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TO STUDY THE ANALGESIC ACTIVITY OF A POLY-HERBAL EXTRACT IN SWISS ALBINO MICE

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INTRODUCTION

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases.

India is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. India officially recognizes over 3000 plants for their medicinal value.

It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the third world countries. Medicinal herbs have been in use in one form or another, under indigenous systems of medicine like Ayurveda, Sidha and Unani. India, with its traditional background, needs to increase its share in the world market. India is also one of the twelve mega biodiversity countries in the world. The total number of plant species of all groups recorded from India is 45,000.[1]

Pain, heat, redness, and swelling are the classic manifestations of the inflammatory process. Abnormalities of the joints of the spine, associated muscles, tendons, ligaments and bone structural abnormalities can all result in pain and need for neurosurgical consultations. Typically, patients will not require immediate surgical intervention, and therefore require treatments to reduce pain and enhance quality of life activities.[2]

In most cases, the genesis of pain is inflammatory, regardless of the etiology. With the elucidation of the role of inflammatory cytokines, there is now a clear understanding of the pathways by which many anti-inflammatory drugs can alleviate inflammation and relieve pain.

The use of non-steroidal anti-inflammatory drug (NSAID) medication is still the mainstay of most classically taught clinicians for joint and spine related inflammatory pain, despite their commonly known side effects.[3]

NSAIDs are effective in the temporary treatment of moderate pain but have the potential for long-term side effects, as follow.[4]

- Cause stomach disorders.
- Long-term use may cause kidney disorders.
- May induce high blood pressure.
- Over the long term they may even accelerate the course of joint degeneration.
- The NSAIDs typically cause some stomach upset. Cause ulceration and bleeding in the stomach.
- Cause tiny pinpoint perforations in the surface of the small intestine. This can induce "leaky gut syndrome," which is thought to be part of the mechanism of allergy, autoimmune disease, and even arthritis itself.
- Long-term use of NSAIDs may weaken the intestinal barrier. This allows allergenic substances to pass that may actually promote inflammation in the joints.

The opioid analgesics are employed to alleviate severe pain conditions such as acute myocardial infarction, fractures of long bones, burns, terminal stages of malignancy, pulmonary embolism, acute pericarditis and spontaneous pneumothorax. But there are some potential adverse effects with these drugs. They are as follow.[5]

- Intolerance
- CNS effects like dysphoria, mental clouding, vertigo, nausea and vomiting, headache, fatigue, and paraesthesiae.
- Respiratory depression

- Constipation
- Hypotension
- Urinary retention
- Tolerance
- Drug dependence

As the above drugs are used in management of pain but they cause some adverse effects. So, It is worthwhile to look for an alternative for the management of pain, therefore phytotherapy is being sought.

In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants[6]. These plant products are reported to be effective in decreasing the recurrence rate of pain with no side effects.

AIM AND OBJECTIVE

Aim

To study the Analgesic activity of a poly-herbal extract in Swiss albino mice.

Objective

- To prepare the aqueous extracts of dried bark powder of *Mangifera indica* and dried flower buds of *Syzygium aromaticum.*
- To formulate the poly-herbal formulation of above two extracts.
- To study the Analgesic activity of the poly-herbal extract in Swiss albino mice.

LITERATURE REVIEW

Pain[7]

The international association for the study of pain has defined pain as "an unpleasant and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".

Neural Mechanisms of Pain[8]

Pain is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia), or persist long after the precipitating injury has healed (e.g. phantom limb pain). It can also occur as a consequence of brain or nerve injury (e.g. following a stroke or herpes infection). Painful conditions of the latter kind, not directly linked to tissue injury, are very common and a major cause of disability and distress, and in general they respond less well to conventional analgesic drugs than do conditions where the immediate cause is clear. In these cases, pain may be considered in terms of disordered neural function, comparable with schizophrenia or epilepsy, rather than simply as a 'normal' response to tissue injury. Therefore it is useful to distinguish two components, either or both of which may be involved in pathological pain states.

- The peripheral nociceptive afferent neuron, which is activated by noxious stimuli.
- The central mechanisms by which the afferent input generates a pain sensation.

Nociceptive Afferent Neurons

Under normal conditions, pain is associated with impulse activity in small-diameter primary afferent fibres of peripheral nerves. These nerves have sensory endings in peripheral tissues and are activated by stimuli of various kinds (mechanical, thermal, chemical). They are distinguished from other sorts of mechanical and thermal receptors by their higher threshold, because they are normally activated only by stimuli of noxious intensity-sufficient to cause some degree of tissue damage. Recordings of activity in single afferent fibres in human subjects have shown that stimuli sufficient to excite these small afferent fibres also evoke a painful sensation. Many of these fibres are non-myelinated C fibres with low conduction velocities (< 1 m/s); this group is known as *C polymodal nociceptors*. Others are fine myelinated (Aδ) fibres, which conduct more rapidly but respond to similar peripheral stimuli. Although there are some species differences, the majority of the C fibres are associated with polymodal nociceptive endings. Afferents from muscle and viscera also convey nociceptive information. In the nerves from these tissues, the small myelinated Aδ fibres are connected to high-threshold mechanoreceptors, while the non-myelinated C fibres are connected to polymodal nociceptors, as in the skin.

