**Different Models Used to Screen Inflammation: An Overview**

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**ABSTRACT**

Inflammation is a crucial part of the body's defence system, but chronic inflammation can contribute to the development of various diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammation, but they can have associated side effects. To address this, pharmaceutical research employs various screening methods, including in vitro assays, animal models, computational modelling, and high-throughput screening techniques, to identify and evaluate potential anti-inflammatory compounds. This paper provides an overview of acute and chronic inflammation, highlighting their characteristics, immune responses, and resolution. It also discusses the molecular targets of NSAIDs, such as the cyclooxygenase (COX) enzymes, and the different types of NSAIDs available. Furthermore, the paper explores the causes of inflammation, including infections, injuries, autoimmune disorders, environmental factors, and metabolic factors. The importance of screening methods in anti-inflammatory drug development is emphasized, with a focus on in vitro assays, animal models, and computational modelling. These methods enable researchers to understand the complex mechanisms underlying inflammation and identify promising candidates for further development. Finally, the paper stresses the need for effective anti-inflammatory therapies that target specific molecular pathways to manage chronic inflammation and improve patient outcomes. In conclusion, this paper provides a comprehensive overview of inflammation, anti-inflammatory drugs, causes of inflammation, and screening methods in drug development. It highlights the importance of addressing chronic inflammation and developing safer and more potent anti-inflammatory therapies for better patient care.

**Keywords:** Screening Method, Anti-Inflammatory, Animal Model, Inducing Agent, COX Enzyme.

**INTRODUCTION**

Inflammation is a natural response of the body's defence system to protect against various internal and external stimuli. It can be categorized as acute or chronic, depending on its duration [1]. Acute inflammation is a short-term response to tissue injury, infection, or exposure to harmful agents. It is a protective mechanism that aims to eliminate the cause of injury, remove damaged tissues, and initiate the healing process [2]. Acute inflammation typically lasts for a few days to a few weeks. On the other hand, chronic inflammation is a prolonged and persistent inflammatory response that can last for months to years. Inflammation is an essential part of the healing process, aiding in the removal of foreign substances from the body [3]. Common symptoms of inflammation include pain, swelling, redness, heat, and limited mobility. This process involves changes in blood vessels, such as increased blood flow and dilation, as well as cellular changes where white blood cells migrate to combat bacteria. Monitoring these changes helps assess the anti-inflammatory properties of compounds [4].

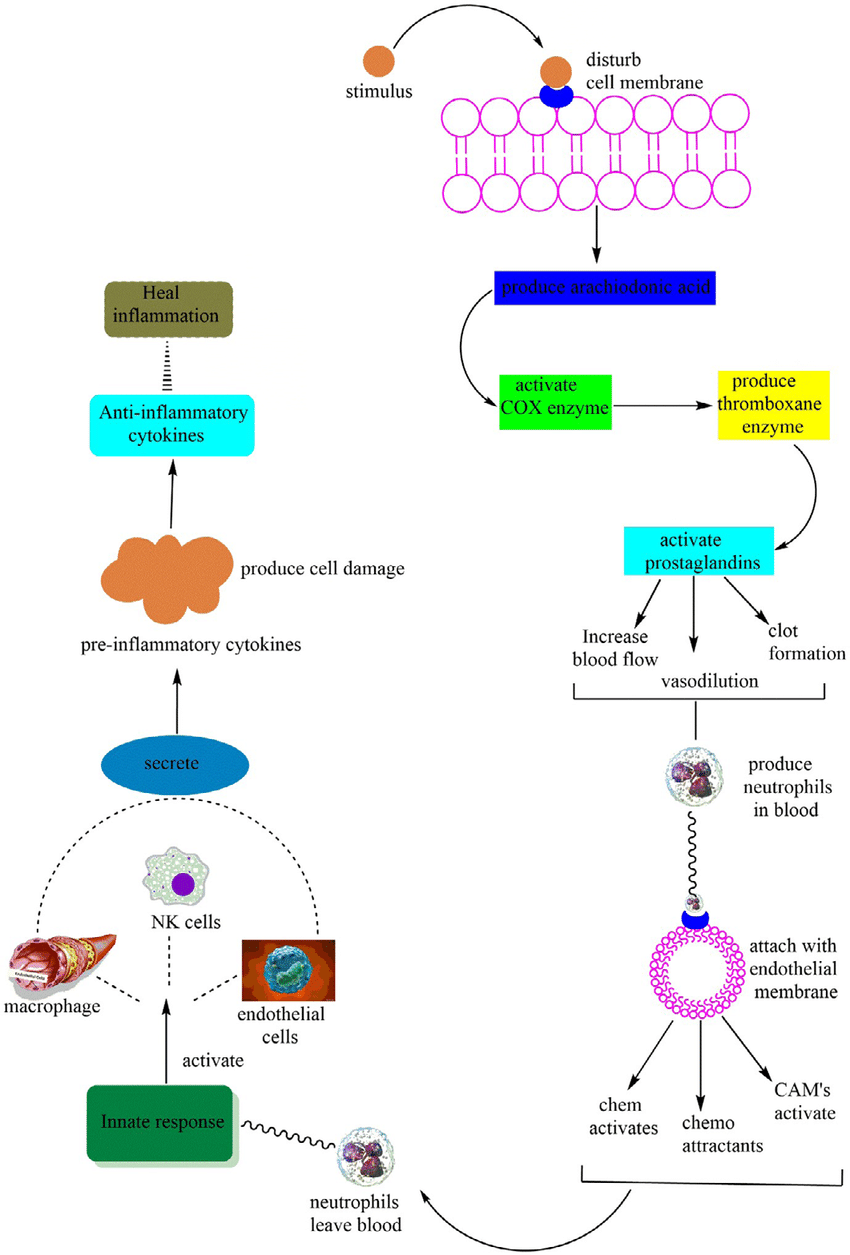
**TABLE 1: ACUTE INFLAMMATION Vs CHRONIC INFLAMMATION [5].**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Characteristics** | **Acute inflammation** | **Chronic inflammation** |
| 1. | Onset | It usually starts rapidly after tissue injury or infection. | It may develop as a result of unresolved acute inflammation, persistent exposure to irritants or pathogens, or an autoimmune response where the immune system mistakenly attacks healthy tissues. |
| 2. | Duration | It is a relatively short-lived response that lasts for a short period of time. | It is a long-lasting inflammatory state that persists for an extended period of time. |
| 3. | Symptoms | Common symptoms of acute inflammation include redness, swelling, heat, pain, and loss of function. These symptoms are often localized to the site of injury or infection. | Chronic inflammation may have milder or less apparent symptoms compared to acute inflammation. However, it can lead to persistent low-grade symptoms such as fatigue, joint pain, and mild fever. Over time, chronic inflammation can cause tissue damage and lead to the development of various diseases. |
| 4. | Immune response | Acute inflammation involves the activation of the immune system, with the release of inflammatory mediators such as histamine, cytokines, and chemokines. This leads to increased blood flow, dilation of blood vessels, and recruitment of immune cells to the affected area. | In chronic inflammation, immune cells, particularly macrophages and lymphocytes, infiltrate the affected tissues and release pro-inflammatory molecules. This sustained immune response can cause damage to healthy tissues and contribute to the progression of diseases like rheumatoid arthritis, inflammatory bowel disease, and atherosclerosis. |
| 5. | Resolution | Acute inflammation typically resolves once the injurious agent is eliminated and the healing process begins. The damaged tissues are repaired, and normal function is restored. | Unlike acute inflammation, chronic inflammation often lacks a resolution phase. The immune response persists, leading to ongoing tissue damage and impaired tissue repair. |

While anti-inflammatory drugs are available to alleviate symptoms like pain, swelling, and fever, many of them have associated side effects. The molecular targets of non-steroidal anti-inflammatory drugs (NSAIDs) are the cyclooxygenase (COX) enzymes. These enzymes play a crucial role in the initial step of arachidonic acid metabolism. NSAIDs primarily inhibit the COX enzymes, thereby reducing the production of prostaglandins and thromboxane involved in inflammation and pain. There are three isoforms of COX: COX-1, COX-2, and COX-3 [6]. COX-1 is constitutively produced and found in platelets, kidneys, and the gastrointestinal (GI) tract. It is responsible for maintaining kidney and GI tract homeostasis. Inhibition of COX-1 by NSAIDs can lead to adverse effects on the GI tract, including the development of ulcers. COX-2 is an inducible isoform, produced by various cell types in response to endotoxins, mitogens, and cytokines released after tissue damage [7]. It is overexpressed at the site of injury and contributes to the production of prostaglandins, triggering an inflammatory and painful response. COX-3, the third isoform, is mainly expressed in the brain and the heart. Acetaminophen (paracetamol) is believed to target this isoform. The development of COX-2 selective inhibitors aimed to reduce GI side effects associated with non-selective NSAIDs [8]. These selective inhibitors target the COX-2 enzyme more specifically and have been found to be well-tolerated and safe in terms of GI adverse effects. They are commonly used for the treatment of arthritic pain and inflammation [9].

NSAIDs are widely used for the management of acute and chronic pain associated with inflammation. They inhibit COX enzymes, interfering with the production of prostaglandins and thromboxane. COX-1 is present in most cells and has regulatory and protective effects, while COX-2 is activated during inflammation. Different classifications of NSAIDs exist based on their selectivity for COX-1 or COX-2, concentration needed for clinical effects, and other factors. Non-selective NSAIDs, preferential COX-2 inhibitors, and highly selective COX-2 inhibitors (coxibs) are examples of different types of NSAIDs. Adverse effects are often related to the inhibition of COX-1, which is involved in various physiological systems [10].

Therefore, there is a need to develop medications with reduced gastric irritation. In addition to using living organisms (in vivo) for testing, in vitro assays performed outside the body are valuable in investigating the anti-inflammatory activity of new drugs. These simpler assays can mimic the responses seen in vivo, making them widely used in the development and evaluation of new anti-inflammatory agents.



