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Analgesic and Anti-Inflammatory Activity: A Comprehensive Review on *Invitro* and *In Vivo* Screening Methods

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Abstract:- Modern pharmacotherapy includes analgesic and anti-inflammatory medicines as essential components to relieve pain and inflammation brought on by a variety of medical diseases. Robust screening techniques are essential for the identification of possible candidates with appropriate safety and effectiveness profiles in the search and development of new analgesic and anti-inflammatory medications. This study looks at the many screening methods used in preclinical studies to assess new drugs' analgesic and anti-inflammatory quality. Conventional techniques like the tail flick, hot plate, and writhing's tests measure analgesic activity by having animals respond to unpleasant stimuli. Comparably, anti-inflammatory activity is frequently assessed using assays like the cotton pellet granuloma test, which gauges tissue granuloma formation, and the carrageenan-induced paw edema model, which measures inflammation. These traditional techniques offer insightful information about the pharmacological effects of test substances. Despite the wide range of screening techniques available, each strategy has advantages and disadvantages. Preclinical studies are more reliable and have higher predictive value when various assays and techniques are integrated into a tiered screening strategy. Furthermore, the successful translation of preclinical findings to human applications depends on taking into account translational variables including species differences and clinical relevancies. As a result, choosing the right screening techniques is critical to the effective identification and characterization of new analgesic and anti-inflammatory drugs.

Keywords:- *Analgesic; Anti-Inflammmatory; Hot Plate; Writing Test; Carrageenan Induced Paw Edema; Prostaglandins; Arthriti.*

I. INTRODUCTION

- *Analgesics*

Pain is termed as "a distressing mental and sensory state accompanied by or expressed in terms of actual or possible harm to tissues," so physiological and perceptual information are both involved in the pain experience [1].

Bradykinin, TNFα, and ILs cause analgesia by blocking the pain nerve sensitizing mechanism [2]. Without substantially affecting awareness, analgesics selectively alleviate pain by acting on peripheral pain mechanisms or the central nervous system. While pain serves mostly as a protective mechanism, it may also be a source of great discomfort and even disability if not managed properly. Additional symptoms such as a sinking feeling, anxiety, perspiration, queasy stomach, palpitations, changes in blood pressure (high or low), and tachypnea may accompany severe pain. Rather of addressing the underlying source of pain, analgesics treat the symptom [3].

- *Anti Inflammatory*

When tissue injury occurs, inflammation is the body's normal physiological reaction. Injuries can result from trauma, mechanical damage, microbial infections, burns, and inflammatory reactions. Inflammation may be either short-lived or long-lasting [4-8]. Molecular and cellular systems work together during an initial inflammatory response to lessen the likelihood of harm or infection.

A strong correlation exists between inflammation and the efficiency of the immune system. Bradykinin, histamine, prostaglandins, lipoxins, leukotrienes, lymphokines, and platelet activator factor are all examples of inflammatory intermediaries, also known as autacoids [9].

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II. MECHANISM OF ACTION

➢ *Anaglesics*

The term "pain" encompasses a wide range of subjective experiences, from mildly uncomfortable to completely unbearable. The least morphologically distinct physiological receptors, namely free nerve endings, are responsible for detecting pain impulses. Dorsal root ganglions house the bodies of bipolar afferent first-order neurons. The transmission of nociceptive impulses occurs via both myelinated and unmyelinated axons, with the former having a conduction velocity of 0.2-2.0 m/s and the latter 5-30 m/s. Intense pressure or heat activates the free ends of Aδ fibers, whereas chemical stimuli [H+, K+, histamine, bradykinin, etc.] resulting from tissue damage activate the free ends of C-fibers.

