(J I D P T S)
ISSN(O):2581-6934

ASSESSMENT OF CAYENNE PEPPER ANTI - INFLAMMATORY PROPERTIES IN RATS.

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Abstract: Inflammation is a complex biological response to harmful stimuli such as infection, injury, and irritants, often associated with tissue damage and metabolic disturbances when excessive or prolonged. The present study was designed to evaluate the effects of Cayenne pepper (Capsicum annuum) extract on experimentally induced acute inflammation in rats. The anti-inflammatory potential was assessed using carrageenan-, histamine-, and dextran-induced rat paw edema models. Rats were divided into five groups: normal control, inflammation control, Cayenne pepper extract-treated groups (200 mg/kg and 400 mg/kg, p.o.), and a standard group treated with diclofenac sodium (10 mg/kg, p.o.). Paw volume was measured at different time intervals, and serum biochemical parameters including total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analyzed to assess systemic effects. The results showed that Carrageenan, histamine, and dextran administration produced a significant increase in paw edema compared to the normal control group. Treatment with Cayenne pepper extract significantly reduced paw swelling in a dose-dependent manner, with the higher dose showing greater efficacy and comparable effects to diclofenac sodium. Biochemical analysis revealed that inflammation caused a significant decrease in total protein and albumin levels, along with elevated ALP, AST, and ALT activities. Treatment with the extract significantly restored these parameters toward normal values, indicating protection against tissue injury and metabolic imbalance. The observed effects may be attributed to bioactive constituents such as capsaicin, flavonoids, and phenolic compounds present in the extract. In conclusion, Cayenne pepper (Capsicum annuum) extract demonstrated significant protective effects against experimentally induced inflammation and associated biochemical alterations in rats. These findings suggest its potential as a natural agent for managing inflammatory conditions, warranting further investigation into its active components and mechanisms of action.

Keywords: Capsicum annuum, Cayenne pepper, inflammation, carrageenan, histamine, dextran, biochemical parameters, diclofenac.

INTRODUCTION

Inflammation is a complex biological response of the body's vascular tissues to harmful stimuli such as pathogens, damaged cells, toxic compounds, and physical injury. It is an essential component of the innate immune system and serves as a protective mechanism designed to eliminate the initial cause of cell injury, remove necrotic tissues, and initiate the healing process. Without inflammation, infections and injuries would remain untreated, leading to severe tissue damage and impaired survival. The inflammatory response involves a highly coordinated interaction among immune cells, blood vessels, and various molecular mediators. When tissue injury occurs, resident immune cells such as macrophages, mast cells, and dendritic cells recognize harmful stimuli and release chemical mediators that trigger the inflammatory cascade. These mediators help recruit additional immune cells to the affected area and facilitate tissue repair. One of the earliest events in inflammation is vasodilation, which increases blood flow to the site of injury. This increased blood flow is responsible for the redness and warmth observed in inflamed tissues. Simultaneously, blood vessels become more permeable, allowing plasma proteins and leukocytes to move from the

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bloodstream into the damaged tissue. This process contributes to swelling and provides essential components for host defense. Leukocyte migration is a critical step in the inflammatory process. Neutrophils are usually the first cells to arrive at the site of injury during acute inflammation. They are followed by monocytes and macrophages, which engulf and destroy microorganisms, cellular debris, and foreign particles through the process of phagocytosis. These cells also release cytokines and growth factors that regulate the inflammatory response and promote tissue healing. Inflammation is characterized by five cardinal signs: redness (rubor), heat (calor), swelling (tumor), pain (dolor), and loss of function (functio laesa). These signs result from vascular changes, fluid accumulation, and the action of inflammatory mediators on sensory nerves. While these manifestations may cause discomfort, they represent the body's attempt to contain and eliminate harmful agents [1-3].

Based on duration and characteristics, inflammation is classified into acute and chronic forms. Acute inflammation develops rapidly and lasts for a short period, usually ranging from a few hours to several days. It is characterized by the accumulation of neutrophils and the formation of inflammatory exudates. Acute inflammation generally resolves once the offending stimulus is eliminated and tissue repair begins. Chronic inflammation occurs when the inflammatory response persists for weeks, months, or even years. It is characterized by the presence of macrophages, lymphocytes, and plasma cells, along with ongoing tissue destruction and repair. Chronic inflammation may result from persistent infections, autoimmune diseases, prolonged exposure to toxic agents, or unresolved acute inflammation. Conditions such as rheumatoid arthritis, inflammatory bowel disease, and tuberculosis are examples of chronic inflammatory disorders. Numerous chemical mediators regulate inflammation, including histamine, prostaglandins, leukotrienes, cytokines, nitric oxide, and bradykinin. These substances coordinate vascular changes, leukocyte recruitment, pain sensation, and tissue repair. Advances in understanding these mediators have led to the development of anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and biologic agents that target specific inflammatory pathways. Although inflammation is primarily beneficial and necessary for survival, excessive or uncontrolled inflammation can contribute to the development of many diseases. Chronic inflammatory processes have been implicated in cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, cancer, and metabolic syndrome. Therefore, a thorough understanding of inflammatory mechanisms is essential for the prevention, diagnosis, and treatment of a wide range of pathological conditions and remains a major focus of biomedical research [4-6].

