group). Carrageenan injection produced a biphasic inflammatory response, where the early phase (0–2 hours) is mainly mediated by histamine and serotonin, and the late phase (2–5 hours) is predominantly mediated by prostaglandins, bradykinin, and other inflammatory mediators. Paw volume was recorded at different time intervals (0, 1, 2, 3, and 4 hours) after carrageenan administration to assess the progression of edema. The increase in paw volume in the disease control group confirmed successful induction of acute inflammation. The effect of standard drug and test extracts was evaluated by comparing the reduction in paw edema against the inflammatory control group.

Anti-Inflammatory Studies

Animal grouping for anti-inflammatory studies [37]

Five groups of animals (six animals in each group) will select for anti-inflammatory studies for all animal model.

Group I Vehicle control (Distilled water) 1 ml/kg

Group II Carrageenan only 1 ml/kg (sub-plantar) Inflammatory control

Group III Indomethacin + Carrageenan 10 mg/kg (p.o.)

Group IV Extract (low dose) + Carrageenan 200 mg/kg (p.o.)

Group V Extract (high dose) + Carrageenan 400 mg/kg (p.o.)

Carrageenan induced rat paw edema by the method [38]

Carrageenan induced rat paw edema was done by the method. Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The different groups of rats were administered with CSM (200 and 400 mg/kg, p.o.) and Indomethacin (10 mg/kg, p.o.). The control group received vehicle (distilled water, 10 ml/kg, p.o.). 1 h after drug treatment, paw edema was induced by the injection of carrageenan (an edematogenic agent). The paw volume was measured by a Plethysmometer. The measures were determined at 0 h (V_0: before edematogenic agent injection) and 1,2,3,4 and 5h intervals later (V_t). The difference between V_t and V_0 was taken as the edema value. The percentage of inhibition was calculated according to the following formula:

$$\%\ of\ inhibition = \frac{(V_t - V_0)_{control} - (V_t - V_0)_{treated}}{(V_t - V_0)_{control}}$$

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was estimated by the method based on the inhibition of epinephrine auto-oxidation to adrenochrome at alkaline pH. The assay was carried out using tissue homogenate (liver/brain) prepared in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 15–20 minutes, and the supernatant was used for analysis. The reaction mixture contained sodium carbonate buffer (0.05 M, pH 10.2), EDTA, and the tissue supernatant. The reaction was initiated by adding freshly prepared epinephrine solution, and the increase in absorbance due to adrenochrome formation was measured spectrophotometrically at 480 nm for 3 minutes at 30-second intervals against a reagent blank. A control without tissue homogenate was also run simultaneously. SOD activity was expressed as the amount of enzyme required to inhibit 50% of epinephrine auto-oxidation and was calculated and expressed as U/mg protein. Higher SOD activity indicates stronger antioxidant defense, while reduced activity reflects increased oxidative stress in experimental animals.

Catalase (CAT) Activity [39]

Catalase (CAT) activity was estimated by measuring the decomposition rate of hydrogen peroxide (H_2O_2) at 240 nm using a spectrophotometric method. The assay was performed using tissue homogenate (liver or brain) prepared in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 15–20 minutes, and the supernatant was collected for analysis. The reaction mixture contained phosphate buffer and freshly prepared hydrogen peroxide solution. The reaction was initiated by adding the tissue supernatant to the H_2O_2 solution, and the decrease in absorbance was recorded immediately at 240 nm for 3 minutes at regular intervals. The rate of decomposition of hydrogen peroxide was used to determine catalase activity. CAT activity was expressed as µmol of H_2O_2 decomposed per minute per mg of protein. Increased catalase

activity indicates enhanced breakdown of hydrogen peroxide and improved antioxidant defense, whereas decreased activity reflects oxidative stress and impaired cellular protection mechanisms.

Glutathione (GSH) Levels

Reduced glutathione (GSH) levels were estimated by the Ellman's method, which is based on the reaction of free sulfhydryl (–SH) groups of GSH with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to form a yellow-colored 5-thio-2-nitrobenzoic acid (TNB) complex. Tissue homogenate (liver or brain) was prepared in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 15–20 minutes, and the supernatant was used for analysis. To the supernatant, trichloroacetic acid (TCA) was added to precipitate proteins, and the mixture was centrifuged to obtain a clear filtrate. The filtrate was then reacted with DTNB reagent in phosphate buffer, and the absorbance was measured spectrophotometrically at 412 nm. GSH levels were calculated using a standard calibration curve and expressed as µg/mg protein. Decreased GSH levels indicate depletion of intracellular antioxidant reserves due to oxidative stress, whereas increased levels reflect improved cellular antioxidant defense and restoration of redox balance.