**FIGURE 1: Mechanism of Inflammation [11].**Top of Form

**TABLE 2: Some NSAIDs Drugs [12, 13].**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Salicylates** | **Aryl acetic derivatives** | **Enolic acids** | **Arylpropionic derivaives** | **Phenemates** | **Others** | **Coxibs** |
| Acetyl salicylic acid  Lysine clinixinate  Berorilate  Diflunisal  Salicylamide  Etersalate  Salsalate  Salicylic acid  Aspirin | Aceclofenac  Bufexamac  Lonazolac  Alclofenac  Zomepirac diclofenac  Etodolac  Fetiazac  Ketorolac | **Oxicans**  Droxicam  Meloxicam  Piroxicam  Tenoxicam  Oxaprozin  Lornoxicam | Butibufen  Phenoprofen  Phenobufen  Flurbiprofen  Benoxaprofen  Suprofen  Ibuprofen  Ketoprofen  Dexetoprofen  Pyprophen  Indoprofen  Naprofen  Oxaprozin  Tiaprofen  Dexibuprofen  Flunoxaprofen  Alminoprofen | Meclofenamic acid  Mefenamic acid  Flufenamic acid  Tolipanic acid  Niflumic acid  Etofenamates | Nabumetone  Glucosamine  Diacerhein  Nimesulide  Proquazone  Azapropazone  Benzidamine  Orgotein  Feprazone  Morniflumato  Tenidap  Glucosaminoglcan | Celecoxib  Rofecoxib  valdecoxib  Etoricoxib  Parecoxib  4-aminophenol  Paracetamol |
| **Pyrazolones**  Phenylbutazone  Mofebutazone  Oxyphenbutazone  Kebuzone  Metamizone  Feprazone  Nifenazone  Suxibuzone  Aminophenazone |

Inflammation plays a significant role in various diseases, making it a focal point of research and drug development. Screening methods are crucial for identifying and assessing potential anti-inflammatory compounds. The pharmaceutical industry employs various screening methods, including in vitro assays that examine cellular and molecular responses related to inflammation. These methods measure the release of inflammatory mediators or evaluate the inhibition of specific enzymes or pathways. Animal models also play a vital role in evaluating the overall effectiveness and systemic effects of anti-inflammatory compounds. Furthermore, advances in computational modelling and high-throughput screening techniques have facilitated the identification of novel anti-inflammatory agents in a more efficient and cost-effective manner.

By combining in vitro, in vivo, and computational screening methods, researchers gain a better understanding of the complex mechanisms underlying inflammation. This approach enables the identification of promising candidates for further development as potential anti-inflammatory therapies.Top of Form

**CAUSES OF INFLAMMATION:**

It's important to note that while acute inflammation is a normal and necessary part of the body's defence mechanism; chronic inflammation can be harmful and contribute to the development of various diseases. Managing chronic inflammation and addressing its underlying causes is crucial for maintaining overall health and well-being. Inflammation can arise from various sources and triggers within the body. These causes include [14, 15, 16, 17]:

1. **Infection:** Pathogens like bacteria, viruses, or fungi can prompt an immune response, leading to inflammation. Examples encompass respiratory, urinary tract, and skin infections.
2. **Injury or trauma:** Physical harm, such as cuts, burns, fractures, or sprains, can induce inflammation as the body initiates the healing process to repair damaged tissues.
3. **Autoimmune disorders:** Conditions such as rheumatoid arthritis, lupus, and multiple sclerosis involve the immune system mistakenly attacking healthy tissues, resulting in chronic inflammation.
4. **Allergic reactions:** Some individuals may experience inflammation as a response to exposure to allergens like pollen, pet dander, or certain foods. Allergic reactions can manifest as hay fever, asthma, or food allergies.
5. **Chronic diseases:** Certain long-term conditions like inflammatory bowel disease (Crohn's disease, ulcerative colitis), psoriasis, and atherosclerosis involve persistent inflammation as a component of their underlying pathological mechanisms.
6. **Environmental factors:** Exposure to environmental pollutants, toxins, or irritants such as cigarette smoke, air pollution, or certain chemicals can trigger inflammation within the body.
7. **Metabolic factors:** Factors such as obesity, excessive consumption of sugar, and an unhealthy diet can contribute to chronic low-grade inflammation known as meta-inflammation, which is associated with conditions like type-2 diabetes and metabolic syndrome.
8. **Stress:** Prolonged or chronic stress can disrupt immune system regulation and promote inflammation in the body.

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By combining in vitro, in vivo, and computational screening methods, researchers gain a better understanding of the complex mechanisms underlying inflammation. This approach enables the identification of promising candidates for further development as potential anti-inflammatory therapies.Top of Form

**TABLE 3: TttTop of Form**

**DIFFERENT MODELS USED TO STUDY INFLAMMATION.**

|  |  |  |
| --- | --- | --- |
| **S. No.** | **SCREENING METHOD** | **REFERENCES** |
| 1. | Acetic Acid-Induced Vascular Permeability | **18, 19, 20, 21, 22, 23, 24** |
| 2. | Carrageenan-Induced Edema | **18, 19, 24, 25, 26, 27, 28** |
| 3. | Carrageenan Induced Air Pouch Model | **18, 19, 28** |
| 4. | Cotton Pellet-Induced | **18, 19, 25, 28** |
| 5. | Migration Induced   * Leucocyte Migration * Neutrophil Migration Into the Peritoneal Cavity | **18, 19, 29, 30** |
| 6. | Ear Edema Induced Model   * Croton Oil-Induced Mouse Ear Edema * Ethyl Phenyl Propionate-Induced Ear Edema * Topical Acute Edema of the Mouse Ear * TPA-Treated Mouse Ear Edema * Xylen or Croton Oil-Induced Mouse Ear Edema * Xylene Induced Ear Edema | **18, 19, 22, 31, 32, 33, 34, 35, 36** |
| 7. | Paw Edema Induced Model   * Carrageenin- And Arachidonic Acid-Induced Paw Edema * Egg White Induced Hind Paw Edema * Formalin Induced Edema in Rat Paw * Histamine- Induced Hind Paw Oedema * Hot Scald-Induced Rat Paw Edema * Paw Edema Induced By Histamine And Serotonin * Paw Oedema Induced By Egg Albumin In Rat | **18, 19, 20, 21, 22, 29, 32, 34, 35, 38** |
| 8. | Pleurisy Induced Model   * Carrageenan-Induced Pleurisy * LPS-Induced Pleurisy | **18, 19, 39** |
| 9. | Cutaneous Inflammation Induced to the Inner Surface of Right Ear | **18, 19, 40** |
| 10. | Formalin-Induced Edema in the Rat Paw | **18, 19, 20, 21, 22, 38** |
| 11. | Granuloma Air Pouch Model | **18, 19, 29** |
| 12. | HRBC Membrane Stabilization | **18, 19, 23, 35** |
| 13 | Human Keratinocyte HaCaT Cells | **18, 19, 41** |
| 14. | Lps-Activated Raw264.7 Cells | **18, 19, 32** |
| 15. | Mouse Model of Acute and Chronic Inflammation | **18, 19, 23** |
| 16. | Zymosan-Induced Edema in Mice | **18, 19, 42, 43, 44, 45** |
| 18. | Carrageenan And Dextran-Induced Pedal Edema | **18, 19, 46, 47, 48** |
| 19. | Freund's Adjuvant-Induced Inflammation Model | **18, 19, 49, 50, 51, 52** |
| 19. | Lipopolysaccharide (LPS)-Induced Septic Shock | **18, 19, 53, 54** |
| 20. | Plasma Leakage in The Mouse Skin | **18, 19, 57, 58** |

1. **ACETIC ACID-INDUCED VASCULAR PERMEABILITY [18, 19, 20, 21, 22, 23, 24]:**

Acetic acid-induced vascular permeability is a commonly used method to evaluate inflammation in laboratory experiments, particularly in animal models. It assesses the leakage of fluid and proteins from blood vessels into surrounding tissues, which is a characteristic feature of inflammatory responses.

**PRINCIPLE:**

Acetic acid injection induces local vasodilation and increases the permeability of blood vessels, leading to the extravasation of plasma proteins and other macromolecules into the surrounding tissues.

**GENERAL PROCEDURE:**

1. Animal preparation: Select an appropriate animal model and divide them into control and experimental groups.
2. Acetic acid injection: Administer a small volume of acetic acid solution (usually 0.6-1.2% diluted in sterile saline) via subcutaneous or intradermal injection.
3. Control injection: Inject a similar volume of sterile saline at a separate site on the animal.
4. Incubation period: Allow the animals to recover and provide a specific incubation period (e.g., 15-60 minutes) for acetic acid-induced inflammation to develop.
5. Assessment of vascular permeability: After the incubation period, sacrifice the animals and measure the extravasation of plasma proteins or suitable indicators of vascular leakage.
6. Measurement techniques: Various methods can be employed, such as quantifying extravasated protein content using colorimetric assays or measuring fluorescence intensity of intravenously injected dyes.
7. Data analysis: Calculate and compare vascular permeability values between control and experimental groups using statistical analysis.
8. **CARRAGEENAN-INDUCED EDEMA [18, 19, 24, 25, 26, 27, 28]:**

Carrageenan-induced edema is a commonly used method to study inflammation in experimental settings, particularly in animal models. It involves injecting carrageenan, a naturally occurring polysaccharide derived from red seaweed, into a specific tissue site to induce localized inflammation and measure the resulting edema or swelling.

**PRINCIPLE:**

Carrageenan injection promotes an inflammatory response characterized by increased vascular permeability, immune cell infiltration, and the release of inflammatory mediators. These events contribute to the accumulation of fluid and the development of edema at the injection site.