Inflammation and Its Mediators

Inflammation is a complex biological response that occurs when the body is exposed to harmful stimuli such as bacterial, viral, or fungal infections. It may also arise as a consequence of tissue injury, cellular damage, ischemia, cancer, degeneration, or necrosis. The inflammatory process serves as a protective mechanism aimed at eliminating injurious agents, removing damaged tissues, and initiating tissue repair. Both the innate and adaptive immune systems contribute significantly to the development and regulation of inflammatory responses. The innate immune system represents the first line of defense and involves immune cells such as macrophages, mast cells, neutrophils, and dendritic cells, which recognize and respond rapidly to invading pathogens. In contrast, the adaptive immune system consists of specialized lymphocytes, including B cells and T cells, which provide targeted immune responses through the production of antibodies and antigen-specific receptors. During inflammation, a wide variety of chemical mediators are synthesized and released by activated immune and non-immune cells. These mediators can be broadly classified into pro-inflammatory and anti-inflammatory molecules, although some exhibit dual functions depending on the physiological context. Among the most important inflammatory mediators are cytokines, chemokines, eicosanoids, reactive nitrogen species, and transcription factors. These substances coordinate the recruitment and activation of immune cells, regulate vascular permeability, and influence tissue repair processes. Cytokines play a central role in the initiation and progression of inflammation. Tumor necrosis factor-alpha (TNF-α) is one of the most potent pro-inflammatory cytokines and is produced primarily by activated macrophages, lymphocytes, and other immune cells. TNF-α promotes leukocyte recruitment, stimulates the production of additional inflammatory mediators, and has been implicated in various pathological conditions,

including autoimmune diseases, chronic inflammatory disorders, cancer, and neuropsychiatric illnesses. Interleukin-1 alpha (IL-1α) is another important cytokine that enhances inflammatory responses by stimulating the release of TNF-α and IL-1β. Similarly, interleukin-6 (IL-6) functions predominantly as a pro-inflammatory cytokine, although it can also exert anti-inflammatory effects under certain conditions. Members of the interleukin-12 family, including IL-12, IL-23, IL-27, and IL-35, display both pro-inflammatory and immunoregulatory activities. In contrast, interleukin-10 (IL-10) acts as a potent anti-inflammatory cytokine that suppresses the production of inflammatory mediators and limits excessive immune responses, thereby maintaining tissue homeostasis and preventing collateral tissue damage. Among lipid-derived inflammatory mediators, prostaglandin E_2 (PGE_2) has been extensively investigated due to its involvement in both physiological and pathological processes. Under normal conditions, PGE_2 contributes to the regulation of body temperature, gastric mucosal protection, renal function, and reproductive physiology. However, excessive production of PGE_2 is associated with inflammatory diseases, fever, pain, and various cancers. The synthesis of prostaglandins begins with the release of arachidonic acid from membrane phospholipids by phospholipase A_2, followed by its conversion into prostaglandins through the action of cyclooxygenase enzymes. Among the cyclooxygenase isoforms, cyclooxygenase-2 (COX-2) is particularly important during inflammatory conditions due to its inducible nature and increased expression at sites of inflammation [7-8]. Leukotrienes are another class of arachidonic acid metabolites that contribute significantly to inflammatory responses. These compounds are synthesized through the action of the enzyme 5-lipoxygenase and are involved in leukocyte recruitment, bronchoconstriction, and vascular permeability. Leukotriene B_4 (LTB_4) is recognized as a potent chemotactic factor and has been implicated in inflammatory disorders, asthma, and other chronic diseases. In addition, nitric oxide (NO), generated by nitric oxide synthase enzymes, plays a critical role in inflammation. Inducible nitric oxide synthase (iNOS) is particularly associated with inflammatory conditions and contributes to tissue injury when produced in excessive amounts. The transcription factor nuclear factor-kappa B (NF-κB) serves as a master regulator of inflammatory and immune responses. Under resting conditions, NF-κB remains inactive in the cytoplasm through its association with inhibitory proteins. Upon stimulation by pathogens, cytokines, oxidative stress, or other inflammatory signals, NF-κB translocates to the nucleus, where it activates the expression of numerous genes involved in inflammation, immunity, cell proliferation, and survival. Dysregulation of NF-κB signaling has been linked to chronic inflammatory diseases, autoimmune disorders, and cancer [9].

Natural Products and Anti-Inflammatory Therapy

The use of medicinal plants and plant-derived preparations for the treatment of inflammatory conditions dates back thousands of years. Traditional systems of medicine across different cultures have relied extensively on herbal remedies to alleviate symptoms associated with inflammation, pain, fever, and tissue injury. For example, extracts obtained from the carob plant (Ceratonia siliqua L.) have historically been used in traditional Arab medicine for the treatment of oral inflammatory disorders. Over the past several decades, scientific investigations have provided substantial evidence supporting the anti-inflammatory potential of numerous medicinal plants and their bioactive constituents. Natural products continue to play a pivotal role in modern drug discovery and development. Despite remarkable advances in synthetic and combinatorial chemistry, many clinically important drugs are either derived directly from natural sources or inspired by naturally occurring compounds. Inflammation is now recognized as a contributing factor in numerous chronic diseases, including cardiovascular disorders, diabetes mellitus, cancer, and neurodegenerative diseases, further increasing interest in natural anti-inflammatory agents. Historically, the anti-inflammatory properties of willow bark led to the discovery of salicylic acid and subsequently aspirin, one of the most widely used anti-inflammatory drugs worldwide. Today, a variety of non-steroidal anti-inflammatory drugs (NSAIDs) are available for the management of inflammatory conditions. Nevertheless, concerns regarding adverse effects associated with long-term NSAID use have stimulated renewed interest in safer alternatives from natural sources. Numerous phytochemicals, including terpenoids, flavonoids, polyphenols, and sulfur-containing compounds, exhibit significant anti-inflammatory activities by modulating inflammatory mediators, inhibiting oxidative stress, and

regulating intracellular signaling pathways. Consequently, medicinal plants and dietary phytochemicals remain promising sources for the development of novel anti-inflammatory therapeutics [10-12].