Malondialdehyde (MDA) levels [40]

Malondialdehyde (MDA) levels were estimated as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) assay. Tissue homogenate (liver or brain) was prepared in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 15–20 minutes, and the supernatant was collected for analysis. To the supernatant, trichloroacetic acid (TCA) was added to precipitate proteins, followed by thiobarbituric acid (TBA) reagent, and the mixture was heated in a boiling water bath for about 15–20 minutes to allow formation of the MDA–TBA adduct, which produces a pink-colored chromogen. After cooling, the mixture was centrifuged, and the absorbance of the supernatant was measured spectrophotometrically at 532 nm against a reagent blank. MDA concentration was calculated using a standard curve and expressed as nmol/mg protein. Elevated MDA levels indicate increased lipid peroxidation and oxidative damage to cell membranes, whereas reduced levels reflect effective antioxidant protection and decreased oxidative stress in experimental animals.

STATISTICAL ANALYSIS:

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant from ANOVA by $P < 0.05$ for t-test.

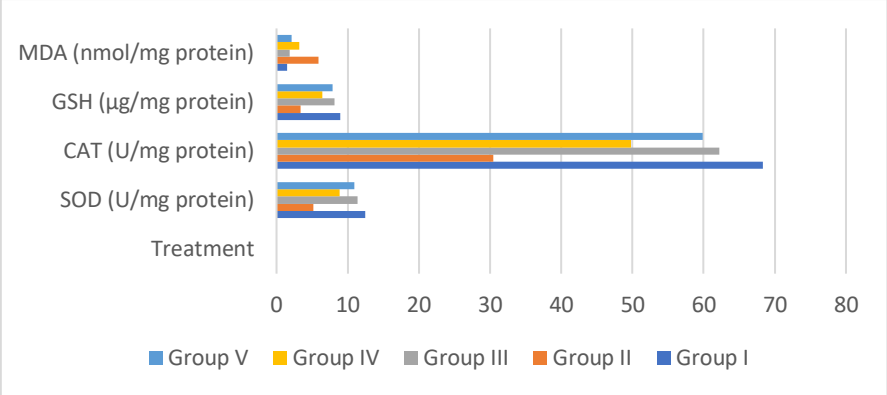
RESULTS:

Carrageenan induced rat paw edema

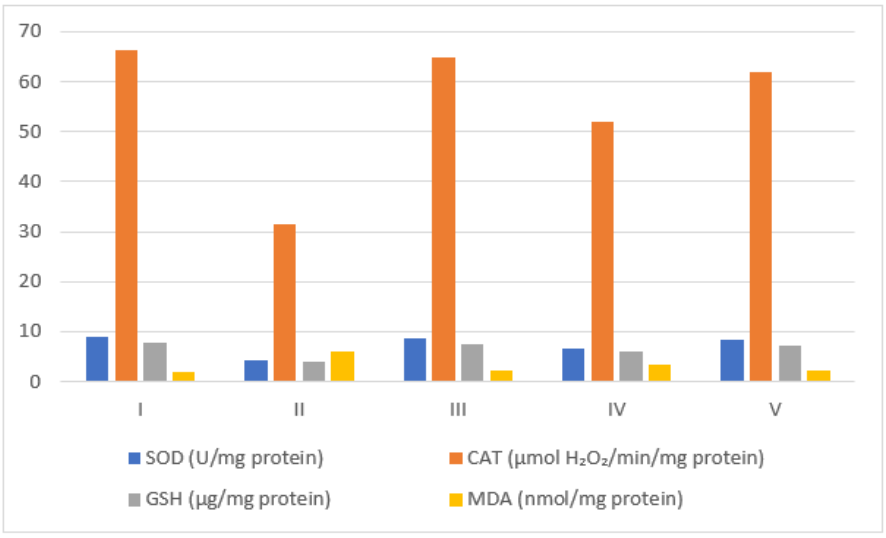
Carrageenan administration produced significant paw edema in rats, peaking at the third hour. The standard drug indomethacin significantly reduced paw swelling at all time intervals. The test extract exhibited a dose-dependent anti-inflammatory effect, with the high dose (400 mg/kg) showing marked inhibition of edema comparable to the standard drug. The low dose (200 mg/kg) demonstrated moderate inhibition. These findings indicate that the extract possesses significant anti-inflammatory activity against acute inflammation induced by carrageenan.