**GENERAL PROCEDURE:**

1. Animal preparation: Select an appropriate animal model and divide them into control and experimental groups.
2. Carrageenan injection: Inject a solution of carrageenan into the subcutaneous tissue of the paw, typically the hind paw. Inject the carrageenan into one paw, while the contralateral paw receives a control injection of sterile saline.
3. Measurement of paw volume: Measure the baseline paw volume of both the injected and control paws before injection using a plethysmometer or a similar device.
4. Time course evaluation: Monitor and measure the paw volume at predetermined time intervals after carrageenan injection (e.g., 1, 2, 3, 4, and 24 hours) using the plethysmometer. Record the paw volume, which reflects the degree of edema or swelling.
5. Data analysis: Calculate and compare the changes in paw volume between the control and experimental paws at different time points. Perform statistical analysis to determine the significance of the observed differences.
6. **CARRAGEENAN INDUCED AIR POUCH MODEL [18, 19, 28]:**

The carrageenan-induced air pouch model is an experimental technique used to study inflammation and immune responses in laboratory settings, particularly in rodents. It involves the creation of an air-filled pouch in the subcutaneous tissue and subsequent stimulation with carrageenan to induce inflammation. This model allows for the investigation of inflammatory parameters and the evaluation of anti-inflammatory compounds or interventions.

**PRINCIPLE:**

The injection of carrageenan into the created air pouch leads to an acute inflammatory response characterized by the recruitment of immune cells, release of inflammatory mediators, and changes in the pouch fluid. Various inflammatory parameters can be measured to assess the inflammation in the air pouch.

**GENERAL PROCEDURE:**

1. Animal preparation: Select an appropriate animal model and divide them into control and experimental groups.
2. Air pouch creation: Anesthetize the animals, shave and disinfect the dorsal area, make a small incision, and create a subcutaneous pouch by injecting sterile air or fluid into the dissected area. Close the incision.
3. Allow pouch maturation: Allow the air pouch to mature for a specific period, typically 3-7 days, to develop a well-vascularized pouch lining.
4. Carrageenan stimulation: Anesthetize the animals again and inject a solution of carrageenan into the air pouch through a small needle. The volume of carrageenan solution injected can vary depending on the desired concentration.
5. Control injection: Inject a similar volume of sterile saline into the air pouch of the control group animals.
6. Incubation period: Allow the animals to recover and provide a specific incubation period, typically 4-6 hours or longer, to allow the inflammatory response to develop.
7. Collection of pouch fluid: Euthanize the animals, excise the air pouch, and collect the pouch fluid by flushing it with a suitable buffer (e.g., phosphate-buffered saline).
8. Measurement of inflammatory parameters: Analyse the pouch fluid for various inflammatory parameters using appropriate laboratory techniques or assays, such as total protein content, leukocyte counts, levels of inflammatory cytokines or chemokines, and enzymatic activity (e.g., myeloperoxidase activity).
9. Data analysis: Compare the inflammatory parameters between the control and experimental groups using statistical analysis.
10. **COTTON PELLET-INDUCED [18, 19, 25, 28]:**

The cotton pellet-induced inflammation model is a widely used preclinical method to evaluate chronic inflammation and assess the anti-inflammatory potential of compounds or interventions. In this model, sterile cotton pellets are implanted subcutaneously, and the resulting tissue response is measured over time.

**PARAMETERS:**

1. Wet weight of cotton pellets: Measures the extent of tissue granulation and edema formation.
2. Dry weight of cotton pellets: Reflects the degree of tissue fibrosis and cellular infiltration.
3. Histological assessment: Evaluates cellular infiltration, tissue remodelling, and neovascularization.
4. Inflammatory mediator levels: Measurement of cytokines, chemokines, or other inflammatory markers in the tissue.

**PRINCIPLE:**

The cotton pellet-induced inflammation model mimics chronic inflammation characterized by fibroblast proliferation, cellular infiltration, and angiogenesis. The implanted cotton pellets serve as foreign bodies, triggering a host immune response and leading to the recruitment of inflammatory cells, tissue remodelling, and the release of pro-inflammatory mediators.

**PROCEDURE:**

1. Animal preparation: Select the appropriate animal model, typically rats or mice, and divide them into control and experimental groups based on your study design.
2. Sterilization of cotton pellets: Sterilize the cotton pellets in an autoclave or other appropriate method to ensure they are sterile before implantation.
3. Anaesthesia and pellet implantation: Anesthetize the animals using suitable anaesthesia protocols. Make a small incision in the subcutaneous tissue, typically in the dorsal region. Implant the sterilized cotton pellets into the subcutaneous pockets created.
4. Control implantation: In the control group, implant sterile cotton pellets or sham implants without any treatment.
5. Closure and recovery: Close the incision with sutures or wound clips. Allow the animals to recover from anaesthesia in a warm and quiet environment.
6. Experimental endpoint: Determine the appropriate time point(s) for evaluating the tissue response. Common time points include 7, 14, or 21 days after implantation. Sacrifice the animals at the designated time points.
7. Harvesting of cotton pellets and tissue assessment:

* Wet weight: Excise the implanted cotton pellets along with the surrounding tissues. Blot off excess fluid, and weigh the wet cotton pellets using an analytical balance. Record the weight.
* Dry weight: Place the wet cotton pellets in a drying oven or desiccator to remove moisture until a constant weight is achieved. Weigh the dried cotton pellets and record the weight.
* Histological assessment: Fix the harvested tissues, embed them in paraffin, section them, and stain with appropriate histological stains (e.g., hematoxylin and eosin). Evaluate the tissue sections for cellular infiltration, fibrosis, and neovascularization.

1. Measurement of inflammatory mediators: If desired, collect tissue samples from the harvested area and measure the levels of specific inflammatory cytokines, chemokines, or other markers using suitable techniques such as ELISA or PCR.
2. Data analysis: Compare the parameters between the control and experimental groups. Perform statistical analysis to determine the significance of observed differences.
3. **MIGRATION INDUCED [18, 19, 29, 30]:**

Leucocyte migration and Neutrophil migration into the peritoneal cavity are important processes of the immune response that occur in the context of inflammation and infection.

**PARAMETERS:**

1. Cell Count: Measuring the total number of leukocytes (white blood cells) and neutrophils that have migrated into the peritoneal cavity.
2. Chemotaxis: Assessing the directional movement of leukocytes and neutrophils towards a chemotactic stimulus.
3. Adhesion Molecules: Studying the expression and role of adhesion molecules that facilitate leukocyte and neutrophil attachment to endothelial cells.
4. Cytokine and Chemokine Levels: Measuring the levels of cytokines and chemokines in the peritoneal fluid that attract and activate leukocytes and neutrophils.
5. Phagocytic Activity: Evaluating the ability of neutrophils to engulf and eliminate pathogens or foreign particles.
6. ROS Production: Assessing the production of reactive oxygen species (ROS) by neutrophils, which aids in pathogen destruction.

**PRINCIPLES:**

1. **Inflammation and Infection:** Leukocyte and neutrophil migration into the peritoneal cavity are essential components of the immune response to inflammation and infection.
2. **Chemotaxis:** Cells are attracted by chemical gradients, guiding their movement towards sites of inflammation or infection.
3. **Adhesion and Transmigration:** Leukocytes and neutrophils adhere to endothelial cells in the blood vessels and then transmigrate through the endothelial lining to reach the site of injury or infection.
4. **Immune Activation:** The presence of pathogens or foreign particles triggers the release of cytokines and chemokines, activating leukocytes and neutrophils.

**PROCEDURE:**

1. Peritoneal Lavage: Inject a sterile saline solution into the peritoneal cavity and then aspirate it to collect peritoneal fluid containing the migrated leukocytes and neutrophils.
2. Cell Count: Perform a cell count using a haemocytometer or flow cytometry to determine the total number of leukocytes and neutrophils.
3. Chemotaxis Assay: Use a chemotaxis chamber or a Boyden chamber to study the directional migration of leukocytes and neutrophils towards a chemotactic stimulus placed in a separate compartment.
4. Adhesion Molecule Expression: Use immunohistochemistry or flow cytometry to analyse the expression of adhesion molecules on endothelial cells and leukocytes/neutrophils.
5. Cytokine and Chemokine Analysis: Employ ELISA or other immunoassays to quantify the levels of cytokines and chemokines in the peritoneal fluid.
6. Phagocytosis Assay: Incubate neutrophils with fluorescently labelled pathogens or particles, then measure phagocytic activity using flow cytometry or fluorescence microscopy.
7. ROS Production Assay: Utilize fluorescent probes like DCFH-DA to measure intracellular ROS levels in neutrophils using flow cytometry.

**TABLE 4: DIFFERENCE BETWEEN LEUCOCYTE MIGRATION AND NEUTROPHILE MIGRATION INTO PERITONEAL CAVITY.**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **PROCESS** | **CELL INVOLVED** | **MECHANISM** |
| 1. | **Leucocyte migration** | Leukocytes are a broad category of white blood cells that include various cell types, such as neutrophils, monocytes, lymphocytes (B cells and T cells), eosinophils, and basophils. Leukocyte migration refers to the collective movement of these different types of white blood cells from the bloodstream into the tissues at the site of inflammation or infection. | The process of leukocyte migration involves several steps:  a. Margination and Rolling: Leukocytes move to the periphery of the blood vessels (margination) and begin to roll along the endothelial lining in response to inflammatory signals.  b. Adhesion: Adhesion molecules on the leukocytes interact with receptors on the endothelial cells, causing the leukocytes to firmly attach to the vessel wall.  c. Transmigration (Diapedesis): The leukocytes then squeeze between endothelial cells and migrate through the vessel wall into the surrounding tissues, guided by chemoattractant released at the site of inflammation or infection. |
| 2. | **Neutrophil migration into peritoneal cavity** | Neutrophils, a type of granulocyte and subset of leukocytes, are the most abundant white blood cells, crucial in the innate immune response against bacterial infections. Neutrophil migration into the peritoneal cavity happens in response to localized inflammation or infection within the abdomen. | The process involves:  a. Chemotaxis: Inflammatory signals or chemoattractant released by damaged tissues or invading pathogens attract neutrophils from the bloodstream to the peritoneal cavity.  b. Diapedesis: Neutrophils adhere to the endothelial cells lining the peritoneal blood vessels and undergo transmigration, crossing the vessel wall to enter the peritoneal cavity.  c. Accumulation: Once in the peritoneal cavity, neutrophils accumulate at the site of inflammation or infection, where they phagocytize and destroy pathogens. |

1. **EAR EDEMA INDUCED MODEL [18, 19, 22, 31, 32, 33, 34, 35, 36]:**

Ear edema induced models are widely used experimental models to study the inflammatory response and evaluate the effects of various compounds or substances on the development of edema (swelling) in the mouse ear. These models involve inducing inflammation by applying specific irritants or agents to the ear, which results in increased vascular permeability and the accumulation of fluid in the tissue.