Experiments on human subjects, in which recording or stimulating electrodes are applied to cutaneous sensory nerves, have shown that activity in the Aδ fibres causes a sensation of sharp, well-localized pain, whereas C fibre activity causes a dull, diffuse, burning pain.

With many pathological conditions, tissue injury is the immediate cause of the pain and results in the local release of a variety of chemicals that act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation. The pharmacological properties of nociceptive nerve terminals are discussed in more detail below.

The cell bodies of spinal nociceptive afferent fibres lie in dorsal root ganglia; fibres enter the spinal cord via the dorsal roots, ending in the grey matter of the dorsal horn (figure 1). Most of the nociceptive afferents terminate in the superficial region of the dorsal horn, the C fibres and some Aδ fibres innervating cell bodies in laminae I and II, while other A fibres penetrate deeper into the dorsal horn (lamina V). Cells in laminae I and V give rise to the main projection pathways from the dorsal horn to the thalamus.

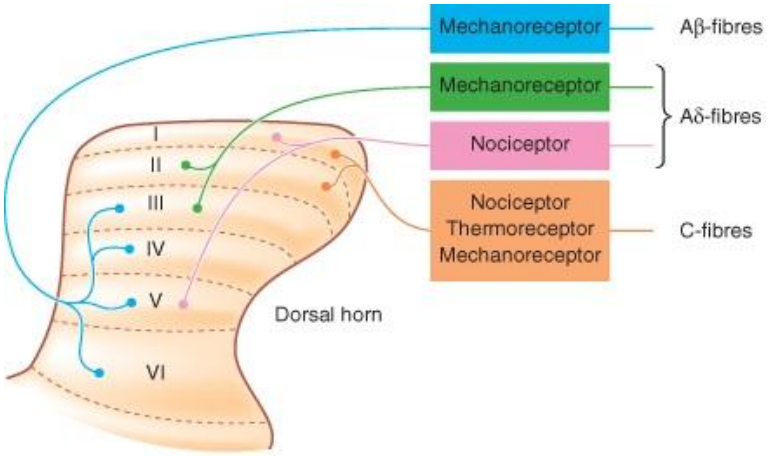


Figure 1:

The non-myelinated afferent neurons contain several neuropeptides, particularly substance P and calcitonin gene-related peptide (CGRP). These are released as mediators at both the central and the peripheral terminals, and play an important role in the pathology of pain.

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.

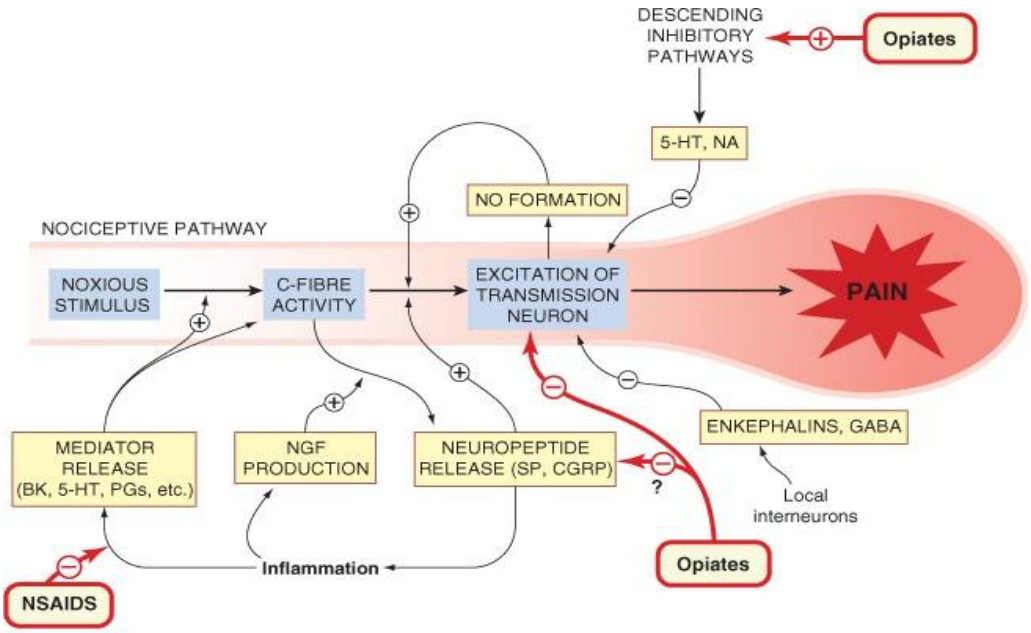


Figure 2:

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.

Chemosensitivity of nociceptive nerve ending

In most cases, stimulation of nociceptive endings in the periphery is chemical in origin. Excessive mechanical or thermal stimuli can obviously cause acute pain, but the persistence of such pain after the stimulus has been removed, or the pain resulting from inflammatory or ischemic changes in tissues, generally reflects an altered chemical environment of the pain afferents. The field was opened up in the 1960s by Keele and Armstrong, who developed a simple method for measuring the pain-producing effect of various substances that act on cutaneous nerve endings. They produced small blisters on the forearm of human subjects, and applied chemicals to the blister base, recording the degree of pain that the subjects reported. Since then, electrical recording from sensory nerves and studies of the membrane responses of neurons in culture, coupled with molecular biology techniques to identify receptors and signal transduction pathways in nociceptive neurons, have produced a wealth of new information, and the humble nociceptive neuron has bathed in a limelight that more aristocratic neurons might envy. Figure 3.