Causes of Inflammation

Inflammation is a protective response of the body that can be triggered by a wide range of harmful stimuli. The most common causes include infections caused by bacteria, viruses, fungi, and parasites, which activate the immune system to eliminate invading pathogens. Physical injuries such as trauma, burns, radiation, and mechanical damage can also initiate inflammation by causing tissue destruction. Exposure to chemical irritants, toxins, pollutants, and certain drugs may damage cells and provoke inflammatory reactions. Tissue necrosis resulting from ischemia, injury, or toxic exposure releases intracellular components that stimulate immune responses. Inflammation may also arise from immune-mediated conditions, including allergic reactions and autoimmune diseases, in which the immune system attacks normal tissues. Foreign bodies such as splinters, surgical implants, and dust particles can trigger localized inflammatory responses as the body attempts to remove or isolate them. Additionally, metabolic disorders such as obesity and diabetes mellitus contribute to chronic low-grade inflammation through the continuous release of inflammatory mediators. Oxidative stress caused by excessive production of reactive oxygen and nitrogen species further promotes inflammatory processes by damaging cellular structures. Cancer and malignant tumors can also induce inflammation by producing cytokines and growth factors that attract immune cells to the tumor microenvironment. Together, these factors activate complex cellular and molecular pathways that result in the characteristic inflammatory response aimed at protecting the body and restoring tissue integrity [13].

Microbial Infections

Microbial infections are among the most common causes of inflammation and occur when pathogenic microorganisms such as bacteria, viruses, fungi, and parasites invade the body and multiply within tissues. These microorganisms are recognized by immune cells through specialized receptors that detect pathogen-associated molecular patterns (PAMPs). Upon recognition, immune cells such as macrophages, neutrophils, dendritic cells, and mast cells become activated and release various inflammatory mediators, including cytokines, chemokines, prostaglandins, and histamine. These mediators increase blood flow and vascular permeability, facilitating the migration of leukocytes to the site of infection. The recruited immune cells engulf and destroy invading pathogens through phagocytosis and other antimicrobial mechanisms. Acute infections typically produce a rapid inflammatory response characterized by redness, heat, swelling, and pain, while persistent or unresolved infections may lead to chronic inflammation, resulting in tissue damage and fibrosis. Therefore, inflammation induced by microbial infections plays a crucial role in host defense by limiting the spread of pathogens and promoting tissue repair.

Physical Injury

Physical injury is a major cause of inflammation and occurs when tissues are damaged by mechanical trauma, burns, frostbite, radiation, surgical p procedures, or other physical agents. Such injuries disrupt the integrity of cells and tissues, leading to the release of intracellular molecules known as damage-associated molecular patterns (DAMPs). These molecules are recognized by immune cells, including macrophages and dendritic cells, which initiate the inflammatory response by releasing cytokines, chemokines, and other inflammatory mediators. As a result, blood vessels dilate and become more permeable, allowing immune cells and plasma proteins to migrate to the site of injury. This process helps remove damaged tissue, prevent infection, and promote healing and tissue regeneration. The characteristic signs of inflammation, including redness, heat, swelling, pain, and temporary loss of function, commonly develop following physical injury. While acute inflammation is essential for tissue repair, excessive or prolonged inflammatory responses may delay healing and contribute to further tissue damage [14].

Chemical Irritants and Toxins

Chemical irritants and toxins are important causes of inflammation and can induce tissue damage through direct or indirect effects on cells and organs. Exposure to harmful substances such as acids, alkalis, industrial chemicals, environmental pollutants, cigarette smoke, toxic gases, pesticides, heavy metals, and certain drugs can disrupt cellular integrity and trigger inflammatory responses. These agents

cause cell injury and death, leading to the release of damage-associated molecular patterns (DAMPs) that activate immune cells such as macrophages and neutrophils. Activated immune cells subsequently release inflammatory mediators, including cytokines, prostaglandins, leukotrienes, and reactive oxygen species, which amplify the inflammatory process. The resulting inflammation is characterized by increased blood flow, vascular permeability, leukocyte infiltration, and tissue edema. Depending on the intensity and duration of exposure, chemical-induced inflammation may be acute and reversible or progress to chronic inflammation, causing persistent tissue damage, fibrosis, and functional impairment. Therefore, inflammation induced by chemical irritants and toxins serves as a protective mechanism to eliminate harmful substances and promote tissue repair, although prolonged exposure can lead to serious pathological consequences [15].

Tissue Necrosis

Tissue necrosis is a significant cause of inflammation and refers to the premature death of cells or tissues resulting from factors such as ischemia, trauma, infections, toxins, or severe physical injury. Unlike programmed cell death (apoptosis), necrosis leads to the rupture of cell membranes and the release of intracellular contents into the surrounding tissues. These released substances, known as damage-associated molecular patterns (DAMPs), are recognized by immune cells such as macrophages and neutrophils, which initiate an inflammatory response. The activation of these immune cells results in the production of cytokines, chemokines, prostaglandins, and other inflammatory mediators that promote vasodilation, increased vascular permeability, and leukocyte recruitment to the affected area. The inflammatory response helps remove dead tissue, eliminate potential pathogens, and initiate tissue repair and regeneration. However, extensive or persistent necrosis can lead to excessive inflammation, further tissue destruction, fibrosis, and impaired organ function. Thus, inflammation associated with tissue necrosis plays a crucial role in clearing damaged cells and facilitating the healing process.