**PARAMETER:**

Measurement of ear edema (swelling) is a common parameter assessed in these methods. The degree of edema is often quantified by measuring the increase in ear thickness or weight compared to a control group.

**PRINCIPLE:**

The principle behind these methods is to induce an inflammatory response in the mouse ear, typically by applying a specific inflammatory agent or irritant topically. This leads to an increase in vascular permeability, recruitment of immune cells, and the release of inflammatory mediators, resulting in edema formation.

**PROCEDURE:**

1. **Animal Preparation:** Experimental animals, typically mice, are selected and acclimated to the laboratory environment. The mice are often of the same strain and age to minimize biological variations.
2. **Treatment Groups:** Mice are divided into different treatment groups, including control and experimental groups. The experimental groups receive the agent or treatment intended to induce inflammation (e.g., croton oil, ethyl phenyl propionate, TPA, xylene, or croton oil/xylene), while the control group receives a vehicle or placebo.
3. **Induction of Inflammation:** The inflammatory agent or irritant is applied topically to the surface of the mouse ear. The specific dosage and duration of application may vary depending on the protocol and experimental design. This can involve a single application or multiple applications over a specified time period.
4. **Observation Period:** After the induction of inflammation, the mice are typically observed for a specific period to allow the inflammatory response to develop fully. This can range from a few hours to several days, depending on the study objectives.
5. **Measurement of Edema:** At the end of the observation period, the mice are euthanized, and the ears are collected. The degree of edema is measured by assessing the increase in ear thickness or weight compared to the control group. Ear thickness is measured using callipers, while ear weight is determined using a sensitive balance.
6. **Statistical Analysis:** The data obtained from measuring ear edema are statistically analysed to determine significant differences between the control and experimental groups. This analysis helps evaluate the effectiveness of the inflammatory agent or potential anti-inflammatory interventions.

**TABLE 5: VARIOUS INDUCING AGENT USE FOR DIFFERENT EAR EDEMA INDUCED MODEL.**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Model Name** | **Inducing agent** |  |
| 1. | Croton oil-induced mouse ear edema | Croton oil is a potent inflammatory agent derived from the seeds of Croton tiglium. |  |
| 2. | Ethyl phenyl propionate-induced ear edema | Ethyl phenyl propionate is a compound that can induce inflammation when applied topically to the mouse ear. |  |
| 3. | Topical acute edema of the mouse ear | Specific agent used may vary depending on the study or experimental design. |  |
| 4. | TPA-treated mouse ear edema | TPA (12-O-tetradecanoylphorbol-13-acetate) is a widely used agent to induce inflammation in various models. |  |
| 5. | Xylen or croton oil-induced mouse ear edema | Involves using either xylene or croton oil to induce ear edema in mice. Xylene is a solvent that can irritate the skin and induce inflammation, while croton oil, as mentioned earlier, is a potent inflammatory agent |  |
| 6. | Xylene induced ear edema | Xylene is applied topically to the ear, resulting in inflammation |  |

1. **PAW EDEMA INDUCED MODEL [18, 19, 20, 21, 22, 23, 32, 34, 35, 38]:**

Paw edema-induced models are commonly used in experimental research to study the inflammatory response and assess the effects of various compounds or substances on the development of edema (swelling) in the paws of rodents, such as rats. These models involve the administration of specific irritants or agents to the paws, which trigger an inflammatory response, increased vascular permeability, and the accumulation of fluid in the tissue.

**PARAMETER:**

The common parameter assessed in these methods is the measurement of paw or hind paw edema (swelling). The degree of edema is typically quantified by measuring the increase in paw thickness or weight compared to a control group.

**PRINCIPLE:**

To induce an inflammatory response in the paw or hind paw of animals, typically rats or mice. Various agents or substances are used to initiate the inflammatory process, leading to increased vascular permeability, recruitment of immune cells, and the release of inflammatory mediators, resulting in paw edema.

**PROCEDURE:**

1. **Animal Preparation:** The experimental animals, usually rats or mice, are selected and acclimated to the laboratory environment. They are often of the same strain and age to minimize biological variations.
2. **Treatment Groups:** The animals are divided into different treatment groups, including control and experimental groups. The experimental groups receive the agent or substance intended to induce inflammation (e.g., carrageenan, arachidonic acid, egg white, formalin, histamine, serotonin, etc.), while the control group may receive a vehicle or placebo.
3. **Induction of Inflammation:** The specific inflammatory agent or substance is administered to the paw or hind paw. The route of administration may vary, such as injection, topical application, or other appropriate methods based on the specific agent used. The dosage and duration of exposure are determined based on previous studies or established protocols.
4. **Observation Period:** After the induction of inflammation, the animals are typically observed for a specified period to allow the inflammatory response to develop fully. This observation period can range from a few minutes to several hours, depending on the specific experimental design and objectives.
5. **Measurement of Paw Edema:** At the end of the observation period, the degree of paw edema is measured. This can be done by assessing the increase in paw thickness using callipers or measuring the paw weight using a sensitive balance. The measurements are compared to the control group to quantify the extent of paw edema.
6. **Statistical Analysis:** The data obtained from measuring paw edema are statistically analysed to determine significant differences between the control and experimental groups. This analysis helps evaluate the effectiveness of the inflammatory agent or substance and the potential anti-inflammatory effects of test compounds or interventions.

**TABLE 6: VARIOUS INDUCING AGENT USE FOR DIFFERENT PAW EDEMA INDUCED MODEL.**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Model used** | **Inducing agent** |  |
| 1. | Carrageenin- and arachidonic acid-induced paw edema | Carrageenin is a natural polysaccharide derived from certain types of red seaweed.  Arachidonic acid is a fatty acid that can be metabolized into inflammatory mediators, such as prostaglandins and leukotrienes. |  |
| 2. | Egg white induced hind paw edema | Egg white, which contains various proteins and inflammatory components, is injected into the hind paw of an animal. |  |
| 3. | Formalin induced edema in rat paw | Formalin, a chemical compound, can be injected into the paw to induce an inflammatory response. |  |
| 4. | Histamine- induced hind paw oedema | Histamine is a well-known inflammatory mediator involved in allergic and inflammatory reactions. |  |
| 5. | Hot scald-induced rat paw edema | The paw of a rat is subjected to a brief application of hot water or a hot plate to induce thermal injury. |  |
| 6. | Paw edema induced by histamine and serotonin | Involves injecting histamine or serotonin (another inflammatory mediator) into the paw to induce inflammation and edema. |  |
| 7. | Paw oedema induced by egg albumin in rat | Egg albumin, a protein component of egg white, can be injected into the paw to induce inflammation. |  |
| 8. | PGE2 induced hind paw edema | GE2 (Prostaglandin E2) is a bioactive lipid mediator known for its pro-inflammatory properties. It can be used to induce hind paw edema in animal models for studying inflammation. |  |
| 9. | λ- Carrageenan (CARR) - induced paw edema | λ-Carrageenan, a specific type of carrageenan, is used to induce paw edema |  |
| 10. | Kaolin- induced paw oedema | Kaolin, a type of clay mineral, can be injected into the paw to induce an inflammatory response. |  |

1. **PLEURISY INDUCED MODEL [18, 19, 39]:**

Pleurisy is the inflammation of the pleura, the thin membrane that lines the lungs and the inner side of the chest cavity. It can be induced in laboratory animals like mice for research purposes to study the inflammatory response and potential treatments for pleurisy.

**PARAMETERS:**

1. Inflammatory Mediators: Measurement of various inflammatory mediators, such as cytokines (e.g., TNF-α, IL-1β, IL-6) and chemokines, in the pleural fluid or serum to assess the extent of the inflammatory response.
2. Cellular Infiltration: Quantification of infiltrating cells, particularly neutrophils and other immune cells, in the pleural cavity using techniques like cell counting or flow cytometry.
3. Pleural Fluid Accumulation: Measurement of the volume of pleural fluid accumulated in the pleural cavity, which is indicative of the severity of pleurisy.
4. Histopathological Analysis: Examination of pleural tissue sections under a microscope to assess tissue damage, inflammatory cell infiltration, and other pathological changes.

**PRINCIPLE:**

Carrageenan-induced pleurisy and LPS-induced pleurisy both share the common principle of inducing inflammation in the pleural cavity to study the pathophysiology of pleurisy and evaluate potential therapeutic interventions. Both models involve the injection of a specific agent (carrageenan or LPS) into the pleural space of mice, triggering an acute inflammatory response. By measuring inflammatory parameters, researchers can assess the severity and resolution of inflammation and understand the mechanisms involved in the immune response.

**PROCEDURE:**

1. Animal Preparation: Mice are selected for the study and allowed to acclimate to the laboratory environment. They are housed under standard conditions with free access to food and water.
2. Anaesthesia: Before the induction procedure, mice are anesthetized to minimize pain and discomfort. Common aesthetic agents include isoflurane or ketamine-xylazine.
3. Surgical Exposure: A small incision is made in the skin, and the underlying tissues are gently dissected to expose the pleural cavity.
4. Induction of Pleurisy: For carrageenan-induced pleurisy, carrageenan solution is injected into the pleural cavity. For LPS-induced pleurisy, LPS solution is injected into the same area.
5. Control Groups: Typically, there are control groups, including a negative control (e.g., saline injection) and a positive control (e.g., untreated inflammation).
6. Observation Period: After injection, mice are observed for a specific duration (usually a few hours to a day) to allow the inflammatory response to develop.
7. Sample Collection: At the end of the observation period, mice are euthanized, and pleural fluid and/or tissue samples are collected for analysis.
8. Data Analysis: Inflammatory parameters, cell counts, cytokine levels, and other relevant measurements are analysed and compared between control and experimental groups.