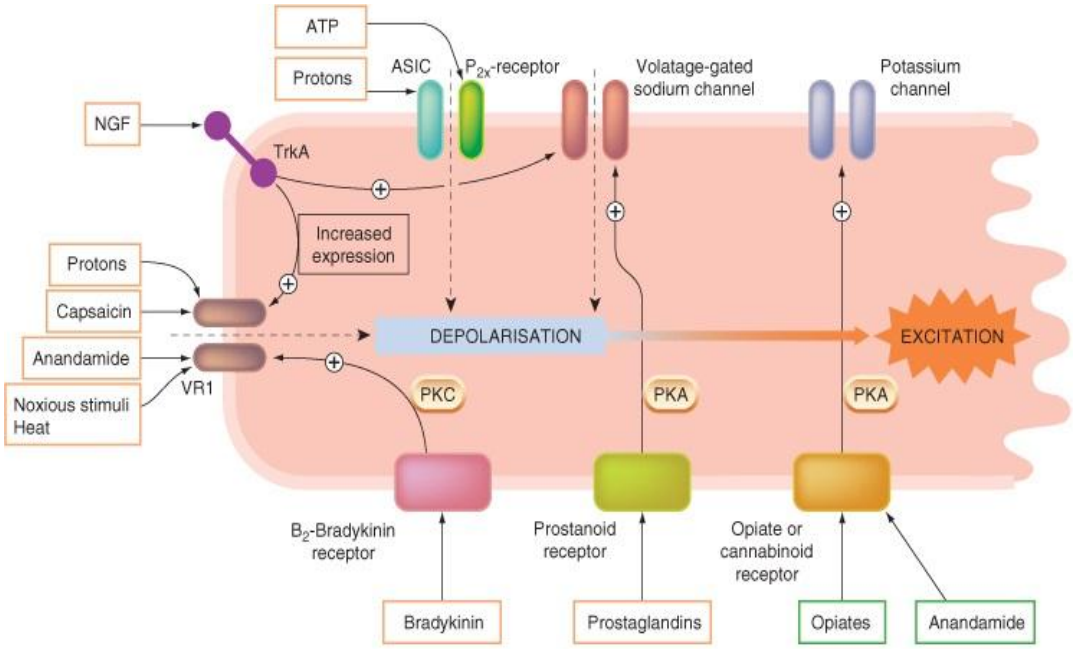


Figure 3:

The main groups of substances that stimulate pain endings in the skin are discussed below.

The vanilloid receptor (TRPV1)

- Capsaicin the substance in chili peppers that gives them their pungency, selectively excites nociceptive nerve terminals, causing intense pain if injected into the skin or applied to sensitive structures such as the cornea. It produces this effect by binding to a receptor expressed by nociceptive afferent neurons. The receptor, originally known as the vanilloid receptor because many capsaicin-like compounds are based on the structure of vanillic acid, is a typical ligand-gated cation channel known as the *transient receptor potential vanilloid receptor 1* (TRPV1). Agonists such as capsaicin open the channel, which is permeable to Na^+, Ca^{2+} and other cations, causing depolarization and initiation of action potentials. TRPV1 responds not only to capsaicin-like agonists but also to other stimuli, including temperatures in excess of about 45°C (the threshold for pain) and proton concentrations in the micromolar range (pH 5.5 and below), which also cause pain. The receptor thus has unusual 'polymodal' characteristics that closely match those of nociceptive neurons, and it is believed to play a central role in nociception. TRPV1 is, like many other ionotropic receptors, modulated by phosphorylation, and several of the pain-producing substances that act through G-protein-coupled receptors (e.g. bradykinin) work by sensitising TRPV1. A search for endogenous ligands for TRPV1 revealed, surprisingly, that **anandamide** (a lipid mediator previously identified as an agonist at cannabinoid receptors is also a TRPV1 agonist, although less potent than capsaicin . Other endogenous lipid mediators, collectively known as *endovanilloids* have since been identified, but their role in nociception is not currently known. Confirming the role of TRPV1 in nociception, it has been found that TRPV1 knockout mice show reduced responsiveness to noxious heat and also fail to show thermal hyperalgesia in response to inflammation. The latter observation is interesting, because TRPV1 expression is known to be increased by inflammation and this may be a key mechanism by which primary hyperalgesia is produced.
- The TRPV1 channels may represent the common pathway through which many pain-producing mediators exert their excitatory effects on nociceptors, and are considered to be a possible target for future analgesic drugs.