Immune Reactions

Immune reactions are an important cause of inflammation and occur when the immune system responds excessively or inappropriately to foreign or self-antigens. These reactions may arise during allergic responses, autoimmune diseases, or hypersensitivity disorders. In allergic reactions, harmless substances such as pollen, dust, food allergens, or drugs trigger the activation of immune cells, leading to the release of histamine, cytokines, leukotrienes, and other inflammatory mediators. In autoimmune diseases, the immune system mistakenly recognizes the body's own tissues as foreign and attacks them, resulting in chronic inflammation and tissue damage. Examples include rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis. Immune-mediated inflammation involves the activation of T lymphocytes, B lymphocytes, macrophages, mast cells, and other immune cells that produce pro-inflammatory cytokines and antibodies. These mediators increase vascular permeability, promote leukocyte infiltration, and amplify the inflammatory response. While immune reactions are essential for protecting the body against pathogens, abnormal or prolonged immune activation can cause persistent inflammation, tissue destruction, and the development of various chronic inflammatory disorders [16].

Foreign Bodies

Foreign bodies are substances or materials that enter the body from the external environment and are recognized as non-self by the immune system, thereby triggering an inflammatory response. Common examples include splinters, glass fragments, dust particles, sutures, surgical implants, and other non-biodegradable materials. When foreign bodies become lodged in tissues, they cause local tissue irritation and damage, leading to the activation of immune cells such as macrophages, neutrophils, and giant cells. These cells release inflammatory mediators, including cytokines and chemokines, which promote vasodilation, increased vascular permeability, and leukocyte recruitment to the affected site. If the foreign material cannot be removed or degraded, the body may attempt to isolate it by forming granulomatous tissue, a specialized type of chronic inflammatory response characterized by the accumulation of macrophages and multinucleated giant cells around the foreign object. Although this inflammatory process helps protect surrounding tissues from further damage, persistent foreign bodies can lead to chronic inflammation, fibrosis, and impaired tissue function. Therefore, inflammation induced by foreign bodies serves as an important defense mechanism aimed at containing and eliminating potentially harmful materials.

Ischemia and Reperfusion Injury

Ischemia and reperfusion injury is an important cause of inflammation that occurs when the blood supply to a tissue is temporarily reduced or completely interrupted and then subsequently restored. Ischemia deprives tissues of oxygen and essential nutrients, leading to cellular stress, metabolic disturbances, and cell death. When blood flow is re-established, the sudden influx of oxygen paradoxically causes additional tissue damage through the excessive generation of reactive oxygen species (ROS), activation of inflammatory pathways, and recruitment of immune cells. These events stimulate the release of pro-inflammatory cytokines, chemokines, and adhesion molecules, which promote leukocyte infiltration and amplify the inflammatory response. Neutrophils and macrophages accumulate at the site of injury, releasing enzymes and free radicals that further damage cellular structures and surrounding tissues. Ischemia-reperfusion injury is commonly observed in conditions such as myocardial infarction, stroke, organ transplantation, and severe trauma. Although the inflammatory response is essential for removing damaged cells and initiating tissue repair, excessive inflammation following reperfusion can exacerbate tissue injury, contribute to organ dysfunction, and delay recovery. Thus, ischemia and reperfusion injury represent a complex pathological process in which inflammation plays a central role in both tissue damage and healing [17].

Metabolic Disorders

Metabolic disorders are increasingly recognized as important causes of chronic inflammation and are closely associated with conditions such as obesity, diabetes mellitus, metabolic syndrome, and dyslipidaemia. These disorders lead to persistent metabolic stress, which stimulates the production and release of pro-inflammatory cytokines, adipokines, and reactive oxygen species. In obesity, excessive adipose tissue functions as an active endocrine organ, secreting inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and leptin, which contribute to a state of low-grade systemic inflammation. Similarly, chronic hyperglycemia in diabetes mellitus promotes oxidative stress, endothelial dysfunction, and activation of inflammatory signaling pathways. The continuous inflammatory response associated with metabolic disorders can damage tissues and organs, impair insulin sensitivity, and increase the risk of cardiovascular diseases, kidney dysfunction, and other complications. Immune cells, particularly macrophages and lymphocytes, play a significant role in maintaining this chronic inflammatory state by infiltrating metabolic tissues and producing additional inflammatory mediators. Therefore, inflammation associated with metabolic disorders is a key factor in the development and progression of many chronic diseases and represents an important target for therapeutic intervention.

Cancer and Malignancy

Cancer and malignancy are closely associated with inflammation and can both induce and be influenced by inflammatory processes. Tumor cells and the surrounding tumor microenvironment produce a variety of inflammatory mediators, including cytokines, chemokines, growth factors, and prostaglandins, which attract immune cells to the site of the tumor. These immune cells, such as macrophages, neutrophils, and lymphocytes, release additional inflammatory substances that promote angiogenesis, tissue remodeling, and tumor progression. Chronic inflammation creates a favorable environment for the initiation, growth, invasion, and metastasis of cancer cells by inducing DNA damage, stimulating cell proliferation, and inhibiting normal immune surveillance mechanisms. Inflammatory pathways involving tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and nuclear factor-kappa B (NF-κB) are frequently activated in various malignancies and contribute to cancer development. Furthermore, persistent inflammatory conditions such as chronic hepatitis, inflammatory bowel disease, and chronic gastritis have been linked to an increased risk of certain cancers. Although inflammation initially serves as a protective response against abnormal cells, prolonged and uncontrolled inflammation can facilitate tumor growth and disease progression. Therefore, the relationship between cancer and inflammation is complex, with inflammation acting as both a defense mechanism and a contributor to malignancy [18].

5. MATERIAL AND METHODS

Collection and Identification of Plant Material

The Cayenne pepper is collected and identified was purchased from an herbal Market of Hyderabad, Telangana, India, and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, and Tirupati.

