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EVALUATION OF ANTI –INFLAMATORY AND ANALGESIC ACTIVITY OF PAINOFF BY FOLLOWING ANIMAL MODELS

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Abstract:
There have been several attempts to define pain. McCafferey in the (1972) states that "pain is whatever the experiencing person says it is, existing whenever he or she says it does" . Inflammation is the bodies immediate response to damage to its tissues and cell by pathogens Noxious stimuli such as chemicals are physical injury.It is a protect to attempt by the Organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Information can be classified as either acute or chronic status depending upon onset time Inflammation response is a serious off well coordinate dynamic mechanism consisting of specific vascular humoral and cellular events. Though a variety of chemical mediator or signaling molecule such as histamine, serotonin, leucotrienes, prostaglandins, are involved in the inflammatory response the mechanism of inflammatory injury is attributed in Part to release of ROS from activated neutrophils and macro phases.

Keywords: Painoff, Carrageenan, Poly-herbal, Eddy's hot plate, Nociceptive pain.

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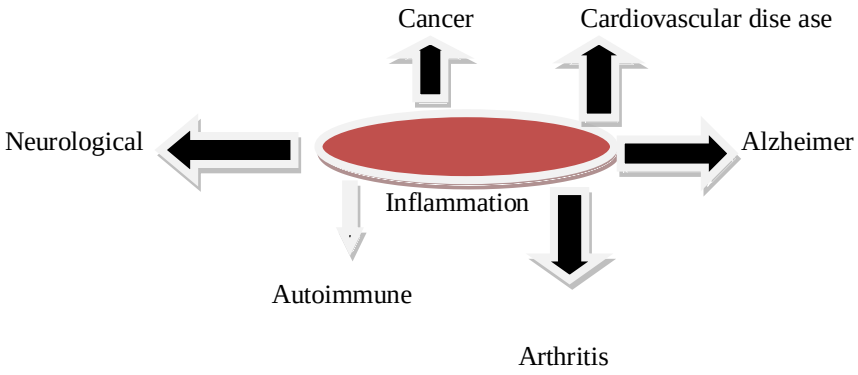
INTRODUCTION:

There have been several attempts to define pain. McCafferey in the (1972) states that "pain is whatever the experiencing person says it is, existing whenever he or she says it does" .The International Association for the study of pain the (IASP) define paint as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage pain is the most common reason for physician consultation it is a major symptom in many medical conditions can significantly interference with a persons quality of life and general functioning. It is a part of the body define system, producing a reflective reaction from the painful stimuli, and tendencies to protect the affected by part while it heals, and avoid that harmful situation in the future. Pain is the most common reason for using complementary and all alarm alternative medicine alternative medicine. Treatment approaches to long term pain include pharmacologic meassures and non pharmacologic measures. The various non pharmacologic measures include interventional procedures physical therapy, physical exercise application of ice and or heat and physiological measures such as biofeedback and cognitive behavioral therapy.

Inflammation is the bodies immediate response to damage to its tissues and cell by pathogens Noxious stimuli such as chemicals are physical injury.It is a protect to attempt by the Organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Information can be classified as either acute or chronic status depending upon onset time acute inflammation is the primary response of the body to injurious steam life and it is involves the local vascular and immune response on the other hand chronic inflammation is a pathological condition characterized by progressive destruction and recovery of the injured tissue from the inflammatory response all the information is a defense mechanism the complex event and mediators involved in formation reaction can induce maintain and aggravate many disorders like arthritis cancer asthma and diabetes.



Inflamation response is a serious off well coordinate dynamic mechanism consisting of specific vascular humoral and cellular events. Though a variety of chemical mediator or signaling molecule such as histamine, serotonin, leucotrienes, prostaglandins, are involved in the inflammatory response the mechanism of inflammatory injury is attributed in Part to release of ROS from activated neutrophils and macro phases. the over production of ROS by macrophages causes oxidative damage to membrane lipids DNA proteins and lipoprotein.

additional neutrophils and macrophages further activate nuclear factor k- β which regulates various celler genes involved in immune and acute phase inflammatory response and in cell survival Survival.

Usually information always associated with pain many allopathic, Ayurvedic drugs were available to treat inflammation and pain separately but they multiple drug therapy cause many side effects all these factors provoked me to research for a drug to treat these symptoms based on the traditional system of usage of medicine a few drugs are available to manage information and pain. Among them pain off is one of the Poly herbal formulations to manage these targets so we selected this Poly herbal formulation painoff for evolution of analgesic and anti-inflammatory activities.

AIM:

Evaluation of anti–inflammatory and Analgesic activity of Pain off by following animal models.

OBJECTIVE:

- To investigate the anti –inflamatory activity of Painoff models of inflammation by Carrageenan induced paw oedema.

- To formulate the poly-herbal formulation of above two extracts.
- To To investigate Analgesic activity of Painoff by Eddy's hot plate method.

Pain:
Pain is a neurobiological phenomenon. It is regulated through Central nervous system and (CNS) Peripheral nervous system (PNS). The drugs which can affect the conduction of pain impulse through this type of nervous system can be used as pain relieving agents list of tricyclic antidepressants (TCA) are reported to process analysis activity by increasing the level of 5- hydroxytryptophan mine(5-HT) and noradrenaline in the brain the available studies about this concept where reviewed.
Definition:

Pain is a complex neurobiological phenomenon. It may also be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage".

