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EVALUATION OF ANALGESIC ACTIVITY OF METHANOLIC EXTRACT EUPLOCAOVALIFOLIA ON SWISS ALBINO MICE

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Abstract:

Pain, a multifaceted and personal sensation, has been a focal point of extensive inquiry spanning various domains such as neuroscience, psychology, and medicine. Traditionally, Euploca ovalifolia has been employed for its analgesic attributes and its capacity to promote wound healing. This study is dedicated to unraveling the analgesic potential of the methanolic extract derived from the entire Euploca ovalifolia (Forssk) plant. The effectiveness of the crude extract in providing peripheral analgesia was evaluated using a mouse model of acetic acid-induced writhing test and central analgesic activity was evaluated using tail flick method.The tail flick method was employed to assess the central analgesic effect of the crude methanolic extract obtained from Euploca ovalifolia. Remarkably, when administered at doses of 200mg/kg and 400mg/kg, the extract exhibited a highly significant analgesic effect, as indicated by a p-value of less than 0.001. This suggests that the extract effectively reduced pain sensitivity in the test subjects, signifying its potential as a therapeutic agent for pain management. The result display the central and peripheral analgesic activity of methanolic extract of Euploca ovalifolia in a dose dependent manner. These findings underscore the promising analgesic properties of the methanolic extract of Euploca ovalifolia and highlight its potential as a natural source of pain relief, warranting further investigation and exploration of its mechanisms of action.

Keywords: Analgesic activity, euplocaovalifolia, methanolic extract, Diclofenac sodium,Tail flick method,Acetic acid induced writhing test.

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1.INTRODUCTION:

1.1. INTRODUCTION TO PAIN:

Algesia(pain) is an unpleasant sensation evoked by internal or external stimulus. Transduction of pain and its perception is complex involving biological events at various levels of nervous system.(1)

Pain, a subset of somatic sensation, is a complex experience characterized by sensory, emotional, and cognitive elements. It emerges as a reaction to actual or perceived tissue injury and initiates autonomic, psychological, and behavioral reactions. Pain, despite its unpleasant nature, serves a crucial purpose. In extreme cases, individuals unable to perceive pain due to conditions like hereditary neuropathies can unknowingly sustain infections, engage in self-mutilation, and experience shortened lifespans. Normally, nociception and the perception of pain are activated when there is a potential for tissue injury. This includes extreme pressures, temperatures, toxic substances, and inflammatory mediators. Specialized peripheral sensory neurons referred to as nociceptors identify these intense physical and chemical stimuli with high thresholds. (2)

Pain experiences vary in qualities and temporal characteristics based on the type and location of the stimulus. First pain is often described as sharp and stabbing, while second pain is more diffuse and includes sensations like burning, throbbing, cramping, and aching. It also incorporates emotional components. Pain perception is highly individual and subjective, contrasting with the relatively objective nature of other senses. Pain can be broadly classified into 3 types (a)Nociceptive pain,(b)Inflammatory pain and (c)Pathological pain Nociceptive pain is linked to the perception and identification of potentially harmful noxious stimuli to protect the body. Inflammatory pain, on the other hand, arises from heightened sensitivity to pain and is part of the healing process following tissue damage due to the infiltration of immune cells.

Pathological pain is associated with any disease state caused due to damage to the nervous system or abnormal functioning of nervous system. [2,3]

Apart from these types of pain, the pain can also be classified based on the duration of time the pain continues. It is as follows:[4]

1.2. CLASSIFICATION OF PAIN:

1. Types of Pain: Pain can be broadly classified into two categories: nociceptive pain and neuropathic pain. Nociceptive pain results from the activation of pain receptors (nociceptors) due to tissue damage or inflammation, while neuropathic pain is caused by damage or dysfunction of the nervous system itself.

2. Pain Pathways: Researchers have identified specific neural pathways that transmit pain signals from the site of injury or damage to the brain. The spinothalamic tract is one of the major pathways involved in transmitting pain signals.

3.Brain Regions: Studies using functional brain imaging techniques, such as fMRI (functional magnetic resonance imaging), have identified brain regions involved in the perception of pain. The primary somatosensory cortex, anterior cingulate cortex, and insula are some of the areas implicated in processing pain.

4.Individual Variability: Pain perception is highly individualized. Factors like genetics, past experiences, and psychological factors can influence how a person perceives and responds to pain. This variability has led to the development of personalized pain management approaches.

5.Pain Management: Pain management techniques have evolved significantly. They range from pharmaceutical interventions like opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) to non-pharmacological approaches such as physical therapy, cognitive-behavioral therapy, and complementary therapies like acupuncture.