When prostaglandins are present, the effectiveness of any kind of stimulus—chemical, mechanical, or thermal—increases dramatically. Pain from inflammation or ischemia (such as angina pectoris or myocardial infarction) or from the overstimulation or spasmodic contraction of smooth muscles in the abdominal organs (such as visceral pain) can also have a chemical basis. Aδ and C-fibers attach to second-order neurons in the dorsal horn after ascending in the dorsolateral funiculus and entering the spinal cord via the dorsal root. A second-order neuron's axon takes the antero-lateral or spinothalamic tract, which crosses the midline, on its route up to the brain. Differentiating neo- and paleospinothalamic tracts is based on their evolutionary ages. Nuclei in the thalamus that receive input from the neospinothalamus project to specific regions of the postcentral gyrus. The sensation of acute, pinpoint pain is a common response to stimuli sent via this pathway. Probably representing the route supporting weakly localizable pain—pain that is dull, aching, or burning—are the nuclear areas that receive input from paleospinothalamic neurons. These regions project to the postcentral gyrus and the frontal and limbic brain. The descending projections from the reticular formation modulate impulse traffic in the neo- and paleospinothalamic pathways by ending at second-order neurons, their synapses with first-order neurons, or at spinal segmental inter neurons [descending antinociceptive system]. The production of opiopeptides (enkephalins) or monoamines (norepinephrine, serotonin) allows this mechanism to block impulse transmission from first- to second-order neurons [10].

➢ *Anti-Inflammatory Drugs*

Nonsteroidal anti-inflammatory drugs (NSAIDs) work by blocking the enzyme cyclooxygenase (COX). The conversion of arachidonic acid to prostacyclins, prostaglandins, and thromboxanes requires cyclooxygenase [11]. Because these eicosanoids are absent, NSAIDs are able to exert their therapeutic benefits. In particular, thromboxanes contribute to platelet adhesion, prostaglandins widen blood vessels, raise the hypothalamic temperature set-point, and have an anti-nociception function.

Both COX-1 and COX-2 are isoenzymes of cyclooxygenase. The body consistently expresses COX-1, which is involved in regulating the lining of the gastrointestinal tract, renal function, and the aggregation of platelets. While the body does not normally produce COX-2, it does so in reaction to inflammation, a process known as induction. Nonselective COX-1 and COX-2 inhibitors make up the bulk of NSAIDs. The adverse effect profile is different, however, for nonsteroidal anti-inflammatory drugs (NSAIDs) that selectively target COX-2, such as celecoxib. Noteworthy, COX-2 selective NSAIDs ought to alleviate inflammation without endangering the gastric mucosa, since COX-1 is mostly responsible for maintaining gastric mucosal integrity whereas COX-2 is primarily associated with inflammation [12].

III. IN VIVO METHODS

A. Analgesic Activity

➢ *Acetic Acid-Induced Writhing Test*

Analgesic efficacy of synthesized compounds was assessed utilizing a model of writhing in response to acetic acid. There were five sets of six 20–25 g b.w. Swiss albino mice utilized. Intraperitoneally, 0.6% acetic acid (dose ¼ 10 ml/Kg) was administered. Following a 5-minute acetic acid injection into each mouse, the number of writhes was tallied for 20 minutes. This reading was used as a benchmark. The next day, the analgesic activity of the identical mouse groups was assessed. The manufactured substances were given orally to each group. One hour before to the acetic acid injection, the animal was given a dose of 100 mg/kg. Mice were observed for 20 minutes to count how many times they writhed following a 5-minute injection of acetic acid. After calculating each group's mean value, it was compared to the control [13-14].

➢ *Hot Plate Test*

There was a set temperature of 55 ± 0.5◦C used for hot plate testing. The animals were already accustomed to the hot plate twice. The response was described as springing [in which case all four paws leave the plate] or licking or biting of a paw. The response latency was defined as the duration in seconds between the platform and the reaction. Mice with latency times longer than thirty seconds or shorter than five seconds were eliminated. Meperidine hydrochloride and saline were administered as test medications, and the latency time was measured at 30, 60, 90, and 120 minutes. Complete analgesia was defined as a latency period of 60 s; if this is the case, we shall cut off its duration to protect the mice. Urine and feces were removed from the hot plate by using damp paper towels to wipe it clean after each test. Female ICR mice were split up in 7 groups. The control group received test medicines and a positive drug for three days, for example, isometrical physiological saline. The positive control group

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was put on a heated plate after receiving an injection of dolatin (25 mg/kg) [15-16].