Types of pain:
Pain is the most common neurobiological phenomenon observed. Although it may be classified in many ways, pain can aften by categorised as nociceptive, neuropathic, mixed and idiopathic pain.

Nociceptive pain:
Pain is termed nociceptive when they clinical evolution suggests that it is sustained primarily by the nociceptivesystem. Nociceptive pain is pain that is proportionate to the degree of actual tissue damage as a more severe injury result in a pain that is perceived to be greater than that caused by a less severe injury.

Neuropathic pain :
Neuropathic pain occurs through peripheral nervous system changes come such as neuroma formation, generation of ectopic discharge from the injured axon or they somata of the dorsal root ganglion neuron , or through central nervous system changes that can lead to enhanced excitability of central pain network in patient with a prolonged exposure noxious stimuli or nerve injury

Mixed pain :
In a given patient components of continued nociceptive to pain May co-exit with a component of neuropathic pain. Patients with persistent back and leg pain after lumbar spine surgery represent a common example.

Idiopathic pain:
Idiopathic pain may be defined as pain that persist without any identifiable organic relations or that is disproportionate to the degree of tissue damage.
Persistent pain is often described as much more bothersome giving rise to depressed mood sleep disturbances and loss of energy. Typically examples of assisting pain in the orofacial region are temporomandibular disorder, burning mouth syndrome and atypical facial pain which covers variety of diagnoses.
Transient pain in the orofacial region is well known to most individuals, and it is general knowledge that dental treatment processes the risk of a transient pain experience which can be termed procedural pain .

Pain has been classified in many ways.
According to this taxonomy a distinction can be made between transient pain, which refers to the response to a noxious stimulus that not produce a long term sequel, tissue injury pain, and nervous system injury pain.

Pain theories.
Theory of pain mechanism must be consistent with both the physiological evidence and the way in which pain is experienced by patient and described to healthcare practitioner.

Specificity theory.
Specificity theory explains pain in terms of a linear relationship between sensory stimulation and outcome, that is, tissue injury activate specific pain receptors and the sensations is then transmitted via an efferent pathway in the spine.

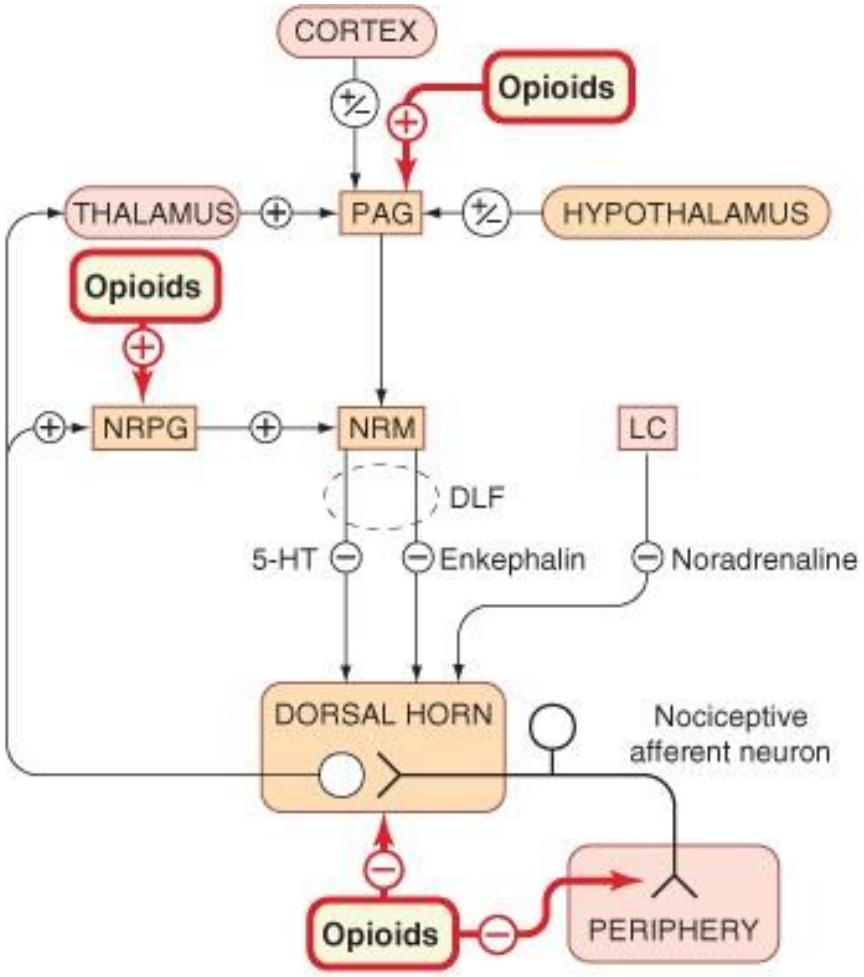
Noxious stimuli activate nociceptor in a in the periphery of the body. According to specificity theory gamma impulses generated by the stimulation of these pain receptors ascending to the brain via multiple pathways in the spinal cord. While the research inspired by the specificity theory provided valuable insight into the physiological mechanism of pain transmission, the theory itself is flawed.

Patterning theories.
In response to the deficiencies of specificity theory . the work of Goldscheidder in 1894 marked the beginning of the development of such theories..