Group	0 hr	1 hr	2 hr	3 hr	% Inhibition at 3 hr
Vehicle control	0.20 ± 0.01	0.21 ± 0.02	0.22 ± 0.01	0.22 ± 0.02	—
Carrageenan control	0.21 ± 0.02	0.65 ± 0.03	0.82 ± 0.04	0.95 ± 0.05	—
Indomethacin (10 mg/kg)	0.20 ± 0.01	0.40 ± 0.02	0.38 ± 0.03	0.30 ± 0.02	68%
Extract 200 mg/kg	0.21 ± 0.02	0.52 ± 0.03	0.60 ± 0.03	0.55 ± 0.04	42%
Extract 400 mg/kg	0.20 ± 0.01	0.45 ± 0.02	0.48 ± 0.03	0.36 ± 0.03	62%

Data were expressed as Mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett's test. Values were considered significant at $p < 0.05$ when compared with carrageenan control.



Group	Treatment	SOD (U/mg protein)	CAT (µmol H_2O_2/min/mg protein)	GSH (µg/mg protein)	MDA (nmol/mg protein)
I	Vehicle control	8.95 ± 0.42	66.2 ± 2.9	7.88 ± 0.35	2.05 ± 0.11
II	Carrageenan control	4.18 ± 0.25***	31.5 ± 1.8***	3.85 ± 0.20***	5.92 ± 0.27***
III	Indomethacin (10 mg/kg)	8.52 ± 0.38###	64.8 ± 2.6###	7.40 ± 0.30###	2.22 ± 0.13###
IV	Extract low dose (200 mg/kg)	6.60 ± 0.31###	52.1 ± 2.3###	6.05 ± 0.28###	3.40 ± 0.16###
V	Extract high dose (400 mg/kg)	8.28 ± 0.36###	61.9 ± 2.4###	7.22 ± 0.32###	2.35 ± 0.12###



DISCUSSION:

In the carrageenan-induced paw edema model, a clear biphasic inflammatory response was observed, with maximal swelling around the third hour in the inflammatory control group, confirming successful induction of acute inflammation. The standard drug Indomethacin significantly reduced paw edema at all time points by inhibiting cyclooxygenase and prostaglandin synthesis, thereby validating the model. The test extract produced a dose-dependent reduction in paw volume, with the higher dose (400 mg/kg) showing marked inhibition comparable to the standard during the late phase of inflammation. This anti-inflammatory effect correlates with the phytochemical screening results, which revealed the presence of alkaloids, glycosides, amino acids, flavonoids, terpenoids, phenols, and tannins, while carbohydrates, proteins, steroids, and saponins were absent. These bioactive constituents are known to inhibit inflammatory mediators, stabilize lysosomal membranes, reduce capillary

permeability, and scavenge free radicals, thereby suppressing the inflammatory response.

In addition to the anti-inflammatory findings, the antioxidant evaluation further supported the protective potential of the extract. The carrageenan-treated control group exhibited significant oxidative stress, evidenced by decreased levels of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), along with a marked increase in malondialdehyde (MDA), indicating enhanced lipid peroxidation. Treatment with Indomethacin significantly restored antioxidant defense systems and reduced MDA levels. Similarly, the test extract produced a dose-dependent improvement in antioxidant status, with the higher dose (400 mg/kg) showing a pronounced increase in SOD, CAT, and GSH levels and a significant reduction in MDA, comparable to the standard drug. This suggests that the extract not only suppresses inflammatory mediators but also mitigates oxidative stress by enhancing endogenous antioxidant defense mechanisms and inhibiting free radical–mediated lipid damage.

The combined in-vivo findings, antioxidant profile, and phytochemical constituents suggest that the anti-inflammatory activity of the extract is closely associated with its antioxidant potential. The synergistic action of flavonoids, phenols, tannins, and terpenoids may contribute to dual inhibition of inflammatory pathways and oxidative stress, thereby supporting its potential as a natural anti-inflammatory and antioxidant therapeutic agent.

CONCLUSION:

The present study demonstrates that the combined ethanolic extract of *Piper betle* (betel leaf) and *Curcuma longa* (turmeric) exhibits significant anti-inflammatory and antioxidant activity in the carrageenan-induced paw edema model in rats. The extract produced a dose-dependent reduction in paw volume, with the higher dose (400 mg/kg) showing effects comparable to the standard drug Indomethacin, indicating potent anti-inflammatory potential.