**TABLE 7: VARIOUS INDUCING AGENT USE FOR DIFFERENT PLEURISY INDUCED MODEL.**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Model Name** | **Inducing agent** |  |
| 1. | Carrageenan-induced pleurisy | Carrageenan-induced pleurisy is triggered by the injection of carrageenan, a seaweed-derived polysaccharide. |  |
| 2. | LPS-induced pleurisy | LPS-induced pleurisy is triggered by injecting lipopolysaccharide from bacteria. |  |

1. **CUTANEOUS INFLAMMATION INDUCED TO THE INNER SURFACE OF RIGHT EAR [18, 19, 40]:**

The cutaneous inflammation induced on the inner surface of the right ear is a commonly used model to study localized inflammatory responses in the skin. An inflammatory agent is applied to the inner surface of the right ear, leading to an immune response and inflammation. The model allows for the evaluation of various parameters to assess the inflammatory reaction.

**PARAMETERS:**

1. Ear thickness: Measured as an indicator of edema or swelling in the treated ear.
2. Erythema score: Visual assessment of redness or erythema in the treated ear compared to the control ear.
3. Leukocyte infiltration: Evaluation of immune cell infiltration into the ear tissue.
4. Cytokine levels: Measurement of specific inflammatory cytokines or chemokines in the ear tissue or serum samples.

**PROCEDURE:**

1. Animal preparation: Select the appropriate animal model (e.g., mice or rats) and divide them into control and experimental groups.
2. Anaesthesia: Anesthetize the animals to minimize discomfort during the procedure.
3. Application of the inflammatory agent: Apply the chosen inflammatory agent to the inner surface of the right ear using an appropriate applicator.
4. Control application: Apply a vehicle control (e.g., the vehicle solution without the inflammatory agent) to the inner surface of the left ear of the control group animals.
5. Observation and scoring: Measure the thickness of both ears before and after the application of the inflammatory agent. Record the measurements and assess the degree of erythema in the treated ear using a scoring system.
6. Evaluation of leukocyte infiltration: At the desired time point, euthanize the animals and excise the treated and control ears. Process the ear tissue for histological analysis to evaluate the extent of leukocyte infiltration.
7. Measurement of cytokine levels: Collect ear tissue samples or serum samples at the desired time point. Analyse the samples for specific inflammatory cytokines or chemokines using techniques like ELISA or multiplex assays.
8. Data analysis: Compare the parameters between the treated and control groups and performs statistical analysis to determine significance.Top of Form
9. **FORMALIN-INDUCED EDEMA IN THE RAT PAW [18, 19, 20, 21, 22, 38]:**

Formalin-induced edema in the rat paw is an experimental model used to study acute inflammation and assess the anti-inflammatory potential of compounds or interventions. In this model, formalin, a chemical irritant, is injected into the paw, resulting in local tissue inflammation and edema.

**PARAMETERS:**

1. Paw volume or thickness: Measured as an indicator of edema development.
2. Paw weight: Used to assess the degree of swelling.
3. Leukocyte infiltration: Evaluation of immune cell migration into the paw tissue.
4. Inflammatory mediator levels: Measurement of specific cytokines, chemokines, or other inflammatory markers in the paw tissue.

**PRINCIPLE:**

Formalin is a strong irritant that induces tissue damage and triggers an inflammatory response when injected into the paw. The injection of formalin leads to the release of inflammatory mediators, activation of immune cells, and increased vascular permeability, resulting in edema formation. This model allows for the assessment of various parameters to evaluate the extent of inflammation and the effectiveness of anti-inflammatory interventions.

**PROCEDURE:**

1. Animal preparation: Select rats and divide them into control and experimental groups.
2. Anaesthesia: Anesthetize the rats to minimize discomfort during the procedure.
3. Formalin injection: Inject formalin solution into the rat paw and a control solution into the contralateral paw of the control group.
4. Measurement of paw edema: Measure paw volume or thickness at regular intervals after formalin injection. Record measurements and weigh the paws at designated time points.
5. Assessment of leukocyte infiltration: Excise the paws at the desired time point and prepare paw tissue samples for histological analysis.
6. Measurement of inflammatory mediators: Collect paw tissue samples and optionally blood samples for cytokine analysis.
7. Inflammatory mediator analysis: Analyse paw tissue or serum samples for specific cytokines, chemokines, or markers using ELISA or multiplex assays.
8. Data analysis: Compare parameters between control and experimental groups and perform statistical analysis to determine significance.

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1. **GRANULOMA AIR POUCH MODEL [18, 19, 28]:**

The granuloma air pouch model is an experimental model used to study chronic inflammation and granuloma formation. It involves the creation of an air-filled pouch in a subcutaneous tissue space, allowing for the injection of inflammatory agents or foreign substances to induce a localized inflammatory response.

**PARAMETERS:**

1. Pouch volume: Measured as an indicator of inflammation and edema development.
2. Inflammatory cell infiltration: Evaluation of immune cell migration into the pouch.
3. Granuloma formation: Assessment of the presence and characteristics of granulomas.
4. Cytokine and chemokine levels: Measurement of specific inflammatory mediators in the pouch fluid or tissue.

**PRINCIPLE:**

The granuloma air pouch model mimics chronic inflammation characterized by granuloma formation, fibrosis, and immune cell infiltration. The air pouch provides a controlled and accessible space to introduce inflammatory agents or foreign substances, thereby inducing a localized inflammatory response. The model allows for the evaluation of various parameters to assess the extent of inflammation, granuloma formation, and the efficacy of anti-inflammatory interventions.

**PROCEDURE:**

1. Animal preparation: Select the appropriate animal model, typically mice or rats, and divide them into control and experimental groups based on your study design.
2. Anaesthesia: Anesthetize the animals using suitable protocols to minimize discomfort during the procedure.
3. Creation of the air pouch: Create a subcutaneous air pouch by injecting a known volume of sterile air into the dorsal area of the animal.
4. Injection of inflammatory agents: Inject the desired inflammatory agent or foreign substance into the air pouch using a fine needle.
5. Experimental endpoint: At the desired time point, sacrifice the animals and collect the pouch fluid by aspirating it into a sterile syringe. Excise the air pouch and surrounding tissue for analysis.
6. Evaluation of parameters: Measure pouch volume and evaluate immune cell infiltration and granulomas through histological analysis. Analyse pouch fluid or tissue samples for specific cytokines, chemokines, or inflammatory mediators using ELISA or multiplex assays.
7. Data analysis: Compare parameters between control and experimental groups and perform statistical analysis to determine significance.
8. **HRBC MEMBRANE STABILIZATION [18, 19, 23, 35]:**

HRBC (Horse Red Blood Cell) membrane stabilization assay is used to evaluate the anti-inflammatory potential of compounds or interventions by measuring the ability of test substances to prevent the lysis or disruption of red blood cell membranes, which can occur in the presence of inflammatory agents or mediators.

**PARAMETERS:**

1. Percentage of HRBC membrane stabilization: Measured as the extent of protection provided by the test substance against membrane disruption.
2. Absorbance: Measured at a specific wavelength to quantify the release of haemoglobin, indicating membrane lysis.

**PROCEDURE:**

1. Preparation of HRBC suspension: Collect fresh horse blood in an anticoagulant solution using aseptic techniques. Centrifuge the blood to separate RBCs from plasma and buffy coat. Wash RBCs with an isotonic buffer to remove residual plasma or anticoagulant.
2. Preparation of test samples: Prepare test samples of the compound or intervention to evaluate anti-inflammatory activity. Include positive and negative controls.
3. Assay procedure: Prepare microplate wells with test substance, HRBC suspension, and inflammatory agent. Incubate the mixture for HRBC lysis. Include blank samples without test substance or inflammatory agent. Centrifuge the reaction mixture to pellet intact RBCs.
4. Measurement of absorbance: Transfer supernatant to a fresh microplate. Measure absorbance at a suitable wavelength using a spectrophotometer with blank samples as a reference.
5. Calculation and data analysis: Calculate the percentage of HRBC membrane stabilization by comparing absorbance of test samples with controls. Analyse data statistically for significance.
6. **HUMAN KERATINOCYTE HaCaT CELLS [18, 19, 41]:**

Human keratinocyte HaCaT cells are commonly used in vitro models to study inflammation and the cellular responses of keratinocytes to inflammatory stimuli.

**PARAMETERS:**

1. Cytokine and chemokine production: Measurement of pro-inflammatory cytokines (e.g., IL-1β, TNF-alpha, IL-6) and chemokines (e.g., CXCL8/IL-8) released by HaCaT cells upon inflammatory stimulation.
2. Cell viability: Assessment of cell viability and cytotoxicity to ensure that the inflammatory stimuli do not cause significant cell death.
3. Gene expression: Analysis of the expression levels of inflammation-related genes (e.g., NF-κB, AP-1) using techniques such as quantitative real-time polymerase chain reaction (qRT-PCR) or RNA sequencing.
4. Protein expression: Detection of protein expression levels of specific inflammatory markers (e.g., COX-2, iNOS) using techniques like Western blotting or immunofluorescence.

**PRINCIPLE:**

HaCaT cells, derived from human keratinocytes, retain several characteristics of normal keratinocytes and exhibit responses similar to primary human keratinocytes upon exposure to inflammatory stimuli. The principle of studying inflammation in HaCaT cells involves exposing the cells to inflammatory stimuli and assessing their inflammatory responses through cytokine production, gene expression, and protein expression.