The first and simplest of these theories was peripherally stimulation. Excessive peripheral stimulation of non specific receptors result in the experience of pain. However empirical evidence indicates that this is not true.

Pertaining the represent several improvements over the linear model assumed by specificity theory.

Gate control theory.
Gate control theory was proposed by Melzack and will in 1965 in response to the limitations of existing pain theories stop Melzack and wall presented a three-stage mechanism of pain transmission and perception The a) Nerve impulses arrive from injured tissue to the dorsal horn of the spinal cord and excite the transmission cells which then transmit the impulse to reflects circuits and the brain b) The above mechanism is modulated by low threshold efferent which exit inhibitory interneuron's decreasing the injury related discharge of the T cells c) Activity of decreasing pathway from the central nervous system further modulates the transmission of the sensory neuron.



The basic assumption of the gate control theory is the presence of a get a central mechanism where sensor input is screened the transmission must pass through this gate for the sensation to be registered as pain . the gate is believed to be located in the dorsal horn of the spinal cord, in the substance diyagelatinosa.

Etiology of pain.
Pen Mein arise from different idiologi ease it can be due to the direct effects of a disease or caused by treatment of the disease. surgery, radiation, and therapy may all result in pain. the patient may also have chronic underlying disease such as osteoarthritis cancer neuropathies and vascular insufficiency can cause chronic .

Recent studies have found that some people with chronic pain may have low level of endorphins in the spinal fluid stop endorphins are neurochemical similar to opitate drugs such as morphine.

Epidemiology of pain :
Pain is the most common reason for a patient to seek medical attention. In fact tama about 2 in 6 of the

visit to the primary care physicians are because of acute pain. Chronic pain effects a 3rd of us population. Unfortunately pain off in remains under under treated and continues to be a problem in hospitals come long term care facilities, and the community.

Pain Physiology.

The 4 major modalities of somatosensory sensibility or as follows:

1. Touch sensation that mediates the shape, texture and size of items touching our surface.
2. The Proprioception that gives information about the position and movement of the body.
3. Sense of temperature that mediates the feeling of warm and cold.
4. Nociception that signals pain and itch.

Nociception. Process of Pain Transmission.

1. **Stimulation**. Noxious stimulus sensitizes and are stimulates noisy sector and cause they release of neural chemicals that also sensitize and all stimulate noisy sector. This activation leads to the production of an action potential.

2. **Transmission**. The action potential continues from the site of Noxious stimulus to the dorsal horn of the spinal cord and then a sense to hire centers in the central nervous system. Transmission takes place in at least 5 pathways

a. Spinothalamic tract
b. Spinoreticular track
c. Spinomesecephalic tract
d. dorsal column post synaptic spinomedullay pathway
e. Proprio spinal multi synaptic ascending system

3. **Perception**. Conscious experience of pain.

4. **Modulation**. Inhibition of Nociceptive impulses. New run from the brain stem descend to the spinal cord and release substance such as endogenous opioids , seratonin and nor epinephrine that inhibit transmission of nociceptive impulses.

MODULATION IN NOCICEPTIVE PATHWAY:

Acute pain is generally well accounted for in terms of *nociception*-an excessive noxious stimulus giving rise to an intense and unpleasant sensation. In contrast, most chronic pain states are associated with aberrations of the normal physiological pathway, giving rise to *hyperalgesia* (an increased amount of pain associated with a mild noxious stimulus), *allodynia* (pain evoked by a non-noxious stimulus) or *spontaneous pain* without any precipitating stimulus. An analogy is with an old radio set that plays uncontrollably loudly (hyperalgesia), receives two stations at once (allodynia), or produces random shrieks and whistles (spontaneous pain spasms). These distortions in the transmission line are beginning to be understood in terms of various types of positive and negative modulation in the nociceptive pathway. Some of the main mechanisms are summarized in Figure 2.