Extraction procedure

Coarsely powdered Cayenne pepper was used for extraction with methanol by using Soxhlet method for 6-8 hours. 50g of dried powder was weighed and 250mL of solvent methanol is used for the extraction. The extracts were evaporated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^{o}C for further analysis.

PHARMACOLOGICAL STUDY [19]

ANIMALS

Wistar albino rats of both sexes (180-220 g) were used for the study. The animals were obtained from 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 feed and water given ad libitum. The use of these animals and the study protocols were approved by CCSEA recognized by ethical committee.

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 is 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.

Animal grouping [20]

Group Treatment

Group I Normal Control – Received vehicle only (distilled water or 0.5% CMC)

Group II Negative Control (Inflammation Control) – Received inflammatory agent (e.g., carrageenan/cayenne pepper-induced inflammation) + vehicle only

Group III Test Group Low Dose – Received Cayenne pepper extract (200 mg/kg, p.o.) + inflammatory agent

Group IV Test Group High Dose – Received Cayenne pepper extract (400 mg/kg, p.o.) + inflammatory agent

Group V Standard Control – Received Diclofenac sodium (10 mg/kg, p.o.) + inflammatory agent

Induction of Inflammation in Rats

For evaluating the anti-inflammatory activity of Cayenne pepper (Capsicum annuum) extract, inflammation can be induced using the Carrageenan-Induced Paw Edema Model, one of the most widely used experimental models for acute inflammation.

Induction of Inflammation [21]

Acute inflammation is induced by injecting 0.1 mL of 1% carrageenan suspension prepared in normal saline into the subplantar region of the right hind paw of each rat. Carrageenan administration produces localized edema through the release of inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, and cytokines. The inflammatory response develops rapidly and reaches its maximum intensity within 3–5 hours after injection. The test extract and standard drug are administered orally one hour before carrageenan injection. Paw volume or paw thickness is measured before carrageenan administration and at regular intervals (1, 2, 3, 4, and 5 hours) after induction of inflammation. The reduction in paw edema compared with the control group is used to assess the anti-inflammatory activity of the test extract.

Carrageenan induced rat paw edema by the method of Winter et al. (1962) [22]

Carrageenan induced rat paw edema was done by the method of Winter et al. (1962). 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 (400 and 600 mg/kg, p.o.) and diclofenac (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 (V0: before edematogenic agent injection) and 1,2,3,4 and 5h intervals later (Vt). The difference between Vt and V0 was taken as the edema value. The percentage of inhibition was calculated according to the following formula:

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

Histamine induced rat paw edema [23]

Inflammation was induced by injection of 0.1 ml of freshly prepared histamine (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats89. The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

Dextran induced rat paw edema [24]

Inflammation was induced by injection of 0.1 ml of freshly prepared dextran (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats90. The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

Biochemical analysis [25-28]

On the eighth day, the animals were sacrificed under mild ether anesthesia and blood was collected in clean centrifuge tubes. The serum was obtained by centrifugation and used for the estimation of various biochemical parameters. The absorbance of all the biochemical parameters was measured in a UV-VIS Spectrophotometer -1601 (Shimadzu, Tokyo, Japan).

Estimation of total protein content

The serum total protein was estimated by modified Biuret method (Yatzidis, 1977) using the total protein test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Biuret Reagent (Copper sulphate - 7 mM/L; sodium hydroxide - 200 mM/L; sodium potassium tartrate - 20 mM/L)

Reagent II: Protein standard (BSA - 6.5 g/dL)

Procedure

3.0 ml of Reagent I was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at 37ºC for 5 minutes. The absorbance was read at 578 nm.

Estimation of albumin content

The serum albumin was estimated by the method given by Corcoran and Durnan (1977)95 using albumin test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Albumin reagent (Succinic acid - 37 mM/L; bromocresol green - 0.15 mM/L; sodium hydroxide - 1 mM/L; buffer pH - 3.68).

Reagent II: Albumin standard (BSA - 4 g/dL)

Procedure

3.0 ml of albumin reagent (Reagent I) was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at room temperature for 1 min. The absorbance was read at 630 nm.

Estimation of acid phosphatase (ACP) activity

The serum acid phosphatase activity was estimated by the method of King and Jagatheesan (1959)96 using ACP test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered substrate, pH 4.9

Reagent II: Sodium hydroxide, 0.5 N

Reagent III: Sodium bicarbonate, 0.5 N

Reagent IV: 4-aminoantipyrine, 0.6%

Reagent V: Potassium ferricyanide, 2.4%

Reagent VI: Stock phenol standard, 10 mg%

Solution I: Reconstitute one vial of reagent I, buffered substrate with 2 ml of purified water.

Solution II: Dissolve reagent 4 in 25 ml of purified water

Solution III: Dissolve reagent 5 in 25 ml of purified water

Working standard: Dilute stock phenol standard 0.5 to 5 with purified water

Procedure

All the test tubes were marked properly as blank (B), standard (S), control (C) and test (T). 0.5 ml of solution I was added in control (C) and test (T). 0.5 ml of purified water was added in control (C) and test (T). 0.6 ml of purified water was added in standard (S) and 1.1 ml of purified water was added in blank (B). All the tubes were mixed well and incubated at 37ºC for 3 min. 0.1 ml of serum was added in test (T), 0.5 ml of working standard was added in standard (S). All the tubes were mixed well and incubated at 37ºC for 60 min. 0.5 ml of reagent II was

added in all the tubes. 0.1 ml of serum was added in control (C). 0.5 ml of reagent III, 0.5 ml of solution II and 0.5 ml of solution III was added in all the tubes. All the tubes were mixed well and absorbance was read at 510 nm. Serum acid phosphatase activity is expressed as KA units.