**PROCEDURE:**

1. Cell culture: Maintain HaCaT cells in DMEM supplemented with FBS and antibiotics.
2. Experimental setup: Plate HaCaT cells in appropriate culture vessels at desired densities and allow them to adhere.
3. Inflammatory stimulation: Choose the inflammatory stimulus (e.g., cytokines, LPS) and prepare it at the desired concentration in suitable media. Add the stimulus to HaCaT cells and include control samples without the stimulus.
4. Incubation: Incubate the cells with the stimulus for the desired duration.
5. Analysis of inflammatory responses: Measure pro-inflammatory cytokine and chemokine levels in the supernatants using ELISA or multiplex assays. Assess cell viability and cytotoxicity with appropriate assays. Analyse gene expression by qRT-PCR or RNA sequencing. Evaluate protein expression with Western blotting, immunofluorescence, or immunocytochemistry.
6. Data analysis: Use statistical methods to determine significance between control and treated groups. Interpret results and draw conclusions about HaCaT cell responses to the inflammatory stimulus.
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8. **LPS-activated RAW264.7 cells [18, 19, 32]:**

LPS-activated RAW264.7 cells are a widely used in vitro model to study inflammation and the immune response. RAW264.7 is a murine macrophage cell line that can be stimulated with lipopolysaccharide (LPS) to induce an inflammatory response.

**PARAMETERS:**

1. Cytokine and chemokine production: Measurement of pro-inflammatory cytokines (e.g., TNF-alpha, IL-1β, IL-6) and chemokines (e.g., MCP-1, CXCL10) released by RAW264.7 cells upon LPS stimulation.
2. Nitric oxide (NO) production: Assessment of NO production by measuring nitrite levels, an indicator of inducible nitric oxide synthase (iNOS) activity.
3. Gene expression: Analysis of the expression levels of inflammation-related genes (e.g., TNF-alpha, IL-1β, iNOS) using techniques such as quantitative real-time polymerase chain reaction (qRT-PCR) or RNA sequencing.
4. NF-κB activation: Evaluation of NF-κB activation, a key transcription factor involved in the inflammatory response.

**PROCEDURE:**

1. Cell culture: Maintain RAW264.7 cells in suitable conditions.
2. Experimental setup: Plate RAW264.7 cells in appropriate culture vessels and allow them to adhere.
3. LPS stimulation: Prepare LPS stock solution and dilute to desired concentration. Stimulate RAW264.7 cells with LPS-containing medium. Include control samples without LPS stimulation.
4. Incubation: Incubate cells with LPS for desired duration, typically up to 24 hours.
5. Analysis of inflammatory responses: Measure pro-inflammatory cytokines and chemokines in cell supernatants using ELISA or multiplex assays. Measure nitrite levels using Griess reagent or specific assays. Analyse gene expression by qRT-PCR or RNA sequencing. Assess NF-κB activation through Western blotting or immunofluorescence.
6. Data analysis: Use appropriate statistical methods to determine significance between LPS-stimulated and control groups. Interpret results and draw conclusions about RAW264.7 cell inflammatory response to LPS stimulation.
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**MOUSE MODEL OF ACUTE AND CHRONIC INFLAMMATION [18, 19, 23]:**

A mouse model of acute and chronic inflammation is commonly used to study the underlying mechanisms, progression, and resolution of inflammatory processes.

**PARAMETERS:**

1. Inflammatory cell infiltration: Evaluation of immune cell recruitment and infiltration at the site of inflammation.
2. Cytokine and chemokine production: Measurement of pro-inflammatory cytokines (e.g., TNF-alpha, IL-1β) and chemokines (e.g., CXCL1/KC, CCL2) in the affected tissues.
3. Tissue damage and fibrosis: Assessment of tissue pathology, including histopathological changes, tissue damage, and fibrotic responses.
4. Resolution of inflammation: Evaluation of the resolution phase, including the reduction in inflammatory mediators and the restoration of tissue homeostasis.

**PRINCIPLE:**

The mouse model of acute and chronic inflammation aims to mimic the key features of human inflammatory diseases by inducing an immune response and subsequent tissue damage. Acute inflammation typically involves a rapid immune response, characterized by neutrophil infiltration and the release of pro-inflammatory cytokines. Chronic inflammation is a sustained inflammatory response involving the recruitment of mononuclear cells, tissue remodelling, and fibrosis. These models allow researchers to investigate the cellular and molecular events associated with inflammation and test therapeutic interventions.

**PROCEDURE:**

1. Animal selection and grouping: Choose the appropriate mouse strain and divide them into experimental groups based on research objectives.
2. Ethical considerations: Obtain ethical approvals and adhere to animal welfare guidelines.
3. Induction of inflammation:

* Acute inflammation model: Administer inducers like LPS, turpentine oil, or carrageenan via various routes (i.p., i.v., s.c., or local injection). Determine optimal dose and timing based on previous studies or pilot experiments.
* Chronic inflammation model: Induce chronic inflammation through repeated administration of a low dose of an inflammatory agent or genetic modifications in transgenic mice. Determine treatment duration and frequency.

1. Sample collection and analysis: Assess immune cell infiltration, cytokine/chemokine production, tissue damage, and fibrosis. Use flow cytometry, ELISA, histopathology, immunohistochemistry, gene expression analysis, and biochemical assays. Include relevant controls for comparison.
2. Data analysis: Analyse data using statistical methods to determine significance. Interpret results, draw conclusions, and relate them to underlying inflammation mechanisms.

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1. **ZYMOSAN-INDUCED EDEMA IN MICE [18, 19, 42, 43, 44, 45]: Top of Form**

Zymosan-induced edema in mice is an experimental model used to study inflammation and evaluate the effects of zymosan, a fungal cell wall component, on edema formation.

**PARAMETERS:**

1. Paw thickness: Measured as an indicator of edema development using a calliper or micrometre.
2. Paw weight: Used to assess the degree of swelling.
3. Inflammatory mediators: Measurement of pro-inflammatory cytokines (e.g., TNF-alpha, IL-1β) or other inflammatory mediators in the paw tissue or serum.
4. Histopathological analysis: Examination of paw tissue sections for signs of inflammation, including cellular infiltration and tissue damage.

**PRINCIPLE:**

In the zymosan-induced edema model, zymosan is injected into the paw of mice, triggering an immune response and subsequent inflammatory reactions. Zymosan stimulates the activation of immune cells, such as macrophages and neutrophils, leading to the release of pro-inflammatory mediators. This results in increased vascular permeability, leukocyte recruitment, and edema formation in the paw tissue. The model allows for the evaluation of various parameters to assess the extent of inflammation and the effects of anti-inflammatory interventions.

**PROCEDURE:**

1. Animal selection and grouping: Choose the appropriate number and strain of mice. Divide them into control and experimental groups.
2. Ethical considerations: Obtain ethical approvals and follow animal welfare guidelines.
3. Edema induction: Anesthetize mice and inject zymosan solution into the paw. Control mice receive a vehicle injection (e.g., saline, phosphate-buffered saline).
4. Measurement of paw edema: Measure paw thickness at regular intervals after zymosan injection. Record measurements and weigh excised paw tissue.
5. Analysis of inflammatory mediators: Collect paw tissue samples and optionally blood samples. Measure pro-inflammatory cytokines or other mediators using ELISA or multiplex assays.
6. Histopathological analysis: Process paw tissue samples for histological examination. Stain sections with appropriate dyes and examine for signs of inflammation.
7. Data analysis: Compare parameters between control and experimental groups. Perform statistical analysis to determine significance. Interpret results and draw conclusions on the inflammatory response.
8. **CARRAGEENAN AND DEXTRAN-INDUCED PEDAL EDEMA [18, 19, 46, 47, 48]:**

Carrageenan and dextran-induced pedal edema is an experimental model used to study inflammation and evaluate the effects of carrageenan and dextran on edema formation in the paw of rodents. This model involves the injection of carrageenan or dextran into the paw, which leads to localized inflammation and edema.

**PARAMETERS:**

1. Paw thickness: Measured as an indicator of edema development using a calliper or micrometre.
2. Paw weight: Used to assess the degree of swelling.
3. Inflammatory mediators: Measurement of pro-inflammatory cytokines (e.g., TNF-alpha, IL-1β) or other inflammatory mediators in the paw tissue or serum.
4. Histopathological analysis: Examination of paw tissue sections for signs of inflammation, including cellular infiltration and tissue damage.

**PRINCIPLE:**

In the carrageenan and dextran-induced pedal edema model, carrageenan or dextran is injected into the subcutaneous tissue of the paw, triggering an immune response and subsequent inflammatory reactions. Carrageenan and dextran cause tissue damage and induce vascular changes, leading to increased vascular permeability and the release of inflammatory mediators. This results in edema formation in the paw tissue. The model allows for the evaluation of various parameters to assess the extent of inflammation and the effects of anti-inflammatory interventions.

**PROCEDURE:**

1. Animal selection and grouping: Choose the appropriate number and strain of animals (e.g., rats, mice). Divide them into control and experimental groups based on research objectives.
2. Ethical considerations: Obtain necessary ethical approvals and follow animal welfare guidelines.
3. Edema induction: Anesthetize animals and inject carrageenan or dextran solution into the subcutaneous paw tissue. Control animals receive a vehicle injection (e.g., saline, mineral oil).
4. Measurement of paw edema: Measure paw thickness at regular intervals after injection. Record measurements and weigh excised paw tissue.
5. Analysis of inflammatory mediators: Collect paw tissue samples and optionally blood samples. Measure pro-inflammatory cytokines or other mediators using ELISA or multiplex assays.
6. Histopathological analysis: Process paw tissue samples for histological examination. Stain sections and examine under a microscope for signs of inflammation.
7. Data analysis: Compare parameters between control and experimental groups. Perform statistical analysis to determine significance. Interpret results and draw conclusions regarding the inflammatory response in the paw.
8. Top of Form

**FREUND'S ADJUVANT-INDUCED INFLAMMATION MODEL [18, 19, 49, 50, 51, 52]:Top of Form**

Freund's adjuvant-induced inflammation model is a widely used experimental model to study chronic inflammation and evaluate the effects of Freund's adjuvant, an immunostimulatory substance, on the development of inflammation. Freund's adjuvant is typically composed of killed or inactivated mycobacteria, which elicit a strong immune response and induce long-lasting inflammation.