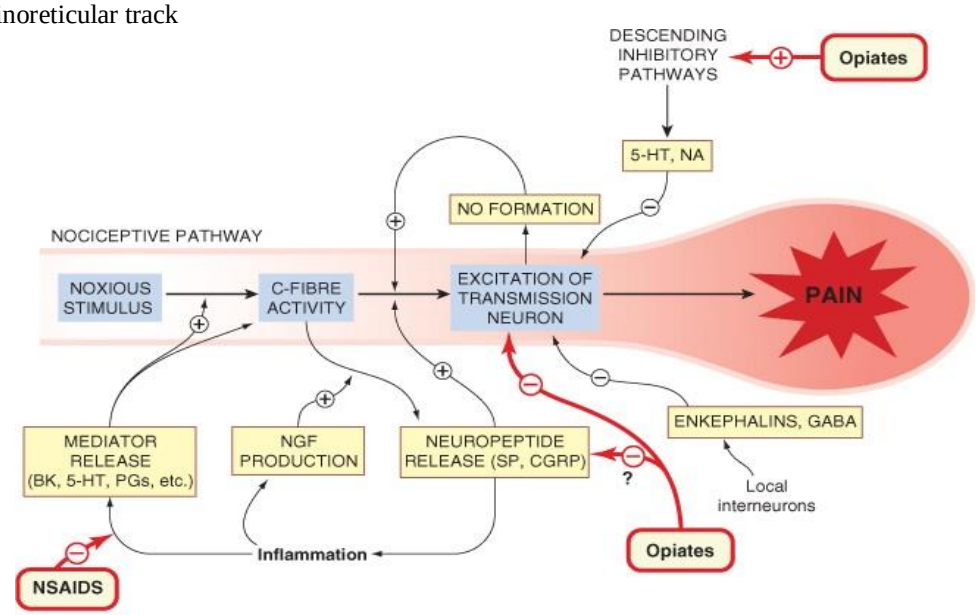


Figure 2

NEUROPATHIC PAIN

Neurological disease affecting the sensory pathway can produce severe chronic pain-termed *neuropathic pain*-unrelated to any peripheral tissue injury. This occurs with central nervous system (CNS) disorders such as stroke and multiple sclerosis, or with conditions associated with peripheral nerve damage, such as mechanical injury, diabetic neuropathy or herpes zoster infection (shingles). The pathophysiological mechanisms underlying this kind of pain are poorly understood, although spontaneous activity in damaged sensory neurons, due to over expression or redistribution of voltage-gated sodium channels, is thought to be a factor. The sympathetic nervous system also plays a part, because damaged sensory neurons can express α adrenoceptors and develop sensitivity to nor-adrenaline (nor-epinephrine) that they do not possess under normal conditions. Thus physiological stimuli that evoke sympathetic responses can produce severe pain, a phenomenon described clinically as *sympathetically mediated pain*. Neuropathic pain, which appears to be a component of many types of clinical pain (including common conditions such as back pain and cancer pain, as well as amputation pain), is generally difficult to control with conventional analgesic drugs.

PAIN AND NOCICEPTION:

As emphasized above, the perception of noxious stimuli (termed *nociception*) is not the same thing as pain, which is a subjective experience and includes a strong emotional (affective) component. The amount of pain that a particular stimulus produces depends on many factors other than the stimulus itself. A stabbing sensation in the chest will cause much more pain if it occurs spontaneously in a middle-aged man than if it is due to a 2-year-old poking him in the ribs with a sharp stick. The nociceptive component may be much the same, but the affective component is quite different. Animal tests of analgesic drugs commonly measure nociception and involve testing the reaction of an animal to a mildly painful stimulus, often mechanical or thermal. Such measures include the tail flick test (measuring the time taken for a rat to withdraw its tail when a standard radiant heat stimulus is applied) or the paw pressure test (measuring the withdrawal threshold when a normal or inflamed paw is pinched with increasing force). Similar tests can be used on human subjects, who simply indicate when a stimulus begins to feel painful, but the pain in these circumstances lacks the affective component. Clinically, spontaneous pain and allodynia of neuropathic origin is coming to be recognised as particularly important, but this is more difficult to model in animal studies. It is recognised clinically that many analgesics, particularly those of the morphine type, can greatly reduce the distress associated with pain even though the patient reports no great change in the intensity of the actual sensation. It is much more difficult to devise tests that measure this affective component, and important to realize that it may be at least as significant as the antinociceptive component in the action of these drugs. There is often a poor correlation between the activity of analgesic drugs in animal tests (which mainly assess antinociceptive activity) and their clinical effectiveness.

TRANSMITTERS AND MODULATORS IN THE NOCICEPTIVE PATHWAY:

The family of opioid peptide plays a key role in nociceptive transmission; its role in descending inhibitory controls is summarized in Figure 4. Opiate analgesics act on the various receptors for these peptides.

Another peptide family thought to play a key role is the tachykinin family, of which substance P is the best-known member. Substance P is expressed by nociceptive afferent neurons and released at their peripheral and central terminals. In the periphery, it produces some of the features of neurogenic inflammation, and in the dorsal horn it may be involved in wind-up and central sensitisation. In animal models, substance P antagonists are effective analgesic drugs, but clinical trials have failed to confirm this in humans, so the high hopes for developing a new type of analgesic for clinical use have been dashed. The reason for this failure is not clear, but it may imply that substance P is less important as a pain mediator in humans than in rats.