➢ *Tail Flick Test*

The immersion water heater with circulating circulation was apparatus used in tail flick test. The thermostat maintained a steady 54±1 ◦C temperature in water bath. Just before the treatment, we put the final three centimeters of each mouse's tail in the water bath and measured how long it took for each mouse to flick its tail in seconds. All mice included in the study had to have a reaction time of three seconds or less before treatment. The five-mice groups were then injected orally with solvents, plant extracts, or reference substances after the measurement of baseline latency. Reaction times of the mice were again measured one and two hours later. The tail flick measurements were subject to a 6-second cutoff duration in order to minimize tissue injury [17-18].

➢ *Formalin Test*

The control group had five percent formalin. The right hind paw's dorsal surface was injected with twenty microliters of 5% formalin 60 minutes after WET [0.1, 0.5, 1.0, and 2.0 g/kg, p.o.] and 30 minutes after Indo [10 mg/kg, *i.p.*] administration. The length of time the mice spent licking the formalin-injected hind paw was noted during the 30-minute observation period that followed the injection. The first five minutes following a formalin injection are known as the "early phase," while the next fifteen to forty minutes are known as the "late phase." A stop watch was used to record amount of time wounded paw was bit or licked (pain behavior). Five-minute intervals were used to capture the activity [19-21].

➢ *Tail Pressure Test*

The tail pressure test in this study was conducted, with a few minor modifications, in accordance with the protocols. At a dose of 6 mg/kg, mice were given several alkaloids dissolved in hydrogel topically on their tails. A motor response threshold was assessed in response to an analgesic meter applying pressure to the tail. A plinth held the tail's tip, while a cone-shaped pusher helped apply pressure in a linear fashion. The threshold at which the mice strained or retracted their tails was determined to represent the mechanical nociceptive threshold after 60 minutes after injection. In order to keep the tissue intact, a pressure setting of 500 g was used [16].

B. Anti-Inflammatory Activity:

➢ *Model Involving Carrageenan As Phlogistic Agent:*

A sulfated, jelly-like polysaccharide called carrageenan is taken out of some algae, including red seaweeds. A common systemic model for assessing acute inflammation is the rat paw edema caused by carrageenan. Due to its non-antigenic nature and lack of discernible side effects, carrageenan is the favored phlogistic agent for assessing anti-inflammatory effectiveness [22].There are two known phases to the inflammation caused by carrageenan. Serotone, kinins, and histamine are released in the first hour, while prostaglandins, protease, and lysosome enzymes are released in the third and fourth hours [23-24].

This paradigm uses oral or intraperitoneal administration of the test medicines or controls to different groups of experimental animals. Carefully inject 0.1 mL of a 1% carrageenan suspension into the subplantar area of the hind paw of each rat after 30 minutes of intraperitoneal or oral medication treatment, respectively [25].The paw volumes are measured using a plethysmometer, Vernier caliper, liquid displacement method, or cotton thread assistance six hours after the carrageenan challenge and again at one-hour intervals thereafter [26]. The following formula [23] is used to calculate the percentage inhibition of edema.

$$\% \text{ Inhibition of Edema} = \frac{EV_0 - EV_{t \times 100}}{EV_0} \quad \ldots(1)$$

where
EV0 = edema volume of negative control group,
EVt = edema volume of test group at a particular time.

To get EV0 and EVt, we subtract the volume of the paws before and after the challenge.