Capsaicin and related irritant substances

- Capsaicin is a potent TRPV1 agonist that selectively stimulates nociceptive nerve endings, as described above. Similar substances exist in other pungent plants (ginger, black pepper, etc.), but none are as potent as capsaicin **Resiniferatoxin**, a compound produced by some plants of the *Euphorbia* family, whose sap causes painful skin irritation, is so far the most potent agonist known.
- There are several interesting features of the action of capsaicin .
- The large influx of Ca^{2+} into nerve terminals that it produces results in peptide release (mainly substance P and CGRP), causing intense vascular and other physiological responses. The Ca^{2+} influx may be enough to cause nerve terminal degeneration, which takes days or weeks to recover. Attempts to use topically applied capsaicin to relieve painful skin conditions have had some success, but the initial strong irritant effect is a major disadvantage.
- Capsaicin applied to the bladder causes degeneration of primary afferent nerve terminals, and has been used to treat incontinence associated with bladder hyper-reactivity in stroke or spinal injury patients. C-fibre afferents in the bladder serve a local reflex function, which promotes emptying when the bladder is distended, the reflex being exaggerated when central control is lost.
- Given to neonatal animals, capsaicin causes an irreversible loss of polymodal nociceptors, because the cell bodies (not just the terminals) are killed. The animals grow up with greatly reduced responses to painful stimuli. This has been used as an experimental procedure for investigating the role of these neurons.
- Unlike mammals, birds do not respond to capsaicin because avian TRPV1 differs from mammalian TRPV1. Consequently, birds eat chili peppers and distribute their seeds, while mammals (other than humans-the only masochistic mammal) avoid them.

Kinins

The most active substances are **bradykinin** and **kallidin**, two closely related peptides produced under conditions of tissue injury by the proteolytic cleavage of the active kinins from a precursor protein contained in the plasma. Bradykinin is a potent pain-producing substance, acting partly by release of prostaglandins, which strongly enhance the direct action of bradykinin on the nerve terminals. Bradykinin acts by combining with specific G-protein-coupled receptors, of which there are two subtypes, B_1 and B_2. In nociceptive neurons, B_2-receptors are coupled to activation of a specific isoform of protein kinase C (PKCε), which phosphorylates TRPV1 and facilitates opening of the TRPV1 channel.

Bradykinin acts on B_2-receptors but is converted in tissues by removal of a terminal arginine residue to des-Arg bradykinin, which acts selectively on B_1-receptors. B_2 and B_1-receptors are both involved in the pathogenesis of pain and inflammation. B_1 receptors are unusual in that they are normally expressed at very low levels, but their expression is strongly up-regulated in inflamed tissues. Transgenic knockout animals lacking either type of receptor show reduced inflammatory hyperalgesia. Specific competitive antagonists for both B_1 and B_2- receptors are known, including peptides such as the B_2 antagonist **icatibant**, as well as non-peptides. These show analgesic and anti-inflammatory properties, and may prove suitable for clinical use as analgesics.

Prostaglandins

Prostaglandins do not themselves cause pain, but they strongly enhance the pain-producing effect of other agents such as 5-hydroxytryptamine or bradykinin. Prostaglandins of the E and F series are released in inflammation and also during tissue ischemia. They sensitise nerve terminals to other agents partly by inhibiting potassium channels and partly by facilitating-through second messenger-mediated phosphorylation reactions the cation channels opened by noxious agents. It is of interest that bradykinin itself causes prostaglandin release, and thus has a powerful 'self-sensitising' effect on nociceptive afferents. Other eicosanoids, including prostacyclin, leukotrienes and the unstable hydroxyeicosatetraenoic acid (HETE) derivatives, may also be important. The analgesic effects of NSAIDs result from inhibition of prostaglandin synthesis.

Other peripheral mediators

Various metabolites and substances are released from damaged or ischemic cells, or inflamed tissues, including ATP, protons (produced by lactic acid), 5-hydroxytryptamine, histamine and K^+, many of which affect nociceptive nerve terminals.

ATP excites nociceptive nerve terminals by acting on P_{2X3} receptors, a form of ligand-gated ion channel that is selectively expressed by these neurons. Down-regulation of P_{2X3} receptors, by antisense DNA technology, reduces inflammatory pain. Antagonists at this receptor may be developed for clinical use. ATP and other purine mediators, such as adenosine also play a role in the dorsal horn, and other types of purinoceptor may also be targeted by analgesic drugs in the future.

Low pH excites nociceptive afferent neurons partly by opening proton-activated cation channels (acid-sensitive ion channels) and partly by facilitation of TRPV1.

5-Hydroxytryptamine causes excitation, but studies with antagonists suggest that it plays at most a minor role. Histamine is also active but causes itching rather than actual pain. Both these substances are released locally in inflammation.

Opioid peptides released peripherally inhibit nociceptor excitability, as do cannabinoids. These agents act through G-protein-coupled receptors that are negatively coupled to Adenylate cyclase, and hence their effects oppose those of prostaglandins. The physiological significance of these mediators in the periphery is uncertain.

In summary, pain endings can be activated or sensitised by a wide variety of endogenous mediators, the receptors for which are often up- or down-regulated under pathophysiological conditions. Neuroplasticity plays an important role in persistent pain states, irrespective of their primary cause; not surprisingly, the signaling pathways have much in common with, and are at least as complex as, those involved in other neuroplasticity-based CNS pathologies discussed in earlier chapters. The strategies for developing the next wave of analgesic drugs therefore follow similar lines.[4]

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.