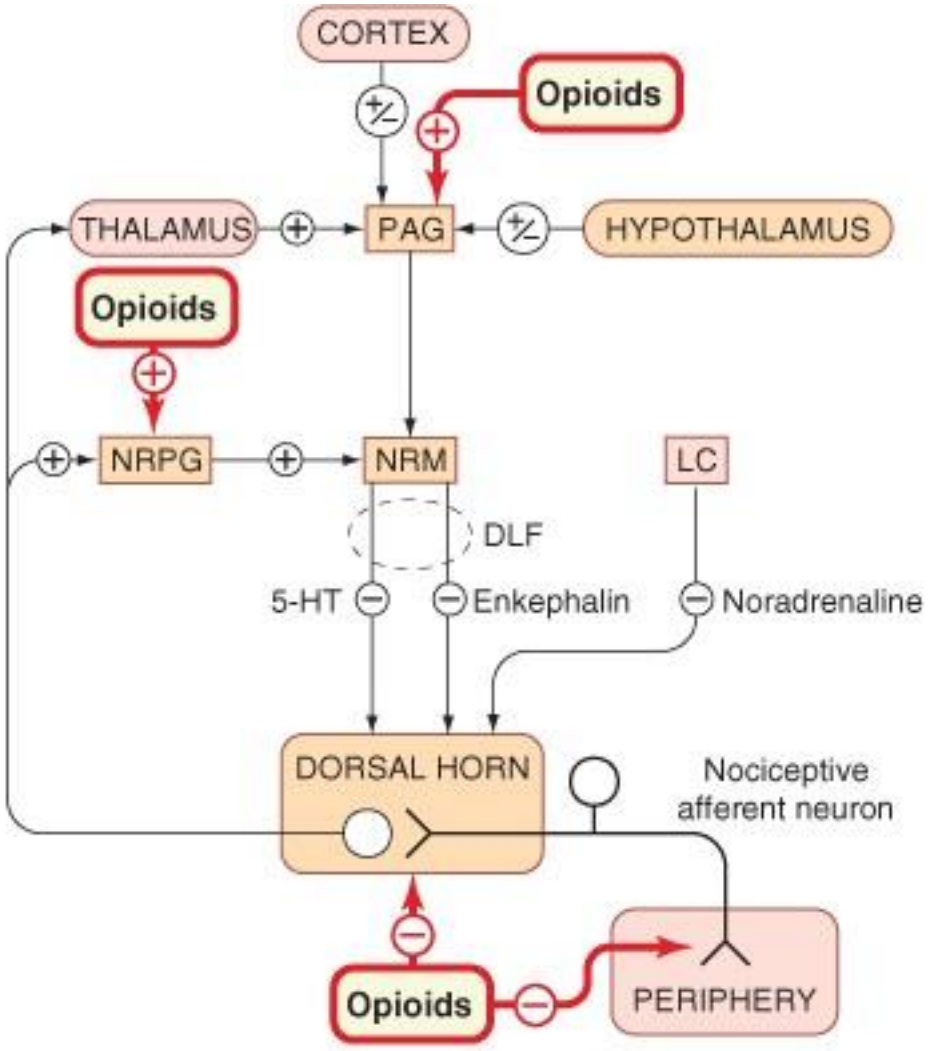


Figure 4

Other mediators include the following:

- Glutamate is released from primary afferent neurons and, acting on AMPA receptors, is responsible for fast synaptic transmission at the first synapse in the dorsal horn. There is also a slower NMDA receptor-mediated response, which is important in relation to the wind-up phenomenon.
- GABA is released by spinal cord interneurons and inhibits transmitter release by primary afferent terminals in the dorsal horn.
- 5-Hydroxytryptamine is the transmitter of inhibitory neurons running from NRM to the dorsal horn.

INFLAMATION:

Inflammation (from Latin: *inflammatio*) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair.

Inflammation can be classified as either *acute* or *chronic*. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by

simultaneous destruction and healing of the tissue from the inflammatory process.

Inflammation has also been classified as Type 1 and Type 2 based on the type of cytokines and helper T cells involved.

Inflammation is not a synonym for infection. Infection describes the interaction between the action of microbial invasion and the reaction of the body's inflammatory response—the two components are considered together when discussing an infection, and the word is used to imply a microbial invasive cause for the observed inflammatory reaction. Inflammation, on the other hand, describes purely the body's immunovascular response, whatever the cause may be. But because of how often the two are correlated, words ending in the suffix *-itis* (which refers to inflammation) are sometimes informally described as referring to infection. For example, the word *urethritis* strictly means only "urethral inflammation", but clinical health care providers usually discuss urethritis as a urethral infection because urethral microbial invasion is the most common cause of urethritis.

Acute inflammation

Acute inflammation occurs immediately upon injury, lasting only a few days. Cytokines and chemokines promote the migration of neutrophils and macrophages to the site of inflammation. Pathogens, allergens, toxins, burns, and frostbite are some of the causes of acute inflammation. Toll-like receptors (TLRs) recognize microbial pathogens. Acute inflammation can be a way tissues are protected from injury. Inflammation lasting 2–6 weeks is designated subacute inflammation.

Chronic inflammation

Chronic inflammation is inflammation that lasts for months or years. Macrophages, lymphocytes, and plasma cells predominate in chronic inflammation, in contrast to the neutrophils that predominate in acute inflammation. Diabetes, cardiovascular disease, allergies, and chronic obstructive pulmonary disease (COPD) are examples of diseases mediated by chronic inflammation. Obesity, smoking, stress, and poor diet.

Mechanism Of Inflammation: Inflammation consists of a tightly regulated cascade of immunological, physiological, and behavioral processes that are orchestrated by soluble immune signaling molecules called cytokines. The first step of the inflammatory cascade involves recognition of infection or damage. This is typically achieved by the detection of pathogen-associated molecular patterns (PAMPs), which are specifically directed toward general motifs of molecules expressed by pathogens that are essential for pathogen survival. Alarmins, or damage-associated molecular patterns (DAMPs), are endogenous molecules that signal damage or necrosis and are also recognized by the innate immune system. An advantage of detecting these signals is that inadvertent targeting of host cells and tissues is minimized. Unlike adaptive immunity, the innate immune system lacks the ability to distinguish among different strains of pathogens and whether such strains are virulent (harmful to the host).