Estimation of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was estimated by the method of Kind and King (1954)97 using ALP test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered substrate, pH 10.0

Reagent II: Chromogen reagent

Reagent III: Phenol standard, 10 mg%

Working solution: Reconstitute one vial of reagent I, buffered substrate with 2.2 ml of purified water.

Procedure

All the test tubes were marked properly as blank (B), standard (S), control (C), and test (T). 0.5 ml of working buffered substrate was added in clean tubes. 1.5 ml of purified water was added in all the tubes. They were mixed well and incubated at 37°C for 3 min. 0.05 ml of serum was added in test (T), 0.05 ml of reagent III (Phenol standard) was added in standard (S) and 0.05 ml of purified water was added in blank (B) tubes. All the tubes were mixed well and incubated at 37°C for 15 min. 1 ml of reagent II was added in all the tubes. 0.05 ml of serum was added in control (C). All the tubes were mixed well and absorbance was read at 510 nm. Serum alkaline phosphatase activity is expressed as KA units.

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 at $F < 0.05$ for ANOVA and $P < 0.05$ for t-test.

6. Results

Results

Percentage of yield

$$Percentage\ of\ yield = \frac{Weight\ of\ crude\ extract}{Weight\ of\ dried\ material} \times 100$$

$$Percentage\ of\ yield = \frac{26g}{200g} \times 100 = 13\%$$

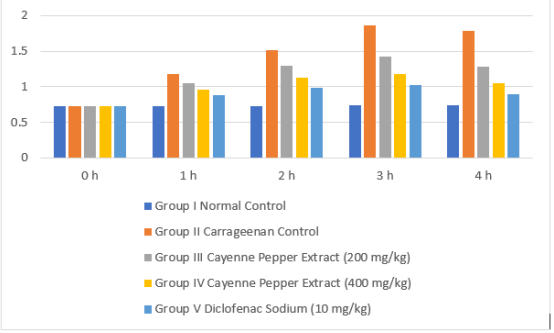
50gms of drug dissolved in 250ml of solvent in 1:4 Ratio

Sl. No.	Phytoconstituents	Test result
1	Alkaloid	-ve
2	Glycosides	-ve
3	Carbohydrate	-ve
4	Protein	-ve
5	Amino acid	-ve
6	Steroids	-ve
7	Flavonoids	+ve
8	Terpenoids	+ve
9	Phenols	+ve
10	Saponins	-ve
11	Tannin	+ve

Carrageenan-Induced Paw Edema in Rats

Group	Treatment	0 h	1 h	2 h	3 h	4 h
Group I	Normal Control	0.72 ± 0.02	0.73 ± 0.02	0.73 ± 0.03	0.74 ± 0.02	0.74 ± 0.02
Group II	Carrageenan Control	0.73 ± 0.03	1.18 ± 0.04	1.52 ± 0.05	1.86 ± 0.06	1.78 ± 0.05
Group III	Cayenne Pepper Extract (200 mg/kg)	0.72 ± 0.02	1.05 ± 0.03*	1.30 ± 0.04*	1.42 ± 0.05**	1.28 ± 0.04**
Group IV	Cayenne Pepper Extract (400 mg/kg)	0.73 ± 0.02	0.96 ± 0.03**	1.12 ± 0.04**	1.18 ± 0.04***	1.05 ± 0.03***
Group V	Diclofenac Sodium (10 mg/kg)	0.72 ± 0.02	0.88 ± 0.02***	0.98 ± 0.03***	1.02 ± 0.03***	0.90 ± 0.02***

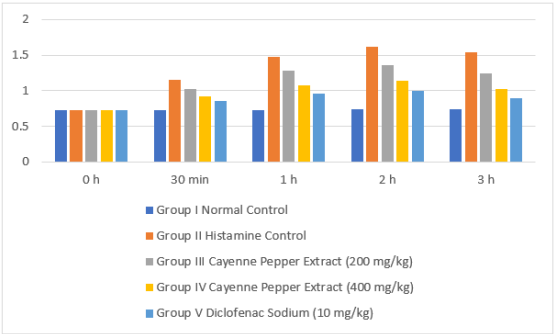
Carrageenan administration produced a significant increase in paw edema in the negative control group, indicating successful induction of acute inflammation. Treatment with Cayenne pepper extract at both 200 mg/kg and 400 mg/kg significantly reduced paw swelling in a dose-dependent manner compared with the carrageenan control group. The higher dose (400 mg/kg) exhibited greater anti-inflammatory activity than the lower dose and showed results comparable to the standard drug diclofenac sodium (10 mg/kg). The maximum inhibition of edema was observed during the late phase of inflammation (3–4 h), suggesting that the extract may inhibit the synthesis or release of inflammatory mediators such as prostaglandins and cytokines. These findings indicate that Cayenne pepper possesses significant anti-inflammatory activity in the carrageenan-induced rat paw edema model.