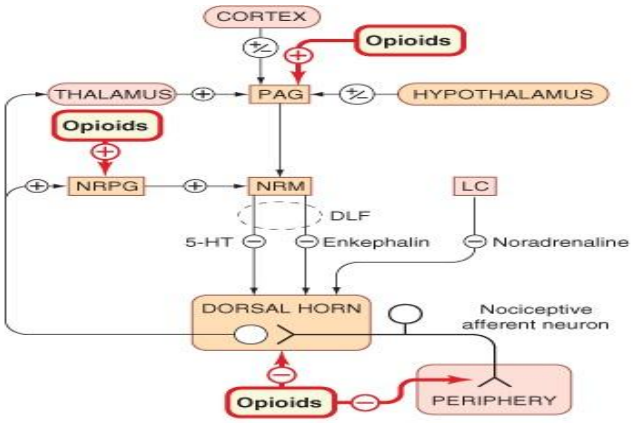


Figure 4:

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.
- Noradrenaline is the transmitter of the inhibitory pathway from the locus coeruleus to the dorsal horn, and possibly also in other antinociceptive pathways.
- Adenosine plays a dual role in regulating nociceptive transmission, activation of A_1 receptors causing analgesia, by acting on both peripheral nerve terminals and dorsal horn neurons, while activation of A_2 receptors in the periphery does the reverse. There is evidence for descending inhibitory purinergic pathways acting on pain transmission through A_1 receptors.

Drugs Used as Analgesics[9]

- Non-steroidal anti-inflammatory drugs, anti-pyretic and analgesics:-
  - Conventional NSAIDs (non-selective COX inhibitors)
    a) Salicylates: aspirin, diflunisal
    b) Pyrazolones: phenyl butazone, oxyphenbutazone
    c) Indoles: indomethacin, sulindac
    d) Propionates: ibuprofen, ketoprofen, flurbiprofen, fenoprofen, Naproxen
    e) Pyrrolo-pyrrole derivatives: ketorolac
    f) Oxicam derivatives: piroxicam, tenoxicam, lornoxicam
    g) Fenamates: mephenamic acid, fluphenamic acid
    h) Aryl-acetate: diclofenac, aceclofenac
  - Relatively selective NSAIDs (preferentially COX-2 inhibitors) Nimesulide, nabumetone, meloxicam
  - Highly selective cox-2 inhibitors Lumiracoxib, etoricoxib, rofecoxib (no longer used)
  - Analgesic-antipyretics with low anti-inflammatory action
    a) Para-aminophenol: paracetamol (acetaminophen)
    b) Pyrazolones: metamizol (dipyrone), propiphenazone
    c) Benzoxazocine derivative: nefopam

- Opioid analgesics:
- Natural opium alkaloids:
- Phenanthrenes: morphine, codeine, thebaine
- Semi synthetic opiates:
- Diacetylmorphine (heroin), pholcodeine
- Synthetic opioids:
- Pethidine (meperidine), methadone, fentanyl, tramadol, levorphanol, dextroprpoxyphene, ethoheptazine
- Based on mode of action on receptors, opioid analgesics are classified as:
- Partial agonists: morphine, fentanyl
- Partial μ agonists: buprenorphine, propiram
- Agonists and antagonists on κ receptors : pentazocine, nalbuphine, Butorphanol

Various Screening Methods For Evaluation Of Analgesic Activity *In-Vivo* Methods[10]

A variety of experimental pain models are available to demonstrate the anti-nociceptive activity of drugs which are used for routine screening of analgesics. Since different classes of analgesics vary in their mechanism of pain relief, it is recommended not to rely on any one form of nociceptive test during the determination of analgesic efficacies.

A great variety of nociceptive tests is currently used differing from each other by the nature of stimuli, parameters, sites of application, nature of responses, quantitation and apparatus. Objectively, depending upon the nature of stimulus, they are classified as follow:-

❖ Chemically induced nociception:
- Writhing method
- Randall-Selitto test
- Intra-arterial bradykinin test
- Formalin tests in rats

❖ Electrical stimulation method
- Electrical stimulation of tail
- Flinch-jump test in mice
- Tooth-pulp stimulation in rabbit

❖ Mechanical stimulation method
- Haffner's tail-clip test in mice

❖ Thermal stimulation method
- Radiant heat method (tail-flick method)
- Tail immersion test
- Hot plate method

Introduction to Mangifera indica Linn


Figure 5:

Plant Profile

Plant	:	*Mangifera indica*
Authority	:	Linn
Synonyms	:	Mango, Aam
Botanical name	:	*Mangifera indica* Linn
Kingdom	:	Plantae
Division	:	Mangnoliophyta
Class	:	Mangnoliopsida
Subclass	:	Rosidae
Order	:	Sapindales
Family	:	Anacardiaceae
Genus	:	Mangifera
Species	:	indica

Vernacular Names

Hindi	:	Aam
Malayalam	:	Amaram
Sanskrit	:	Aamra
Tamil	:	Maangi
Telugu	:	Mamidi
Marathi	:	Amba
Bengali	:	Aama
Gujarati	:	Aambo

Occurrence And Distribution[11]

Mangifera indica L. is a large evergreen tree, long living, 10-45 m high with a strong trunk and heavy crown. Native from tropical Asia, it has been introduced wherever the climate is sufficiently warm and damp and is now completely naturalized in many parts of tropics and
Subtropics.

Morphology of *Mangifera indica* Stem Bark

- Colour: grayish to dark brown externally and yellowish-white to red Internally.
- Odour: pleasant
- Taste: astringent
- Size and shape: bark occurs in pieces of variable size and thickness
- Extra features: surface rough due to longitudinal cracks, fissures and scattered, raised lenticels.