Many damage signals are recognized by germ-line encoded receptors, such as transmembrane Toll-like receptors (TLRs) and intracellular nucleotide binding domain and leucine-rich-repeat-containing receptors (NOD-like receptors or NLRs). Once recognition of ligands occurs, TLRs activate common signaling pathways that culminate in the activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells. This transcription factor is found in virtually all cell types and remains in an inactivated state bound to an inhibitor protein, IκB . Upon transduction of the signal, NF-κB is released from IκB and translocates to the nucleus, where transcription is upregulated through binding to target genes. Importantly, activation of NF-κB does not require new protein synthesis, which permits a rapid response. The NF-κB signaling system is ancient, but there is phylogenetic evidence that regulation of immune function by this pathway in vertebrates evolved independently from invertebrate immune mechanisms. Intracellular NLRs respond to increasing numbers of **DAMPs** that alert the immune system to cell injury and provide a proximate pathway for sensing exposure to possible toxins or pollutants in the environment.

Mediators And Effectors Of Inflammation: Inducers of inflammation triggers the production of numerous inflammatory mediators, which in turn alter the functionality of many tissues and organs via the downstream effectors of the inflammatory pathway. Many of these inflammatory mediators have effects in common on the vasculature and on the recruitment of leucocytes. The cellular mediators can be produced by specialized leucocytes or by cells present in local tissues. Others are performed and stored in the granules of mast cells, basophiles and platelets. Other mediators are produced directly in response to appropriate stimulation by inducers of inflammation. Inflammation can be classified into

seven groups according to their biochemical properties.

Group	Mediators/Effectors	Physiological role
Vasoactive amines	Histamine and serotonin	complex effect on the vasculature, increased vascular permeability, vasodilatation or vasoconstriction.
Vasoactive peptide	Substance P, kinins FibrinopeptideA, Fibrinopeptide B	Mast-cell degranulation vasodilation and increased vascular permeability
Complement fragment	C3a, C4Aand C5a	promote granulocyte ……. etc.

Role Of ROS In Inflammation

Reactive oxygen species (ROS) are key signaling molecules that play an important role in the progression of inflammatory disorders. An enhanced ROS generation by polymorphonuclear neutrophils (PMNs) at the site of inflammation causes endothelial dysfunction and tissue injury. The vascular endothelium plays an important role in passage of macromolecules and inflammatory cells from the blood to tissue. Under the inflammatory conditions, oxidative stress produced by PMNs leads to the opening of inter-endothelial junctions and promotes the migration of inflammatory cells across the endothelial barrier. The migrated inflammatory cells not only help in the clearance of pathogens and foreign particles but also lead to tissue injury. The current review compiles the past and current research in the area of inflammation with particular emphasis on oxidative stress-mediated signaling mechanisms that are involved in inflammation and tissue injury.

ROS are classically defined as partially reduced metabolites of oxygen that possess strong oxidizing capabilities. They are deleterious to cells at high concentrations but at low concentrations (exact concentrations still remaining to be defined), they serve complex signaling functions. They are injurious, because they oxidize protein and lipid cellular constituents and damage the DNA. At "physiological concentrations," ROS function as signaling molecules that regulate cell growth, the adhesion of cells toward other cells, differentiation, senescence, and apoptosis. The concept of chronic or prolonged ROS production is considered central to the progression of inflammatory disease. What are the biologically relevant ROS? The widely studied and understood family members are the superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl) . Although others may be important in signaling and disease , their functions remain poorly understood. ROS are generated as byproducts of cellular metabolism through the electron transport chain (ETC) in mitochondria as well as *via* the cytochrome P450. The other major source, where ROS are not produced as by products, are the NADPH oxidases that are present in a variety of cells, especially the professional phagocytes and endothelial cells , which are central to the genesis of the inflammatory response .

$O_2^{\bullet-}$ is generated by one-electron reduction of O_2 through enzymatic catalysis by NADPH oxidase or xanthine oxidase (XO) or during electron transfer reactions in the ETC of mitochondria. $O_2^{\bullet-}$ has a half life of 10^6 ns , as it undergoes spontaneous dismutation to H_2O_2 (under physiological conditions $k=2\times10^5\ M^{-1}s^{-1}$). This reaction can be accelerated to 10^4-fold by the enzyme SOD ($K=1.6\times10^9\ M^{-1}s^{-1}$) . In the presence of the transition metal iron, $O_2^{\bullet-}$ and H_2O_2, in turn, generate the highly reactive OH^- and $OH^{\bullet}$(Haber–Weiss reaction). In the first step of this reaction, $O_2^{\bullet-}$ reacts with Fe^{3+} to form Fe^{2+} and O_2. However, this reaction is thermodynamically unfavorable under the physiological conditions. The second step of this reaction is also known as Fenton's reaction and occurs under the biological conditions in which Fe^{2+} reacts with H_2O_2 to form both $OH^{\bullet}$ and OH^- .$OH^{\bullet}$ is defined as the most potent oxidizing species of biological membrane proteins and lipids and has an extremely short half-life . At inflammatory sites where PMN are abundant, H_2O_2 and chloride generate HOCl by the enzyme myeloperoxidase, generally considered as being a PMN-specific enzyme. The passage of $O_2^{\bullet-}$ across biological membranes is highly restricted because of its negative charge. However, certain transmembrane proteins, such as voltage-dependent anion channels (VDAC) found in mitochondria, allow trans-

membrane passage of $O_2^{\bullet-}$ produced in ETC . H_2O_2, on the other hand, can cross biological membranes through aquaporin channels such as AQP3 and AQP8 which mediate membrane H_2O_2 uptake, raising the possibility that it can enter cells that are contacting one another .