Histamine-Induced Paw Edema in Rats

Group	Treatment	0 h	30 min	1 h	2 h	3 h
Group I	Normal Control	0.72 ± 0.02	0.73 ± 0.02	0.73 ± 0.03	0.74 ± 0.02	0.74 ± 0.02
Group II	Histamine Control	0.73 ± 0.02	1.15 ± 0.04	1.48 ± 0.05	1.62 ± 0.06	1.54 ± 0.05
Group III	Cayenne Pepper Extract (200 mg/kg)	0.72 ± 0.02	1.02 ± 0.03*	1.28 ± 0.04*	1.36 ± 0.04**	1.24 ± 0.04**
Group IV	Cayenne Pepper Extract (400 mg/kg)	0.73 ± 0.02	0.92 ± 0.03**	1.08 ± 0.03**	1.14 ± 0.04***	1.02 ± 0.03***
Group V	Diclofenac Sodium (10 mg/kg)	0.72 ± 0.02	0.86 ± 0.02***	0.96 ± 0.03***	1.00 ± 0.03***	0.89 ± 0.02***

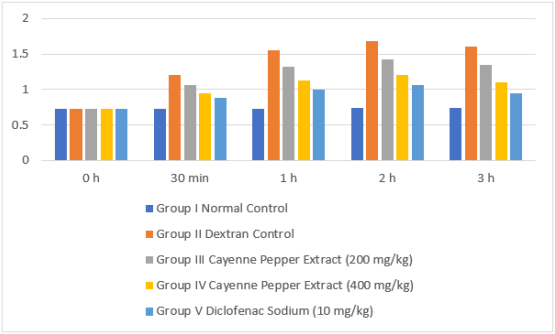
Subplantar administration of histamine produced marked paw edema in the negative control group, confirming successful induction of acute inflammation. Oral treatment with Cayenne pepper extract at doses of 200 mg/kg and 400 mg/kg significantly reduced histamine-induced paw swelling in a dose-dependent manner. The higher dose (400 mg/kg) showed greater inhibition of edema and demonstrated activity comparable to the standard drug diclofenac sodium. The reduction in paw volume suggests that the extract may interfere with histamine-mediated vascular permeability and inflammatory responses. These findings indicate that Cayenne pepper extract possesses significant anti-inflammatory activity against histamine-induced inflammation in rats.



Dextran induced rat paw edema

Group	Treatment	0 h	30 min	1 h	2 h	3 h
Group I	Normal Control	0.72 ± 0.02	0.73 ± 0.02	0.73 ± 0.02	0.74 ± 0.03	0.74 ± 0.02
Group II	Dextran Control	0.73 ± 0.02	1.20 ± 0.04	1.55 ± 0.05	1.68 ± 0.06	1.60 ± 0.05
Group III	Cayenne Pepper Extract (200 mg/kg)	0.72 ± 0.02	1.06 ± 0.03*	1.32 ± 0.04*	1.42 ± 0.05**	1.34 ± 0.04**
Group IV	Cayenne Pepper Extract (400 mg/kg)	0.73 ± 0.02	0.95 ± 0.03**	1.12 ± 0.04**	1.20 ± 0.04***	1.10 ± 0.03***
Group V	Diclofenac Sodium (10 mg/kg)	0.72 ± 0.02	0.88 ± 0.02***	1.00 ± 0.03***	1.06 ± 0.03***	0.95 ± 0.02***

Dextran administration produced a significant increase in paw volume in the negative control group, indicating successful induction of acute inflammatory edema. Pretreatment with Cayenne pepper extract at doses of 200 mg/kg and 400 mg/kg significantly reduced paw swelling compared with the dextran control group. The anti-inflammatory effect was dose-dependent, with the 400 mg/kg dose showing greater inhibition of edema. Diclofenac sodium (10 mg/kg) produced the highest inhibition and served as the standard reference drug. Since dextran-induced edema is primarily mediated by the release of histamine and serotonin from mast cells, the observed inhibition suggests that Cayenne pepper extract may stabilize mast cells or interfere with the actions of these inflammatory mediators. These results demonstrate significant anti-inflammatory activity of Cayenne pepper extract in the dextran-induced rat paw edema model.

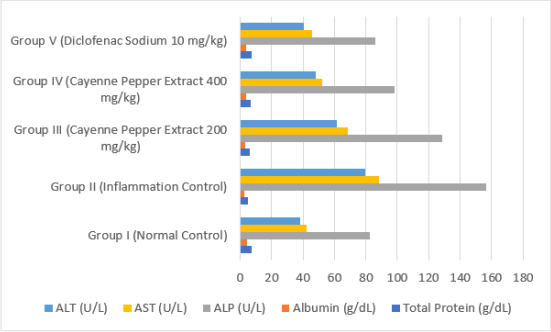


Biochemical Parameters in Anti-Inflammatory Study of Cayenne Pepper Extract

Group	Total Protein (g/dL)	Albumin (g/dL)	ALP (U/L)	AST (U/L)	ALT (U/L)
Group I (Normal Control)	7.42 ± 0.18	4.21 ± 0.12	82.45 ± 3.12	42.36 ± 1.54	38.24 ± 1.42
Group II (Inflammation Control)	5.18 ± 0.16	2.86 ± 0.10	156.82 ± 5.64	88.54 ± 2.86	79.42 ± 2.64
Group III (Cayenne Pepper Extract 200 mg/kg)	6.12 ± 0.17*	3.42 ± 0.11*	128.36 ± 4.82*	68.42 ± 2.24*	61.35 ± 2.18*
Group IV (Cayenne Pepper Extract 400 mg/kg)	6.88 ± 0.19**	3.92 ± 0.12**	98.54 ± 3.68**	52.48 ± 1.86**	48.26 ± 1.74**
Group V (Diclofenac Sodium 10 mg/kg)	7.26 ± 0.18***	4.12 ± 0.11***	86.24 ± 3.24***	45.62 ± 1.62***	40.54 ± 1.48***

The inflammation control group showed significant alterations in serum biochemical parameters, characterized by a decrease in total protein and albumin levels along with a marked increase in alkaline phosphatase (ALP), aspartate

aminotransferase (AST), and alanine aminotransferase (ALT) activities compared with the normal control group. These changes indicate inflammation-associated tissue injury and metabolic disturbances. Treatment with Cayenne pepper extract at doses of 200 mg/kg and 400 mg/kg significantly restored total protein and albumin levels while reducing ALP, AST, and ALT activities in a dose-dependent manner. The higher dose (400 mg/kg) exhibited greater protective effects and produced values closer to those observed in the diclofenac-treated group. These findings suggest that Cayenne pepper extract possesses anti-inflammatory and tissue-protective properties, helping to maintain normal biochemical function during inflammatory conditions.