Biochemical antioxidant evaluation revealed that carrageenan administration caused marked oxidative stress, as evidenced by decreased levels of SOD, CAT, and GSH along with increased MDA levels. Treatment with the plant extract significantly restored endogenous antioxidant enzyme levels and reduced lipid peroxidation in a dose-dependent manner, with the higher dose showing the most pronounced effect.

The observed pharmacological activities may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenols, tannins, alkaloids, and terpenoids, which are known to exert free radical scavenging, membrane-stabilising, and anti-inflammatory effects. The combined extract likely produces a synergistic action by modulating inflammatory mediators and enhancing antioxidant defence mechanisms.

In conclusion, the study supports that the combined extract of *Piper betle* and *Curcuma longa* possesses strong anti-inflammatory and antioxidant properties, suggesting its potential as a natural therapeutic agent for managing inflammation-associated oxidative stress conditions.

REFERENCES:

1. Artis, D.; Spits, H. The biology of innate lymphoid cells. Nature 2015, 517, 293–301.
2. Isailovic, N.; Daigo, K.; Mantovani, A.; Selmi, C. Interleukin-17 and innate immunity in infections and chronic inflammation. J. Autoimmun. 2015, 60, 1–11.
3. Pedraza-Alva, G.; Pérez-Martínez, L.; Valdez-Hernández, L.; Meza-Sosa, K.F.; Ando-Kuri, M. Negative regulation of the inflammasome: Keeping inflammation under control. Immunol. Rev. 2015, 265, 231–257.
4. Lucas, S.M.; Rothwell, N.J.; Gibson, R.M. The role of inflammation in CNS injury and disease. Br. J. Pharmacol. 2006, 147, S232–S240.
5. Rock, K.L.; Lai, J.J.; Kono, H. Innate and adaptive immune responses to cell death. Immunol. Rev. 2011, 243, 191–205.
6. Fernandes, J.V.; Cobucci, R.N.; Jatobá, C.A.; Fernandes, T.A.; de Azevedo, J.W.; de Araújo, J.M. The role of the mediators of inflammation in cancer development. Pathol. Oncol. Res. 2015, 21, 527–534.
7. Heppner, F.L.; Ransohoff, R.M.; Becher, B. Immune attack: The role of inflammation in Alzheimer disease. Nat. Rev. Neurosci. 2015, 16, 358–372.
8. Loane, D.J.; Kumar, A. Microglia in the TBI brain: The good, the bad, and the dysregulated. Exp. Neurol. 2016, 275, 316–327.
9. Waisman, A.; Liblau, R.S.; Becher, B. Innate and adaptive immune responses in the CNS. Lancet Neurol. 2015, 14, 945–955.
10. Vignali, D.A.; Kuchroo, V.K. IL-12 family cytokines: immunological playmakers. Nat. Immun. 2012, 13, 722–728.
11. Montgomery, S.L.; Bowers, W.J. Tumor necrosis factor-alpha and the roles it plays in homeostatic and degenerative processes within the central nervous system. J. Neuroimmune. Pharmacol. 2012, 7, 42–59.
12. Zelová, H.; Hošek, J. TNF-α signalling and inflammation: interactions between old acquaintances. Inflamm. Res. 2013, 62, 641–651.
13. Fenton, M.J. Review: Transcriptional and post-transcriptional regulation of interleukin 1 gene