Figure 6:

Chemical Constituents

Mango bark contains 10-20% tannins, namely protocatechuic acid and catechin. Additionally it also contains mangiferin, alanine, glycine, aminobutyric acid, kinic acid and shikimic acid. The mangiferin aglycone is a phenolic compound that arises from two different aromatization pathways, the shikimate (carbons C4b, C5, C6, C7, C8, and C8a) and the ketate (carbons C1, C2, C3, C4, C4a, and C8b) pathways.

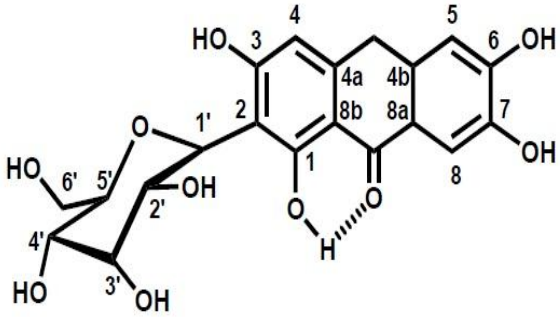


Figure 7: Mangiferin.

Medicinal Uses

Mango bark is used as

- Leucorrhea, bleeding hemorrhoids, lung hemorrhage
- Diabetes
- Astringent, tonic
- Menorrhagia
- Jaundice
- Preventing constipation
- Melancholia, nervous debility

- Anti-oxidant
- Treatment of diarrhea, dysentery and rheumatism

Scientific Work Till Reported

A thorough and complete literature survey on *Mangifera indica* was done from the chemical abstract, biological abstract, and text, national and international journals, Medline, internet and other research materials.

- Seifried *et al*. 2007 studied the anti-oxidant effect.[12]
- Jagetia *et al* 2005 studied radio protective effect.[13]
- Sarkar *et al*. 2004 studied the immunomodulatory activity.[14]
- Rivera *et al*. 2006 studied anti-allergic effect.[15]
- Garrido *et al*. 2004 studied anti-inflammatory and anti-nociceptive activity.[16]
- Yoshimi *et al*. 2001 studied anti tumour activity.[17]
- Muruganandan *et al*. 2005 studied anti-diabetic activity.[18]
- Yoshikawa *et al*. 2002 studied lipolytic activity.[19]
- Stoilova *et al*. 2005 studied anti-bacterial activity.[20]
- Perrucci *et al*. 2006 studied anti-parasitc activity.[21]

Introduction to *Syzygium aromaticum* Linn

Figure 8

Plant Profile

Plant	:	*Syzygium aromaticum*
Authority	:	Linn
Synonyms	:	clove bud, clove flower
Botanical name	:	*Syzygium aromaticum*
Kingdom	:	Plantae
Division	:	Mangnoliophyta
Class	:	Mangnoliopsida
Subclass	:	Rosidae
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	*Syzygium*
Species	:	*aromaticum*

Vernacular Names

Hindi	:	laung
Malayalam	:	lavanga
Sanskrit	:	lavanga
Tamil	:	krambu
Telugu	:	lavangalu
Marathi	:	lavang
Bengali	:	lavanga
Gujarati	:	lavang

Occurance and Distribution[22]

Clove is indigenous to 38mboynas and Moluccas islands. It is now cultivated chiefly in Zanzibar, Pemba, Penang, Madagascar, Caribbean islands, srilanka and India. In India cloves are grown in Nilgiri, tenkasi-hills and in kanyakumari district of tamilnadu state. It is also cultivated in kottayam and Quilon districts of Kerala.

Morphology OF *Syzygium aromaticum* Flower Bud

- Colour: crimson to dark brown
- Odour: slightly aromatic
- Taste: pungent and aromatic followed by numbness
- Size: about 10-17.5 mm in length, 4mm in width, and 2mm thick.
- Shape: hypanthodium is surmounted with 4 thick acute divergent sepels

Surrounded by dome shaped corolla. The corolla consists of Unexpanded membranous petals with several stamens and single Stiff prominent style.

Figure 9:

Chemical Constituents

Clove contains about 15-20 % of volatile oil; 10-13% of tannins (gallotanic acid) resin, chromone and eugenin. The volatile oil of the clove contains eugenol (about 70-90%), eugenol acetate, caryophyllenes and small quantities of esters, ketones and alcohols.

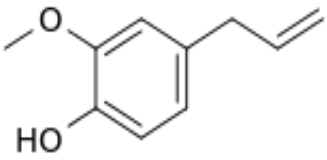


Figure 10: Eugenol.

Medicinal Uses of Clove

Clove is used as:-

- Dental analgesic
- Carminative

- Stimulant
- Flavoring agent
- An aromatic
- Antiseptic

Scientific Work Till Reported

A thorough and complete literature survey on *Syzygium aromaticum* was done from the chemical abstract, biological abstract, and text, national and international journals, Medline, internet and other research materials.

- Gulcin I et al 2004 studied the anti-oxidant activity.[23]
- Devi KP et al 2010 studied the anti-bacterial activity against salmonella typhi.[24]
- Daniel AN et al 2009 studied the anti-inflammatory and anti-nocicetive activity.[25]
- Kurokawa M et al 1998 studied the anti-viral activity against herpes virus.[26]
- Slamenova D et al 2009 studied the cytotoxic activity.[27]
- Sritabutra D et al 2011 studied the pesticidal activity which is used in agriculture.[28]

MATERIALS AND METHODS

Collection of Plant Material

The stem bark of *Mangifera indica* was obtained from the surroundings of chenchinada area in west Godavari district, Andhra Pradesh. The flower buds of *Syzygium aromaticum* were purchased from local market, west Godavari, Andhra Pradesh. These plant materials were shade dried for 1 week and powdered. The powders were stored in an airtight container and kept in a cool, dark and dry place.