DISCUSSION

The present study investigated the effects of Cayenne pepper (Capsicum annuum) extract on acute inflammation using carrageenan-, histamine-, and dextran-induced paw edema models in rats, together with an assessment of serum biochemical parameters. The results demonstrated that the extract produced a significant reduction in paw edema and improved biochemical alterations associated with inflammatory stress, indicating its beneficial effects on tissue function and physiological homeostasis. In the carrageenan-induced paw edema model, a marked increase in paw volume was observed in the inflammation control group, confirming successful induction of acute inflammation. Carrageenan-induced edema is characterized by a biphasic response involving the release of histamine, serotonin, bradykinin, prostaglandins, and other mediators. Treatment with Cayenne pepper extract significantly reduced paw swelling at all observation periods, with the higher dose (400 mg/kg) showing greater efficacy than the lower dose (200 mg/kg). The inhibition of edema during the later phase suggests an ability of the extract to modulate inflammatory processes associated with tissue swelling and vascular changes. The histamine-induced paw edema model further supported the effectiveness of the extract. Histamine is a major mediator responsible for increased vascular permeability and fluid accumulation at sites of tissue injury. Administration of histamine produced significant edema in the control animals, whereas pretreatment with Cayenne pepper extract markedly reduced paw swelling. The higher dose produced a greater reduction in edema, indicating a dose-dependent response. These findings suggest that the extract may influence histamine-mediated physiological events and help maintain normal vascular function. Similarly, dextran-induced paw edema resulted in a substantial increase in paw volume in the inflammation control group. Dextran is known to induce edema through mast cell degranulation and subsequent release of vasoactive substances. Treatment with Cayenne pepper extract significantly attenuated the edema response, indicating its ability to reduce tissue fluid accumulation and maintain cellular stability. The higher dose exhibited effects that approached those of the standard drug diclofenac sodium, demonstrating considerable efficacy. The serum biochemical findings revealed significant alterations following inflammation induction. The inflammation control group showed decreased levels of total protein and albumin along with elevated activities of ALP, AST, and ALT. These changes are indicative of disturbances in protein metabolism, cellular integrity, and tissue function. Reduced total protein and albumin levels may result from increased protein catabolism and altered synthetic activity during inflammatory stress. Elevated ALP, AST, and ALT activities suggest increased membrane permeability and leakage of intracellular enzymes into the circulation due to tissue injury. Treatment with Cayenne pepper extract significantly improved these biochemical parameters in a dose-dependent manner. The restoration of total protein and albumin levels suggests improved metabolic status and preservation of normal physiological functions. Likewise, the reduction in ALP, AST, and ALT activities indicates stabilization of cellular membranes and protection against tissue damage. The higher dose of the extract demonstrated greater efficacy and produced values closer to those observed in the diclofenac-treated group. The beneficial effects observed in the present study may be attributed to the presence of bioactive phytochemicals in Cayenne pepper, including capsaicin, flavonoids, carotenoids,

and phenolic compounds. These constituents are known to support cellular health, maintain membrane integrity, and contribute to normal metabolic functions. The dose-dependent improvements observed in both edema and biochemical parameters suggest that these phytochemicals play an important role in preserving tissue structure and physiological balance under conditions of inflammatory stress. Overall, the findings indicate that Cayenne pepper extract effectively reduced paw edema in carrageenan-, histamine-, and dextran-induced models and improved serum biochemical parameters associated with tissue injury and metabolic dysfunction. The results demonstrate its potential to preserve tissue integrity, support normal biochemical function, and maintain physiological homeostasis in experimental animals subjected to inflammatory conditions.

CONCLUSION

The present study demonstrated that Cayenne pepper (Capsicum annuum) extract possesses significant protective effects against experimentally induced inflammatory conditions in rats. The extract effectively reduced paw edema in carrageenan-, histamine-, and dextran-induced inflammation models, with the higher dose (400 mg/kg) producing greater effects than the lower dose (200 mg/kg). The observed reduction in edema indicates the ability of the extract to attenuate tissue swelling and maintain normal vascular responses during inflammatory stress. Serum biochemical analysis revealed that inflammation induced significant alterations in total protein, albumin, ALP, AST, and ALT levels, reflecting tissue injury, cellular dysfunction, and metabolic disturbances. Treatment with Cayenne pepper extract significantly restored protein levels and reduced elevated enzyme activities toward normal values in a dose-dependent manner. These findings suggest improved cellular integrity, preservation of tissue function, and maintenance of biochemical homeostasis. The beneficial effects of the extract may be attributed to its rich content of bioactive phytochemicals, including capsaicin, flavonoids, phenolic compounds, and carotenoids, which contribute to the maintenance of normal physiological and metabolic functions. The higher dose of the extract exhibited results comparable to those of the standard drug diclofenac sodium, indicating substantial efficacy. Overall, the findings of this study suggest that Cayenne pepper extract has considerable potential as a natural therapeutic agent for supporting tissue health, maintaining biochemical balance, and protecting against inflammation-associated physiological disturbances. Further studies are warranted to isolate the active constituents, investigate their molecular mechanisms, and evaluate their long-term safety and

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