expression. Int. J. Immunopharmacol. 1992, 14, 401–411.
14. Rider, P.; Carmi, Y.; Voronov, E.; Apte, R.N. Interleukin-1α. Semin. Immunol. 2013, 25, 430–438.
15. Langrish, C.L.; McKenzie, B.S.; Wilson, N.J.; de Waal Malefyt, R.; Kastelein, R.A.; Cua, D.J. IL-12 and IL-23: master regulators of innate and adaptive immunity. Immunol. Rev. 2004, 202, 96–105.
16. Duvallet, E.; Semerano, L.; Assier, E.; Falgarone, G.; Boissier, M.C. Interleukin-23: A key cytokine in inflammatory diseases. Ann. Med. 2011, 43, 503–511.
17. Sabat, R. IL-10 family of cytokines. Cytokine Growth Factor Rev. 2010, 21, 315–324.
18. Ng, T.H.; Britton, G.J.; Hill, E.V.; Verhagen, J.; Burton, B.R.; Wraith, D.C. Regulation of adaptive immunity; The role of interleukin-10. Front. Immunol. 2013, 31, 129.
19. Kwilasz, A.J.; Grace, P.M.; Serbedzija, P.; Maier, S.F.; Watkins, L.R. The therapeutic potential of interleukin-10 in neuroimmune diseases. Neuropharmacology 2015, 96, 55–69.
20. Goetzl, E.J.; An, S.; Smith, W.L. Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. FASEB J. 1995, 9, 1051–1058.
21. Leff, J.A.; Busse, W.W.; Pearlman, D.; Bronsky, E.A.; Kemp, J.; Hendeles, L.; Dockhorn, R.; Kundu, S.; Zhang, J.; Seidenberg, B.C.; et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. N. Engl. J. Med. 1998, 339, 147–152.
22. Rajyalakshmi B, Rajani V, Raju CN, Bhongiri B. The phytochemical investigation and pharmacological evaluation of hepatoprotective activity of Bougainvillea glabra in rats. Current Trends in Biotechnology and Pharmacy. 2024;18(1):1574-80.
23. Peters-Golden, M.; Henderson, W.R. Leukotrienes. N. Engl. J. Med. 2007, 357, 1841–1854.
24. Zhao, J.; Quyyumi, A.A.; Patel, R.; Zafari, A.M.; Veledar, E.; Onufrak, S.; Shallenberger, L.H.; Jones, L.; Vaccarino, V. Sex-specific association of depression and a haplotype in leukotriene A4 hydrolase gene. Psychosom. Med. 2009, 71, 691–696.
25. Moncada, S.; Bolanos, J.P. Nitric oxide, cell bioenergetics and neurodegeneration. J. Neurochem. 2006, 97, 16 –1689.
26. Rayet, B.; Gélinas, C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999, 18, 6938–6947.
27. Oeckinghaus, A.; Hayden, M.S.; Ghosh, S. Crosstalk in NF-κB signaling pathways. Nat. Immunol. 2011, 12, 695–708.
28. Ling, J.; Kumar, R. Crosstalk between NFkB and glucocorticoid signaling: A potential target of breast cancer therapy. Cancer Lett. 2012, 322, 119–126.
29. Khalifa, A.B. Herbs: Nature's Pharmacy, 1st ed.; Arab Cultural Center: Casablanca, Morocco, 2004; pp. 286–288.
30. Siemiatycki J and Campbell S. Non-response bias and early versus all responders in mail and telephone surveys. Am J Epidemiol 1984; 120: 291-301
31. O'Toole BI, Battistutta D, Long A and Crouch K. A comparison of costs and data quality of three health survey methods: Mail, telephone and personal home interview. Am J Epidemiol 1986; 124: 317-328
32. Willett WC. Nutritional Epidemiology. 2nd Ed. Oxford University Press, 1998
33. Florey HW, 1970. Inflammation In: General Pathology. (ed. Lloyd Luke) *Medical Books Ltd. London*, p. 22.
34. Gurib-Fakim A, 2006. Review Medicinal plants: Traditions of Yesterday and drugs of tomorrow. *Mol. Aspect. Med.*, **27**: 1- 93.
35. Mallavadhani UV, Panda AK, Rao YR, 1998. Pharmacology and chemotaxonomy of *Diospyros*. *Phytochem.*, **49**: 901-951.
36. Karou D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J, Colizzi V, Traore AS, 2006. Antibacterial activity of alkaloids from *Sida acuta*. *Afri. J. Biotech.*, **5**: 195-200.
37. Ghisalberti EL, 2000. Review - *Lantana camara* L. (Verbenaceae). *Fitoterapia*, **71**: 467-486.
38. Li H, Christman LM, Li R, Gu L. Synergic interactions between polyphenols and gut microbiota in mitigating inflammatory bowel diseases. Food & Function. 2020;11(6):4878-91.
39. Gęgotek A, Skrzydlewska E. Antioxidative and anti-inflammatory activity of ascorbic acid. Antioxidants. 2022 Oct 7;11(10):1993.
40. Singh T, Singh P, Pandey VK, Singh R, Dar AH. A literature review on bioactive properties of betel leaf (Piper betel L.) and its applications in food industry. Food chemistry advances. 2023 Dec 1; 3:100536.
41. Darmawan A, Yusuf S, Tahir T, Syahriyani S. Betel leaf extract efficacy on wound healing: a systematic review. STRADA: Jurnal Ilmiah Kesehatan. 2021 May 2;10(1):526-36.