Preparation of Extract[29]

About 200 gm of powdered material was taken individually in a clean, flat bottomed glass container and soaked in 500 ml of distilled water. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate (aqueous extract) obtained was evaporated using rotary evaporator. It rendered a concentrate of brownish-yellow color. The concentrate was designated as crude extract of aqueous (AEMS). The extracts were transferred to closed containers for further use and protection.

Requirements

- **Chemicals:** diclofenac sodium tablets, acacia, extracts and distilled water.
- **Apparatus:** eddy's hot plate apparatus, beaker, thermometer, Bunsen burner, oral feeding tube, syringes and stop watch.

Animals

Young Swiss-albino mice of either sex aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDRB). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and were fed with pellet diet and water ad libitum.

Group treatment

The animals were divided into 4 groups, each group containing 5 animals. The group animals were treated as follow:

- Group I: control group (only vehicle was given)
- Group II: positive group (diclofenac treated)
- Group III: test group (low dose-500mg/kg B.wt)
- Group IV: test group (high dose-1000mg/kg B.wt)

Preparation of standard dose and extract dose

Standard dose: diclofenac sodium tablets (50mg) were purchased from pharmacy. As it was the human dose, the doses for mice were calculated according to the mice dose factor. And dose was calculated as follow:

Mice dose factor[30] = 0.0026

Mice dose = human dose X mice dose factor

(This will give mice dose for 20gm weight mice)

Thus, dose for 20gm weight mice = 50mg diclofenac X 0.0026 = 0.13mg/20gm body wt. or 6.5mg/kg b.wt

The tablet was powdered and required quantity containing required diclofenac sodium was weighed and suspended in distilled water containing 1% acacia.

Extract dose: both the extracts of MI and EC were weighed and mixed in 1:1 ratio. These extracts were suspended in water containing 1% acacia. The doses were prepared according to their body weight for both group III and group IV.

Methods

Eddy's hot plate method[31]

Each animal group received a particular treatment i.e. control (1% acacia solution, 10ml/kg, p.o.), positive control (Diclofenac sodium 6.5mg/kg b.wt) and the test sample (aqueous extract of 500mg/kg b.wt & 1000mg/kg b.wt. respectively). The animals were positioned on Eddy's hot plate maintained at a temperature of 55±0°C. A cut off period of 15 sec was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 90, 120 min after oral administration of the samples.

Tail immersion method[32]

Each animal group received a particular treatment i.e. control (1% acacia solution, 10ml/kg b.wt), positive control (Diclofenac sodium 6.5mg/kg b.wt) and the test sample (aqueous extract of 500mg/kg b.wt & 1000mg/kg b.wt respectively). The lower portion of the tail was immersed in a water bath maintained at 55±0.5°C. The time in seconds for tail withdrawal from the water was

taken as the reaction time, with a cut-off time of immersion set as 15 s. the reaction time was measured 30 min prior to and 0, 30, 60, 90, 120 min after oral administration of the samples.

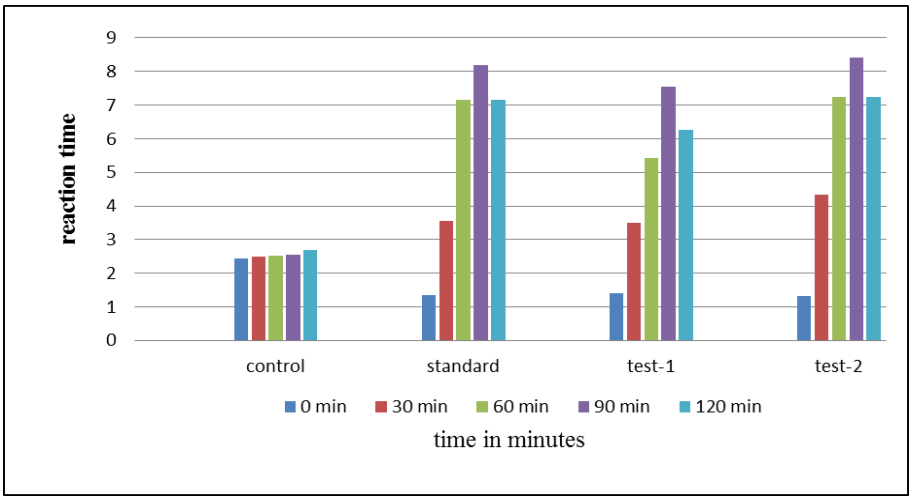
RESULTS

Eddy's Hotplate Method

Results of the eddy's hotplate method are presented in Table 1 for control group, standard group, test-1 group, and test-2 group. The mixture of extracts of both the plants (*Mangifera indica* and *Eugenia caryophyllus*) was found to exhibit a dose dependent increase in reaction time when compared with control. At 90 minutes, the mean reaction time of two different doses (500 and 1000 mg/kg body weight) was 7.56 sec and 8.40sec respectively. The results were found to be significant when compared with control group.