Role Of Antioxidants In Inflammation

A free radical is a molecule or atom that carries one or more unpaired electrons and is able to exist independently. Meanwhile, free radicals have an odd number of electrons; this makes them short lived, highly reactive, and unstable. Consequently, it can react quickly with other substances trying to catch the required electron to obtain stability. Free radical can become balanced by attacking the closest stable molecule and "stealing" its electron. Meanwhile the attacked molecule can become a free radical by losing its electron and start a chain reaction cascade causing damage to the living cell . Examples of free radicals are hydroxyl free radical, superoxide free radical anion, lipid peroxyl, lipid peroxide, and lipid alkoxyl. Reactive oxygen species (ROS) are radical derivatives such as singlet oxygen and hydrogen peroxide

Normal cellular metabolism produces ROS and these play crucial roles in activation of signaling pathways in animal and plant cells which alter the intra- and extracellular metabolism. Almost most of the ROS are produced in cells through the mitochondrial respiratory chain . During endogenous metabolic reactions, aerobic cells generate ROS (e.g., superoxide anion, hydrogen peroxide (H_2O_2), and hydroxyl radical and organic peroxides) as the usual products of biological diminution of molecular oxygen. Within hypoxic situation, the mitochondrial respiratory chain also generates nitric oxide (NO), which can produce other reactive nitrogen species (RNS) . RNS can produce other additional reactive species, for example, reactive aldehydes-malondialdehyde and 4-hydroxynonenal, by inducing excessive lipid peroxidation. Lipids and proteins are important targets for oxidative attack and alteration of these molecules can enhance the mutagenesis process

Review of painoff formulation:

The painoff formulation has the following composition and past literature on these individual compounds have the different pharmacological activities. The these individual compounds were screened for different activities and proved for those screened activities successfully. the composition and pharmacological activities individual constituents of the painoff formulation based on the literature as follows.

S.no	Name of constituent	Quantity	Pharmacological activities
01	Shudda guggulu	250mg	Anti-inflammatory, hypolipidemic activites
02	musthak	5mg	Hypoglycemic, immune modulatory, anti inlamatory activities.
03	shunti	20mg	immune modulatory and anti-fungal activities.
04	maricha	20mg	Anticonvulsant and analgesic activities.
05	pippali	20mg	Anti-inflammatory and analgesic activities.
06	arjuna	10mg	cardiotonic
07	suranjan	50mg	immune modulatory and anti-oxidant activities
08	cashun	10mg	analgesic and anxiolytic activities.
09	rasna	10mg	immune modulatory and anti-oxidant activities.
10	aswagandha	10mg	Anti-stress and immune modulatory activities

MATERIALS AND METHOD:

Drug And Chemicals:

1. Pentazocin inj.(Ranbaxy laboratories limited)
2. Indomethacin capsule .(Ranbaxy laboratories limited)

All other chemicals used for this investigation were of analytical grade from S.D fine chemicals, Mumbai,india.

Experimental Animals and Housing of Animals:

Albino wister rats weighing 150±25g of either sex and swiss albino mice weighing 20±5 gm was used for the study in different models. The animals were procured from national institute of nutrition (Hyderabad) 2 weeks prior to the study, so that animals could acclimatize to the new environment.

Animals kept in well-maintained room under standard hygienic conditions. Commercial pellet diet and water were made available ad libitum . they were housed in propylene cages (32x24x16cm) with stainless steel grill top, bedded with rice husk.

Dosing calculation:

the dosage for the Poly Herbal Formulation (PHF) painoff for an adult human is given as 06 tablets per a day each tablet weighs 500mg.

the human adult dose of PHF painoff is 6X500mg=3,000mg/day

conversion factor for human (70kg) dose to rat(200gm) dose is 0.018

from the above factor for dose of PHF painoff for 200gms rat is as follows

3000x0.018=54mg/200gm rat and it will be converted for 01 kg body weight rat then **270mg/kg body weight rat**.

In the similar manner the conversion factor for human (70kg)) dose to mice (20gm) dose is 0.0026

from the above factor for dose of PHF painoff for 20gms mice is as follows

3000x0.0026=7.8mg/20gm mice and it will be converted for 01 kg body weight rat then **390mg/kg body weight rat**.

Screening of anti-inflammatory activity:

Carageenan induced hind paw edema in rats

Albino wister rats weighing between 150-200gms werec divided into 4 groups of 6 rats each:three animals being housed in labeled cage each.

GROUP TREATMENT:

The animals were divided into 4 groups, each group containing 6 animals. The group animals were treated as follow:

Group I : Animals were administered 0.1ml saline p.o

Group II: Animals were administered 0.1ml saline p.o

Group III : Animals were administered standard(indomethacin10mg/kg) p.o

Group IV : Animals were administered painoff (270 mg/kg) p.o

Procedure:

All the rats of II,III,IV(except I group) groups were injected with 0.1 ml of carageenan (1%) in normal salineinto sub planter of right hind paw.

Paw volume was measured by mercury plethysmograph at 0,1,2,3,6 hrs after the carageenan injection.

SCREENING OF ANALGESIC ACTIVITY:

EDDY'S HOT PLATE METHOD

swiss albino mice weighing between 20±5 gms were divided into 3 groups of 6 mice each:three animals being housed in labeled cage each.