➢ *Egg Albumin Edema Model in Animals*

Although both models are similar, the phlogistic agent in the egg-albumin edema model is raw, undiluted egg albumin, as opposed to carrageenan. Experiment isconducted as previously described and is used to evaluate sub-acute inflammation. Animals are made edematous by subplantarly administering 0.1 mL of fresh egg albumin into each hind paw 30 minutes (or 1 hour if the medicine is administered orally). Paw thickness or volume is calculated properly. Egg albumin causes subacute inflammation, or persistent edema. It's linked to the release of 5-hydroxytryptamine and histamine [23]. Histamine is released as a result of mast cell degranulation stimulated by egg albumin. Blood artery dilatation and permeability have both been related to histamine [27]. The readily available and inexpensive egg albumin, which is simple to extract from raw eggs using a syringe and needle, is one benefit of this approach versus carrageenan edema.

➢ *Dextran-Induced Paw Edema in Rats*

The glucose molecules that make up dextran form a complicated polymer. Some lactic acid bacteria utilize sugar as a precursor to make it. The development of dextran-induced edema is due to an increase in vascular permeability, kinins, and the generation of histamine and 5-hydroxytryptamine by mast cells. Because of the low numbers of neutrophils and protein, osmotic edema develops. It is possible to evaluate the effect of experimental medications on systemic acute inflammation using either the dextran model or the

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carrageenan model. In order to cause inflammation in the subplantar area of the animal paws, test samples and controls are injected with 0.2 mL of 2% dextran solution in normal saline one hour after drug treatment [28].

➢ *Serotonin Paw Edema Model*

The neurotransmitter molecule serotonin, also known as 5-hydroxytryptamine, is connected to mood and overall health. It is a phlogistic agent and one among the messengers of inflammation. In this technique, experimental rats are divided into groups and given oral treatment with test medicines and controls. Following an hour, 0.1 mL of serotonin is injected into the paws of the rats [29-30]. Hence, the first measurement of the paw volume or circumference is taken at 0 hours, and subsequent measurements are taken hourly. As is customary, we use Equation 1 to determine the level of edema inhibition. Due to its serotonin specificity, this method may be used as a confirmatory test for serotonin involvement. But getting your hands on pure serotonin is no picnic.

➢ *Method Involving Histamine as Phlogistic Agent*

One chemical mediator linked to both inflammatory and allergic reactions is histamine, a vasoactive amine. It is a medication that dilates blood vessels. It plays a role in hypersensitivity reactions that are both acute and subacute [30]. Paw edema is thought to develop earlier in chemically induced inflammation, primarily as a result of histamine release. Some other autacoids degranulate the mast cells, which causes histamine release. In the histamine model, the animals' left hind paw's subplantar region is injected with approximately 0.1 ml of a 1% histamine solution (in 0.9% saline solution) one hour after oral therapy. The edema volume is measured prior to the histamine challenge, half an hour later, and then every hour for six hours [30]. The volume of paw edema is determined based on the level of inflammation. It is possible to verify that the test sample is inhibiting histamine using this histamine-specific approach.

➢ *The Formalin-Induced Edema Model*

This approach, which uses formalin as the phlogistic agent, is suitable for assessing medications that prevent chronic inflammation. Formacine has two stages of its nociceptive effect. Tissue-mediated reaction is involved in the later phase, whereas neurogenic component is involved in the early phase. Histamine, 5-HT, and kinin are said to be released during the early phase, and prostaglandin is thought to be involved during the later period [31]. This method involves injecting the rat's paw with a 20 µL new solution of 2% formalin subplantarly in order to induce swelling. The drug is given orally six days in a row, and the volume of the paw is measured every time, one hour after the treatment. Previous descriptions [31] outline the procedure for calculating percentage edema inhibitions. This model's main selling point is that it provides a way to evaluate the effects of drugs on chronic inflammation. It does, however, take a lot of time.

➢ *Agar-Induced Inflammation*

Agarose and agaropectin are two complex carbohydrates combined to form agar. It is linked to vascular inflammation and has several applications in biochemical and microbiological research. For studies involving acute inflammation, the agar model is appropriate. The phlogistic material in this model is a 2% agar suspension (0.1 mL) [32]. Volume of paw edema is calculated correctly. The method's apparent benefits include agar's readily available nature and safety. However, because the right sterile, non-irritating solvent is not easily accessible, agar is injected as a suspension.