Table: 1, results of eddy's hotplate method.

	Mean reaction time (in seconds)				
Treatment	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
CONTROL	2.45	2.5	2.52	2.56	2.7
STANDARD	1.35	3.56	7.15	8.19	7.17
TEST-1	1.41	3.49	5.43	7.56	6.26
TEST-2	1.32	4.33	7.25	8.4	7.24



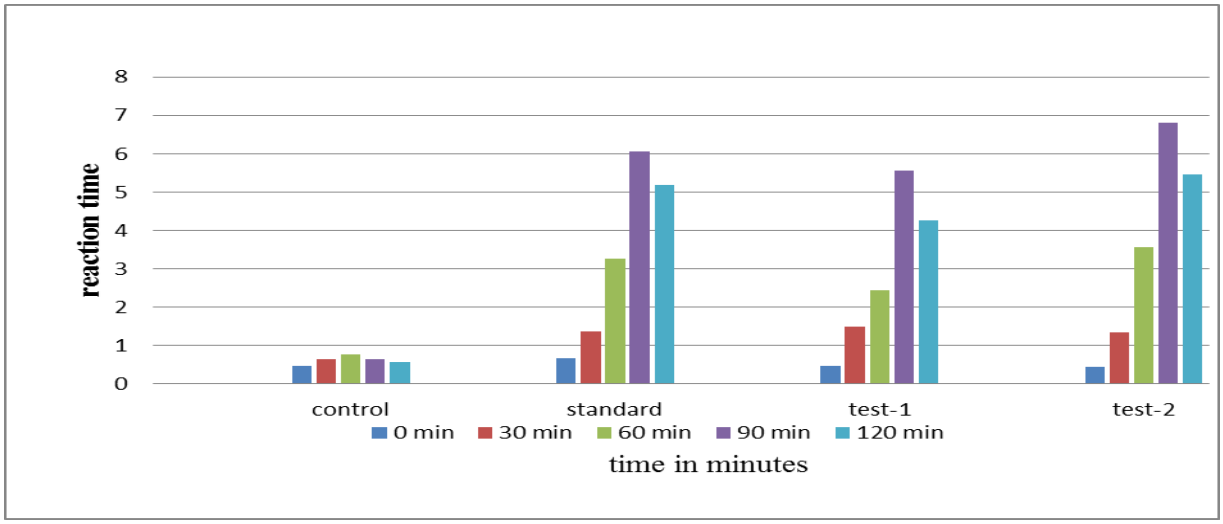
Graph: 1.

Tail Immersion Method

Results of the tail immersion method are presented in Table 2 for control group, standard group, test-1 group, and test-2 group. The mixture of extracts of both the plants (*Mangifera indica* and *Eugenia caryophyllus*) was found to exhibit a dose dependent increase in reaction time when compared with control. At 90 minutes, the mean reaction time of two different doses (500 and 1000 mg/kg body weight) was 5.55 sec and 6.81 sec respectively. The results were found to be significant when compared with control group.

Table 2: results of tail immersion method.

	Mean reaction time (in seconds)				
Treatment	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control	0.47	0.65	0.76	0.65	0.56
Standard	0.66	1.36	3.25	6.07	5.18
TEST-1	0.47	1.49	2.43	5.55	4.26
TEST-2	0.45	1.33	3.57	6.81	5.47



Graph: 2

DISCUSSION

The collected bark of *Mangifera indica* Linn. And flower buds of *Syzygium aromaticum* Linn were shade dried and then powdered to coarse size. About 200 gm of bark powder of *Mangifera indica* Linn. And *Syzygium aromaticum* Linn was subjected to extraction with distilled water through maceration. After maceration for 7 days, the slurry was filtered and the filtrate was dried. The present study explores the Poly-herbal formulation for Analgesic activity of aqueous extract of bark of *Mangifera indica* Linn and flower buds of *Syzygium aromaticum* Linn against experimental model of Eddy's hot plate method And tail immersion method in mice. Poly-herbal suspensions were prepared by the trituration method using a suspending agent (1% acacia) and dried extracts.

The extract of *Mangifera indica* Linn and *Syzygium aromaticum* Linn was evaluated for analgesic activity. In both of Eddy's hot plate method and tail immersion method in mice; the oral administration of extracts induced a significant analgesic activity in a dose-dependent manner in the mice. Test-2 group has shown significant analgesic effect when compared with Diclofenac sodium as a standard drug. The plant may have the phytoconstituents which inhibit cyclooxygenase enzyme or act on central opioid receptors. Based on the results of the present study, it can be concluded that extracts with different doses (500mg/kg b.wt & 1000mg/kg b.wt) showed significant analgesic activity in mice.

CONCLUSION

Treatment with both low dose 500mg/kg b.wt and high dose 1000mg/kg b.wt of poly-herbal aqueous extract of dried bark powder of *Mangifera indica* and dried flower bud powder of *Syzygium aromaticum* to mice increased the reaction time in both the methods (eddy's hot plate method and tail immersion method) confirming analgesic activity. However, comparatively 1000mg/kg b.wt dose of AEMS is more efficacious.

This plant may be providing good scope for use in treating pain. Further research work should be conducted for unveiling the active compound responsible for analgesic activity.

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