**PARAMETERS:**

1. Paw thickness: Measured as an indicator of edema development using a calliper or micrometre.
2. Paw weight: Used to assess the degree of swelling.
3. Inflammatory mediators: Measurement of pro-inflammatory cytokines (e.g., TNF-alpha, IL-1β) or other inflammatory mediators in the paw tissue or serum.
4. Histopathological analysis: Examination of paw tissue sections for signs of inflammation, including cellular infiltration and tissue damage.

**PROCEDURE:**

1. Animal selection and grouping: Choose the appropriate number and strain of mice. Divide them into control and experimental groups.
2. Ethical considerations: Obtain ethical approvals and follow animal welfare guidelines.
3. Edema induction: Anesthetize mice and inject zymosan solution into the paw. Control mice receive a vehicle injection.
4. Measurement of paw edema: Measure paw thickness at regular intervals after zymosan injection. Record measurements and weigh excised paw tissue.
5. Analysis of inflammatory mediators: Collect paw tissue samples and optionally blood samples. Measure pro-inflammatory cytokines or other mediators using ELISA or multiplex assays.
6. Histopathological analysis: Process paw tissue samples for histological examination. Stain sections with appropriate dyes and examine for signs of inflammation.
7. Data analysis: Compare parameters between control and experimental groups. Perform statistical analysis to determine significance. Interpret results and draw conclusions on the inflammatory response.
8. Top of Form

**LIPOPOLYSACCHARIDE (LPS)-INDUCED SEPTIC SHOCK [18, 19, 53, 54]:** Top of Form

Lipopolysaccharide (LPS)-induced septic shock is an experimental model used to study the pathophysiology of septic shock and evaluate the effects of LPS, a component of the outer membrane of Gram-negative bacteria, on the development of systemic inflammation. LPS administration leads to a systemic inflammatory response mimicking sepsis.

**PARAMETERS:**

1. Survival rate: Assessing the mortality rate following LPS administration.
2. Hemodynamic parameters: Monitoring blood pressure, heart rate, and other cardiovascular parameters.
3. Inflammatory markers: Measurement of pro-inflammatory cytokines (e.g., TNF-alpha, IL-1β) or other inflammatory mediators in the blood or tissues.
4. Organ dysfunction: Assessing the function and histopathological changes in vital organs, such as the liver, lung, and kidney.

**PROCEDURE**:

1. Choose appropriate animals; divide into control and experimental groups.
2. Obtain ethical approvals and follow animal welfare guidelines.
3. Anesthetize and administer LPS intravenously or intraperitoneally; control animals receive a vehicle injection.
4. Monitor animals for survival, body temperature, weight, and clinical signs of septic shock. Measure hemodynamic parameters and collect blood samples for inflammatory marker analysis. Sacrifice animals for histopathological analysis.
5. Analyse data, including survival rate, hemodynamic parameters, inflammatory markers, and histopathological findings. Perform statistical analysis to determine significance. Interpret results and draw conclusions about systemic inflammatory response and organ dysfunction in LPS-induced septic shock.Top of Form

**20. PLASMA LEAKAGE IN THE MOUSE SKIN [18, 19, 55, 56]:**

Plasma leakage in the mouse skin is an experimental parameter used to study inflammation and evaluate the increased vascular permeability that occurs during inflammatory processes. It involves the measurement of extravasation or leakage of plasma proteins from blood vessels into the surrounding skin tissue.

**PARAMETERS:**

1. Evans blue dye extravasation: Measurement of extravagated Evans blue dye, which binds to plasma proteins, in the skin tissue.
2. Spectrophotometric analysis: Quantification of the amount of extravagated Evans blue dye by spectrophotometry.
3. Skin edema: Assessment of the degree of edema formation in the skin.

**PROCEDURE:**

1. Choose appropriate number and strain of mice. Divide into control and experimental groups.
2. Obtain ethical approvals and follow animal welfare guidelines.
3. Anesthetize mice and induce inflammation by applying an inflammatory stimulus to the skin. Control animals undergo a similar procedure without the stimulus.
4. Administer Evans blue dye systemically via intravenous injection into the tail vein.
5. Sacrifice mice at desired time point after dye administration. Excise inflamed skin area or site of interest.
6. Homogenize skin tissue to extract Evans blue dye using a suitable solvent.
7. Measure absorbance of extracted dye using a spectrophotometer and quantify the amount of extravagated dye.
8. Measure skin thickness at the site of inflammation using a calliper or micrometre.
9. Compare parameters between control and experimental groups. Perform statistical analysis to determine significance. Interpret results and draw conclusions about plasma leakage and inflammation severity in the mouse skin.

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**20. HYALURONIDASE INHIBITORY ASSAY [18, 19, 57, 58]:**

The hyaluronidase inhibitory assay is a biochemical assay used to evaluate the inhibitory activity of compounds or substances against hyaluronidase, an enzyme involved in the degradation of hyaluronic acid, a major component of the extracellular matrix.

**PARAMETERS:**

1. Enzyme activity: Measurement of hyaluronidase activity, typically assessed by quantifying the hydrolysis of hyaluronic acid substrate.
2. Inhibitory activity: Evaluation of the inhibitory effect of test compounds or substances on hyaluronidase activity.

**PROCEDURE:**

1. Prepare hyaluronidase enzyme from a suitable source or obtain it and purify if needed.
2. Dissolve hyaluronic acid powder in a buffer to create a stock solution and dilute to the desired working concentration.
3. Prepare stock solutions of test compounds to be evaluated for inhibitory activity against hyaluronidase.
4. Set up reaction mixtures containing enzyme, substrate, and test compounds at different concentrations in microplates or test tubes. Include positive and negative control wells.
5. Incubate the reaction mixtures for a specific duration at an appropriate temperature.
6. Stop the reaction and measure released reducing sugars or hyaluronic acid fragments using suitable assays.
7. Calculate hyaluronidase activity in each reaction mixture using a standard curve.
8. Calculate the percentage inhibition of hyaluronidase activity for each concentration of the test compound using the following formula: % Inhibition = [(Control Activity - Sample Activity) / Control Activity] × 100
9. Plot a graph of percentage inhibition against the concentration of the test compound.
10. Determine the IC50 value, representing the concentration that inhibits 50% of hyaluronidase activity.
11. Perform statistical analysis to assess the significance of inhibitory effects, if necessary.

**CONCLUSION**

Inflammation is a complex biological process with a significant role in various diseases, making it a central focus of research and drug development. By employing a combination of in vitro, in vivo, and computational screening methods, researchers can gain a better understanding of inflammation's mechanisms and identify potential anti-inflammatory therapies that may lead to improved treatments for various inflammatory-related conditions.