➢ *Zymosan-Induced Edema in Rat*

Zymosan reagent is a glycoside derived from yeast cell walls that is extensively utilized in research related to immunology and chronic inflammation. Within 48 hours of injection, it causes arthritis in animals by activating complement via a different route. In 14 days, arthritis goes away. The process involves the infiltration of neutrophils, followed by synovial hyperplasia, macrophage infiltration, and pannus development. For both local and systemic inflammation, the zymosan model is a good fit. Both locally (ear swelling) and systemically (rat paw edema) is it utilized for ear-skin inflammation. It is well known that the administration of zymosan stimulates the complement system, increases NO generation, and encourages enhanced COX-2 activity and prostaglandin formation, mostly of the PGE2 type [33]. By injecting 0.1 mL of a 1% zymosan suspension into the paws of the animals, we may induce systemic edema. Injecting it into one ear and measuring the resulting edema in comparison to the other allows researchers to examine local activity [33].

➢ *Croton Oil-Induced Topical Ear Edema in Mice*

A phlogistic substance for topical inflammatory research, croton oil is similar to xylene. Derived from Croton tiglium seeds, the principal ingredients of this irritating oil are phorbol esters, namely 12-o-tetracanoilphorbol-13-acetate, or TPA. In addition to causing systemic diarrhea and skin inflammation and irritation, TPA triggers protein kinase, which in turn enhances the activities of other enzymes such as phospholipase A2, leading to the creation of platelet activation factor. This sequence of events induces the release of histamine and serotonin, as well as the promotion of vasodilation, polymorphonuclear leukocyte movement, and vascular permeability. Moreover, it encourages COX and 5-lipoxygenase to produce inflammatory eicosanoids in a fair amount [34].

In this topical model, one mouse ear is treated with the test medications on both the inner and outer surfaces, whereas the other ear is treated simply with the vehicle. After around fifteen minutes, 5% (v/v) Croton oil in acetone is topically applied to both ears to cause edema [34]. Six hours after applying Croton oil, the animals are humanely killed, and a 7

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mm diameter disk is extracted with a steel punch from each ear. The weight of the untreated ear is compared to the edema volume calculation. Other suitable phlogistic compounds that are frequently employed for topical anti-inflammatory activity studies include tissue plasminogen activator and ethyl phenylpropionate, in addition to xylene, croton oil, or TPA.

➢ *Cotton Pellet-Induced Granuloma Model*

This model is commonly utilized to evaluate the makeup of chemicals linked to chronic inflammation, both proliferative and transudative. Based on the subcutaneous implantation of cotton pellets in the groin area of the animals, granuloma release is generated. Transudate is positively correlated with the wet weight of the pellets, whereas the size of granulomatous tissues is directly correlated with the dehydrated weight. Development of multiplying cells that can spread or take the shape of granulomas is the cause of chronic inflammation. Nonsteroidal anti-inflammatory medications reduce the growth of granulomas that arise from cellular reactions by inhibiting the penetration of granulocytes, preventing the synthesis of collagen fibers, and suppressing mucopolysaccharides [25]. The test protocol entails a meticulous implantation of approximately 30 milligrams of sterile cotton pellets in the groins of the animals while they are sedated, and then they receive medication for approximately four days. The animals are humanely killed on day five, and the pellets with the accompanying granuloma are carefully removed, dried at 60°C to a consistent mass, and weighed. Inflammation is measured by the size of the granuloma mass that develops [25]. For persistent inflammation, this method works well. The main drawbacks of this approach are that it is time-consuming, expert-only, and necessitates the death of numerous animals.

➢ *Prostaglandin E2 (PGE2)-Induced Paw Edema in Rats*

One commonly utilized prostaglandin in medicine is PGE2, also known as dinoprostone, which occurs naturally. It is a potent inflammatory response mediator that can be utilized to induce edema in animals. One hour following the oral administration of the test medications and controls, the animal paws are subplantarly injected with 100 µl of a 0.01 µg/ml solution of PGE2. Paw edema volume is calculated as a function of inflammation at hourly intervals for six hours [35].