GROUP TREATMENT:

The animals were divided into 4 groups, each group containing 6 animals. The group animals were treated as follow:

Group I : Animals were administered 0.1ml saline p.o

Group II : Animals were administered standard(Pentazocin 5mg/kg) I.P

Group III : Animals were administered painoff (390 mg/kg) p.o

the time for licking paws or jumping in hot plate was recorded as response , prior and 0,30,60,90,120 min after administration of respective drugs.

STATISTICAL ANALYSIS:

All the data's were analyzed using One-Way ANOVA method followed by Dunnet's/Tukey's test . all the values were reported as mean ±sSEM .P≤0.05was considered to be statistically significant

RESULTS:

Anti-inflammatory activity:

Carageenan induced paw edema in rats:

After treatment with the respective drugs the paw volumes were measured at different intervals by using plethysmograph and mercury as solvent in Plethysmograph at room temperature the following observation were observed and tabulated in the table -01.

Carageenan induced paw edema painoff significantly inhibited the edema as shown in table .1. paw volume in normal control group rats on 2nd hr was found to be 0.2148±0.0122ml.the paw volume in rats pretreated with painoff (270 mg/kg/day) and indomethacin (10mg/kg/day at 2nd hr were found to be 0.191±0.0064 ml and 0.1369±0.0054** ml. the effectiveness of the tested drug i.e PHF painoff and the standard drug indomethacin also have shown their maximum effect at 02 hrs only when compared with other intervals. The table 021 shows the paw edema volumes at different intervals for all the groups of animals.

Table 1: anti-inflammatory effect of PHF pain off on Carageenan induced paw edema in rats

Treatment	Paw volume in ml at different Hrs (Mean± S.E.M)				
	0	**1**	**2**	**3**	**4**
Normal control	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058
Inflammatory control	0.1225± 0.0079++	0.1876± 0.007	0.2148± 0.012+++	0.2083± 0.0094++	0.165± 0.0076
Indomethacin 10mg/kg,p.o	0.1249± 0.0061	0.1427± 0.0071**	0.1369± 0.0054**	0.1442± 0.007**	0.1449± 0.0060
Painoff(270mg/kg)	0.1210± 0.0186	0.152± 0.008	0.191± 0.0061	0.196± 0.006	0.159± 0.009*

Values are expressed as (mean± S.E.M) n=6; one way ANOVA followed by Tukey's test. +++ P<0.001 V_s normal control & **P<0.01 V_s inflammatory control.

Analgesic activity:

EDDY'S HOTPLATE METHOD:

Painoff showed maximum analgesic activity at 60,90 min for 270mg/kg dose. The reaction time in normal control group at 60,90 min was found to be 3.52±0.002, 4.08±0.0161sec. the reaction time (paw licking/jumping response) in mice pretreated with painoff 270mg/kg and pentazocine (5mg/kg) at 60,90 min were found to be 9.26±0.0851,, 9.82±0.0894 and 8.60±0.0992, 14.12±0.3.182 respectively when compared to control group rats.

The duration of analgesic effect was maximum for painoff 270mg/kg at 60, min when compared with other intervals reference drug pentazocine at 5mg/kg dose significantly increased the reaction time at 90 min as shown in table .2.

Table.2.: Effect of painoff on reaction time (sec) in Eddy's hot plate

Treatment	Rection time in seconds				
	0	**30**	**60**	**90**	**120**
Normal control	3.51± 0.277	3.80± 0.343	3.52± 0.455	4.08± 0.161	3.93± 0.067
Pentazocin 5mg/kg,	4.11± 0.238	6.64± 0.430**	9.82± 0.894**	14.12± 3.182**	9.41± 0.650**
Painoff(100mg/kg)	4.02± 0.194	5.01± 0.332	9.26± 0.851**	8.60± 0.992	6.30± 0.259**

Values are expressed as (mean± S.E.M) n=6; one way ANOVA followed by Dunnet's test. **P< 0.001 V_s normal control & *P< 0.05 V_s inflammatory control.

The development of edema in the paw of the rat after injection of carageenan is a abiphasic event. The initial phase of the edema has been attributed to the release of histamine and serotonin, the edema maintained during the plateau phase to kinin like substance and second accelerating phase of swelling to the release of prostaglandin like substance. Inhibition of edema observed in various inflammatory models induced experimentally in the present study may, therefore be attributed to the ability of the painoff to inhibit various chemical mediators of inlamation like histamine and5-HT during the initial phase.

In the present study painoff significantly increased the reaction time in hot plate test suggesting its central analgesic activity:the probable mechanism could be by inhibition of prostaglandin synthesis. Prostaglandins play significant role in different phases of inflammatory reactions and elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli.

CONCLUSION:

The finding in this study suggest that the painoff possess anti-inflammatory and analgesic activity.
The result have been obtained in carefully controlled experiments with laboratory animals where psychological factors can presumably be ruled out. In all the tests the responses have been assessed by actual measurement and not by subjective comparison which may be influenced by the observer. Therefore the statistical validity of the findings has been proved and they provide a scientific foundation for the use of the biologically active ingredient pof painoff in anxiety, inflammatory and pain conditions and explain the clinical effectiveness of the painoff.

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