**REFERENCES**

1. L. Silva, “A literature review of inflammation and its relationship with the oral cavity,” Glob J Infect Dis Clin Res, 1(2), 2015, 21-7.
2. C. N. Serhan, S. D. Brain, C. D. Buckley, D. W. Gilroy, C. Haslett, L. A. O’Neill,... & J. L. Wallace, “Resolution of inflammation: state of the art, definitions and terms,” FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 21(2), 2007, 325.
3. L. Ferrero-Miliani, O. H. Nielsen, P. S. Andersen, & S. Girardin, “Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation,” Clinical & Experimental Immunology, 147(2), 2007, 227-235.
4. C. Nathan, “Points of control in inflammation,” Nature, 420(6918), 2002, 846-852.
5. D. Furman, J. Campisi, E. Verdin, P. Carrera-Bastos, S. Targ, C. Franceschi, ... & G. M Slavich, “Chronic inflammation in the etiology of disease across the life span,” Nature medicine, 25(12), 2019, 1922-193
6. P. Libby, “Inflammatory mechanisms: the molecular basis of inflammation and disease,” Nutrition reviews, 65(suppl\_3), 2007, S140-S146.
7. R. Medzhitov, “Inflammation 2010: new adventures of an old flame,” Cell, 140(6), 2010, 771-776.
8. A. L Kiss, “Inflammation in focus: the beginning and the end. Pathology and Oncology Research,” 27, 2022, 1610136.
9. P. H. Araújo, R. S. Ramos, J. N. da Cruz, S. G. Silva, E. F. Ferreira, L. R. de Lima,... & C. B. Santos, “Identification of potential COX-2 inhibitors for the treatment of inflammatory diseases using molecular modeling approaches,” Molecules, 25(19), 2020, 4193.
10. Nasybullina, N. M. (1999). Nonsteroidal antiinflammatory drugs and their medicinal forms (A review). Pharmaceutical chemistry journal, 33(2), 88-93.
11. A. Muzamil, H. M.Tahir, S. Ali, I. Liaqat, A. Ali, M. Summer, “Inflammatory process and role of cytokines in inflammation: an overview,” Punjab Univ J Zool, 36, 2021, 237-52.
12. Z. Ju, M. Li, J. Xu, D. C. Howell, Z. Li, & F. E. Chen, “Recent development on COX-2 inhibitors as promising anti-inflammatory agents: The past 10 years,” Acta Pharmaceutica Sinica B, 12(6), 2022, 2790-2807.
13. T. C. Ramalho, M. V. Rocha, E. F. da Cunha, & M. P. Freitas, “The search for new COX-2 inhibitors: a review of 2002–2008 patents,” Expert opinion on therapeutic patents, 19(9), 2009, 1193-1228.
14. P. Libby, P. M. Ridker, & A. Maseri, “Inflammation and atherosclerosis,” Circulation, 105(9), 2002, 1135-1143.
15. A. J. Merched, K. Ko, K. H. Gotlinger, C. N. Serhan, & L. Chan, “Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators,” The FASEB Journal, 22(10), 2008, 3595.
16. L. Chen, H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng,... & L. Zhao, “Inflammatory responses and inflammation-associated diseases in organs,” Oncotarget, 9(6), (2019), 7204.
17. A. U. Ahmed, “An overview of inflammation: mechanism and consequences,” Frontiers in Biology, 6(4), (2011), 274-281.
18. K. R. Patil, U. B. Mahajan, B. S. Unger, S. N. Goyal, S. Belemkar, S. J. Surana,... & C. R. Patil, “Animal models of inflammation for screening of anti-inflammatory drugs: implications for the discovery and development of phytopharmaceuticals,” International journal of molecular sciences, 20(19), 2019, 4367.
19. M. A. Phanse, M. J. Patil, K. Abbulu, P. D. Chaudhari, & B. Patel, “In-vivo and in-vitro screening of medicinal plants for their anti-inflammatory activity: an overview,” Journal of Applied Pharmaceutical Science, 2(7), 2012,19-33.
20. S. S. Huang, G. J. Huang, W. H. Peng, Y. L. Ho, M. J. Chang, H. J. Hung,... & Y. S. Chang, “Analgesic and Anti-inflammatory Activities of an Aqueous Extract of Hydrocotyle batrachium Hance in Mice,” Mid-Taiwan Journal of Medicine, 13(4), 2008,189-195.
21. F. P. Ching, E. K. Omogbai, R. I. Ozolua, & S. O. Okpo, “Analgesic activity of aqueous extract of Stereospermum kunthianum (Cham, Sandrine Petit) stem bark,” Acta Poloniae Pharmaceutica—Drug Research, 66(1), 2009, 83-88.
22. H. Hosseinzadeh, & H. M. Younesi, “Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice,” BMC pharmacology, 2, 2002, 1-8.
23. B. K. Choo, T. Yoon, M. S. Cheon, H. W. Lee, A. Y. Lee, & H. K. Kim, “Anti-inflammatory effects of Asparagus cochinchinensis extract in acute and chronic cutaneous inflammation,” Journal of ethnopharmacology, 121(1), 2009, 28-34.
24. N. Megha Rani, P. K. Shetty, S. N. Rao, R. P. Nayak, “Screening for anti-inflammatory and peripheral analgesic activity of Coleus amboinicus leaves using wistar albino rats,” Indian J Pharm Pharmacol;7(3), 2020, 164-167
25. R. Ilavarasan, M. Malika, & S. Venkataraman, “Anti-inflammatory and antioxidant activities of Cassia fistula Linn bark extracts,” African journal of traditional, complementary and alternative medicines, 2(1), 2005, 70-85.
26. R. N. Kumar, K. Arumugasamy, M. R. Udhayasankar, & H. A. Kaffoor, “In vivo anti-inflammatory activity of methanolic extract of Hydrocotyle conferta Wight (Apiaceae),”.
27. B. S. Nayak, K. N. Patel, “Anti-Inflammatory screening of Jatropha curcas root, stem and leaf in albino rats,” Rom J Biol Plant Biol, 55(1), 2010, 9-13.
28. M. S. Paschapur, M. B. Patil, R. Kumar, & S. R. Patil, “Evaluation of anti-inflammatory activity of ethanolic extract of Borassus flabellifer L. male flowers (inflorescences) in experimental animals,” Journal of Medicinal Plants Research, 3(2), 2009, 049-054.
29. J. Gertsch, M. Leonti, S. Raduner, I. Racz, J. Z. Chen, X. Q. Xie,... & A. Zimmer, “Beta-caryophyllene is a dietary cannabinoid,” Proceedings of the National Academy of Sciences, 105(26), 2008, 9099-9104.
30. G. E. De Souza, & S. H. Ferreira, “Blockade by antimacrophage serum of the migration of PMN neutrophils into the inflamed peritoneal cavity,” Agents and Actions, 18, 1985, 97-103.
31. E. E. Bralley, P. Greenspan, , J. L. Hargrove, L. Wicker, & D. K. Hartle, “Topical anti-inflammatory activity of Polygonum cuspidatum extract in the TPA model of mouse ear inflammation,” Journal of Inflammation, 5(1), 2008, 1-7.
32. Z. B. Dong, Y. H. Zhang, B. J. Zhao, C. Li, G. Tian, B. Niu,... & J. G. Shao, “Screening for anti-inflammatory components from Corydalis bungeana Turcz. based on macrophage binding combined with HPLC,” BMC complementary and alternative medicine, 15, 2015, 1-10.
33. H. Hosseinzadeh, M. Ramezani, & G. A. Salmani, Antinociceptive, anti-inflammatory and acute toxicity effects of Zataria multiflora Boiss extracts in mice and rats. Journal of ethnopharmacology, 73(3), 2000, 379-385.
34. E. Küpeli, I. I. Tatli, Z. S. Akdemir, & E. Yesilada, “Estimation of antinociceptive and anti-inflammatory activity on Geranium pratense subsp. finitimum and its phenolic compounds,” Journal of ethnopharmacology, 114(2), 2007, 234-240.
35. S. C. Chun, S. Y. Jee, S. G. Lee, S. J. Park, J. R. Lee, & S. C. Kim, “Anti-inflammatory activity of the methanol extract of moutan cortex in LPS-activated Raw264. 7 cells,” Evidence-Based Complementary and Alternative Medicine, 4, 2007, 327-333.
36. H. M. Manga, D. Brkic, D. E. P. Marie, & J. Quetin-Leclercq, “In vivo anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg.(Euphorbiaceae),” Journal of ethnopharmacology, 92(2-3), 2004, 209-214.
37. A. Cowan, F. Porreca, & H. Wheeler, “Use of the formalin test in evaluating analgesics,” Problems of Drug Dependence 1989, 116.
38. T. Dimo, A. L. Fotio, T. B. Nguelefack, E. A. Asongalem, & P. Kamtchouing, “Antiinflammatory activity of leaf extracts of Kalanchoe crenata Andr,” Indian Journal of Pharmacology, 38(2), 2006, 115.
39. V. D. S. Frutuoso, M. M. Monteiro, F. C. Amendoeira, A. L. F. Almeida, D. D. D. Nascimento, A. L. R. Bérenger,... & H. C. Castro-Faria-Neto, “Analgesic and anti-inflammatory activity of the aqueous extract of Rheedia longifolia Planch & Triana,” Memorias do instituto oswaldo cruz, 102, 2007, 91-96.
40. H. M. Manga, D. Brkic, D. E. P. Marie, & J. Quetin-Leclercq, “In vivo anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg.(Euphorbiaceae),” Journal of ethnopharmacology, 92(2-3), 2004, 209-214.
41. H. Kim, T. H. Han, & S. G. Lee, “Anti-inflammatory activity of a water extract of Acorus calamus L. leaves on keratinocyte HaCaT cells,” Journal of ethnopharmacology, 122(1), 2009, 149-156.
42. S. Hunskaar, O. B. Fasmer, & K. Hole, Formalin test in mice, a useful technique for evaluating mild analgesics. Journal of neuroscience methods, 14(1), 1985, 69-76.
43. Y. Kasahara, H. Hikino, S. Tsurufuji, M. Watanabe, & K. Ohuchi, “Antiinflammatory actions of ephedrines in acute inflammations,” Planta Medica, 51(04), 1985, 325-331.
44. J. H. Rosland, A. Tjølsen, B. Mæhle, & K. Hole, “The formalin test in mice: effect of formalin concentration,” Pain, 42(2), 1990, 235-242.
45. D. Salvemini, Z. Q. Wang, D. M. Bourdon, M. K. Stern, M. G. Currie, & P. T. Manning, “Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema,” European journal of pharmacology, 303(3), 1996, 218-220.
46. D. Sarkar, A. Dutta, M. Das, K. Sarkar, C. Mandal, & M. Chatterjee, Effect of Aloe vera on nitric oxide production by macrophages during inflammation. Indian journal of pharmacology, 37(6), 2005, 371.
47. C. G. Van Arman, A. J. Begany, L. M. Miller, & H. H Pless, “Some details of the inflammations caused by yeast and carrageenin (with appendix on kinetics of the reaction),” Journal of Pharmacology and Experimental Therapeutics, 150(2), 1965, 328-334.
48. H. Venegas-Flores, D. Segura-Cobos, & B. Vázquez-Cruz, “Antiinflammatory activity of the aqueous extract of Calea zacatechichi,” In Proceedings of the Western Pharmacology Society (Vol. 45, pp. 110-111). Seattle, Wash.: The Society, 2002.
49. L. M. De Young, J. B. Kheifets, S. J. Ballaron, & J. M. Young, “Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separate and can be differentially modulated by pharmacologic agents,” Agents and actions, 26, 1989, 335-341.
50. R. Koster, “Acetic acid for analgesics screening,” In Fed proc 1959, (Vol. 19, pp. 412-418)
51. M. Li, H. Luo, Z. Huang, J. Qi, & B. Yu, “Screening and Identification of Anti-Inflammatory Compounds from Erdong Gao via Multiple-Target-Cell Extraction Coupled with HPLC-Q-TOF-MS/MS and Their Structure–Activity Relationship,” Molecules, 28(1), 2022, 295.
52. P. F. D'arcy, E. M. Howard, P. W. Muggleton, & S. B. Townsend, “The anti-inflammatory action of griseofulvin in experimental animals,” Journal of Pharmacy and Pharmacology, 12(1), 1960, 659-665.
53. J. H. Rosland, “The formalin test in mice: the influence of ambient temperature,” Pain, 45(2), 1991, 211-216.
54. U. M. Teotino, L. P. Friz, A. Gandini, & D. D. Bella, “Thio derivatives of 2, 3-dihydro-4H-1, 3-benzoxazin-4-one. Synthesis and pharmacological properties,” Journal of medicinal chemistry, 6(3), 1963, 248-250.
55. A. Tjølsen, O. G. Berge, S. Hunskaar, J. H. Rosland, & K. Hole, “The formalin test: an evaluation of the method,” Pain, 51(1), 1992, 5-18.
56. C. A. Winter, E. A. Risley, & G. W. Nuss, “Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs,” Proceedings of the society for experimental biology and medicine, 111(3), 1962, 544-547.
57. J. A. Witkowski, “A screening test for anti-inflammatory activity using human skin. Journal of Occupational and Environmental Medicine, 1(9), 1959, 522.
58. A. Yadav, & S. Mohite, “Screening of In-vitro anti-inflammatory and Antibacterial assay of Malvastrum Coromandelianum. International Journal of Pharma Sciences and Research, 11(4), 2020, 68-70