➢ *Vascular Permeability Test*

The contraction and separation of endothelial cells at the blood vessel's borders causes an increase in cell movement in and out of the vessel, exposing the basement membrane and making it readily porous to fluid and plasma proteins [36]. One of the main features of inflammation is exudation, which is brought on by increased vascular permeability. Medication that performs well in this paradigm is probably going to inhibit neutrophil-mediated myeloperoxidase activity and cytokine synthesis that mediates inflammation. They also probably block TNF-α and interleukin-1β, which keeps leukocytes from building up. Vascular permeability is induced by acetic acid immediately and lasts for 24 hours [37]. One hour after the mice are treated with test samples, each mouse receives an intravenous injection of 0.2 ml of 0.25% Evans blue dye in normal saline through the tail vein. After 30 minutes, 1 ml/100 g of acetic acid (0.6%, v/v) is injected intraperitoneally into each animal. The treated animals are mercifully slaughtered after a further half hour.Each animal's peritoneal cavity is cleaned in a test tube using a certain volume of normal saline before being centrifuged. Using spectrophotometry, the quantity of dye in the supernatant (depending on vascular permeability) is determined at 610 nm [38-39].

➢ *Acetic-Acid Induced Paw Edema*

The synthesis of certain prostaglandins, including thromboxanes and leukotrienes, requires acetic acid as an intermediary. These prostaglandins are crucial mediators in the control of signal of these chemicals is assessed [45]. The process involves injecting 0.1 mL of a solution containing 0.5% AA in a 0.2 M carbonate buffer into the animal's paws via the subplantar injection. It is sufficient to evaluate the edema volume every hour after an AA challenge. An intermediary in prostaglandin formation, arachidonic acid is likely to set off the chain reaction. [40].

IV. IN-VITRO MODELS

A. *Anti-Inflammatory*

➢ *Method Involving Phospholipase A2 Enzyme Activity:*

The enzyme known as phospholipase A2, or PLA2, is a lipolytic (fatty acid cleaving) enzyme that catalyzes the hydrolysis of the sn-2 ester link into a variety of distinct phospholipids. The release of arachidonic acid (AA), a crucial precursor in the manufacture of prostaglandins, depends on it [41]. The premise of the enzyme's anti-inflammatory activity assay is that medications that block enzyme activity can prevent the inflammatory mediators from being produced.

The procedure for conducting the test is based on previous research [42]. The test drugs were incubated with a 0.5 mL portion of re-suspended erythrocytes in normal saline, 2 mM calcium chloride, and the bacterial enzyme preparation for 1 hour at 37°C. The supernatant's absorbance was measured spectrophotometrically at 418 nm after a 10-minute centrifugation at 3000×g. Enzymes may induce erythrocyte membrane permeability, which results in hemoglobin spilling into the medium. The quantity of hemoglobin in the media correlates with the enzyme activity. The hemoglobin λmax is 418 nm. As a positive control, a well-known enzyme inhibitor, such prednisolone, is ideal. [42].

➢ *The Mobility Shift Electrophoresis Method*

The electrophoretic mobility shift test is perhaps the most intricate, high-tech, and expensive method now in use for evaluating anti-inflammatory effects. The test evaluates the

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effectiveness of the drugs in blocking NF-κB. It is based on the principle of electrophoretic separation of protein-DNA or protein-RNA mixtures. Combining Jurkat T cells with different dosages of the test drugs and incubating them for one hour according to Aguilar's approach [43] was the protocol followed. The next step was to generate whole cell extracts after stimulating the cells with 200 U/ml of tumor necrosis factor (TNF)-α, a cytokine associated with systemic inflammation. Totex (20 mM Hepes, pH 7.9, 350 mM sodium chloride, 20% (v/v) glycerol, 1% (w/v) Nonidet P-40, 1 mM magnesium chloride, 0.5 mM EDTA, 0.1 mM egtazic acid, 0.5 mM dithiothreitol, 0.1% phenylmethylsulfonyl fluoride, and 1% aprotinin) was utilized in the preparation of the extracts. Following centrifugal separation, the cells were washed in phosphate-buffered saline and reconstituted in four cell volumes of Totex buffer. Centrifuged was the fluid containing the lysed cells after a 30-minute incubation time at 4°C. After determining the protein content of the supernatant, a reaction mixture was produced according to the instructions [15]. Protein concentrations ranging from 10 to 20 µg were then added to it. The fact that this shift was no longer present indicated that anti-inflammatory effect decreased NF-κB activation [43].

- *Hyaluronidase Inhibitory Assay*

There is an enzyme family called hyaluronidases that breaks down hyaluronic acid (HA). Because it is a part of the extracellular matrix of connective tissues, HA facilitates the translocation of inflammatory mediators. Allergies, cancer cell migration, inflammation, and enhanced vascular permeability are inflammatory disorders that it contributes to [44].carrying out HA using cetylpyridinium chloride. This lays the groundwork for screening hyaluronidase inhibitors at high throughput. Following a previously established procedure [44], the test samples and controls were incubated at 37°C for one hour with 800 U/ml of the enzyme solution and 0.40 mg/mL of HA. By precipitating the undigested substrate (HA) with cetylpyridinium chloride, the enzyme activity may be evaluated spectrophotometrically at 415 nm after the process. It was possible to ascertain the enzyme activity by monitoring the concentration of undigested HA substrate in the precipitate and taking readings at 415 nm after the enzyme reaction. We calculated the enzyme activity % using this formula.

$$\% \text{ Enzyme Activity} = \frac{A_0 - A_e \times 100\%}{A_0} \quad \ldots(1)$$

where
Ao = absorbance of pure HA,
Ae = absorbance of HA after enzyme action.

- *Lipopolysaccharide (LPS)-Stimulated RAW 264.7 Cells*

Raised from BALB/c mice, RAW 264.7 cells resemble macrophages. Many studies use murine macrophages as a model to study cellular responses to microbes and their products. In Dulbecco's Modified Eagle's Medium, which is supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin, 4.5 mg/mL l-glutamine, and 4.5 mg/mL glucose solution, the RAW 264.7 macrophages are cultivated. What follows is an incubation period of 37°C in a humid chamber with 5% CO2 and 95% air. This follows a prior report's instructions [45]. We colorimetrically calculated the proportion of live cells using the MTT assay methodology. The RAW 264.7 cell seeding concentration in 96-well plates was 1×104 cells/well. The cells were grown for eighteen hours at 37°C with (LPS, 1 µg/mL) after the test samples and controls were added two hours later. The wells were treated with about 20 µL of MTT solution (5 mg/mL) and then left to incubate at 37°C for an extra four hours. After dissolving in 200 µL of dimethyl sulfoxide, the formazan crystals that formed in each well were colorimetrically quantified at 570 nm. The procedure for determining NO production included priming RAW 264.7 macrophages (5×10^1 cells/mL), placing the macrophages on 48-well plates for two hours, mixing the cells with the test samples and controls, and finally subjecting the cells to 37°C treatment with LPS (1 µg/mL) for eighteen hours. We assessed the quantity of nitrite in the culture supernatants in relation to the anti-inflammatory activity to quantify the amount of NO production using the Griess reagent [45]. The reaction between NO and oxygen produces nitrite.

V. CONCLUSION

There are advantages and disadvantages to every screening method. Although *in vitro* tests are more efficient and cost less, they may not capture the complexities of the human body's physiological milieu. *In vivo* animal models give a more thorough knowledge of the effects of medications, but they may also provide ethical and logistical issues. When used together, these screening methods offer a comprehensive strategy for discovering and developing new anti-inflammatory and analgesic medications. The process starts with in vitro tests to verify the action mechanism, continues with *in vivo* investigations to assess the effectiveness and safety in animals, and finally concludes with clinical trials including humans.

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CONFLICT OF INTEREST

Authors does not have conflict of interest in the publication of this manuscript

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