6.Chronic Pain: Chronic pain is a significant area of research. It is defined as pain that persists beyond the normal time of healing (usually 3-6 months). Conditions like fibromyalgia, chronic lower back pain, and neuropathic pain syndromes have been studied extensively to develop effective treatments.

7.Placebo Effect: The placebo effect, where patients experience pain relief from a treatment with no active therapeutic ingredient, highlights the role of psychology in pain perception. Research has shown that expectations, beliefs, and the patient-provider relationship can influence pain outcomes.

8.Neuroplasticity: Studies have demonstrated that the nervous system can undergo changes in response to chronic pain, leading to sensitization or increased pain perception. This concept of neuroplasticity has

implications for understanding and treating chronic pain conditions.

9.Referred Pain: Referred pain is felt in a location different from the actual source of the pain. For example, heart pain may be felt in the left arm.

10.Pain Assessment: Researchers have developed various tools and scales to assess and quantify pain, helping healthcare professionals better understand and manage their patients' pain. These tools often take into account both the sensory and emotional aspects of pain.

11.Emerging Therapies: Ongoing research continues to explore new approaches to pain management, including the use of neurostimulation techniques (e.g., spinal cord stimulation and transcranial magnetic stimulation) and the development of novel pharmacological agents with fewer side effects than traditional opioids.

Research into pain is a dynamic field, with ongoing efforts to better understand its underlying mechanisms, improve pain management strategies, and ultimately alleviate the suffering experienced by individuals with acute and chronic pain conditions.

Acute, Chronic and Breakthrough pain (5)

Acute pain is normal and temporary pain with short duration of time less than 6 months. It gives the body an alert response towards an injury.

Chronic pain have a long duration than acute pain and can be continued without any improvement for months.

Breakthrough pain, the word “breakthough” means a pain relief for short duration of time.It usually occurs when analgesic medicines are taken to treat the chronic pain.

1.3. ANALGESICS:

Analgesia is insensibility to pain caused by the interruption in the nervous system pathway between brain and the particular sense organ.Analgesics ,also known as painkillers are the drugs or medications administered to relief different types of pain. These are the medications used for the treatment and management of pain –from headache to arthritis and injury.(5)

Analgesics are substances that alleviate pain without causing loss of consciousness. The term "analgesic" originates from the Greek words "an-" meaning "without" and "algos" meaning "pain." These drugs function through various mechanisms within the peripheral and central nervous systems. They can be derived from synthetic sources such as Paracetamol, COX-2 inhibitors, NSAIDs, Ibuprofen, and Diclofenac. Additionally, medicinal plants are a rich source of analgesics, including opioid analgesics, Aloe vera (Aloe barbadensis), Andrographis paniculata, Elettaria cardamomum, Punica granatum, Eugenia caryophyllus, Mimosa, Curcuma alismatifolia, Phoenix sylvestris, Stachys schtschegle, Cissus quadrangularis, Menthol, Buxus sempervirens, Burns sempervirens, Fumaria vaillantii, Rumex crispus, Urtica dioica, and Morinda citrifolia. [6]

1.4. CLASSIFICATION OF ANALGESIC DRUGS ACTING BY DIFFERENT PATHWAYS:

A.NON SELECTIVE COX INHIBITOR (traditional NSAIDs):

1. Salicylates: Aspirin
2. Propionic acid derivatives:I buprofen, Naproxen, Ketoprofen, Flurbiprofen.
3. Fenamate: Mephenamic acid.
4. Enolic acid derivatives: Piroxicam, Tenoxicam.
5. Acetic acid derivatives: Ketorolac, Indomethacin, Nabumetone.
6. Pyrazolone derivatives: Phenyl butazone, Oxyphenbutazone.

B. PRFERENTIAL COX -2 INHIBITORS: Nimesulide, Diclofenac, Aceclofenac, Meloxicam, Etodolac.

C.SELECTIVE COX-2 INHIBITORS: Celecoxib, Etoricoxib, Parecoxib.

D.ANALGESIC-ANTIPYRETIC WITH POOR ANTI INFAMMATORY ACTION:

1. Paraaminophenol derivatives:

Paracetamol(acetaminophen)

2. Pyrazolone derivatives: Metamizol (Dipyrone),

Propephenazone.

3. Benzoxazocine derivatives: Nefopam

1.5. PERIPHERAL PATHWAY OF ANALGESIC ACTION:(37)

1. Noxious Stimulus: The pathway begins with the presence of a noxious stimulus in the periphery.

2. Activation of Free Nerve Endings: Unsophisticated free nerve endings associated with small-diameter primary afferent neurons are activated by the noxious stimulus.

3. Transduction: The physical stimulus is transduced into an electrochemical signal by these nerve endings.

4. Transmission along Primary Afferents: The electrochemical signal travels along slowly conducting primary afferent nerves towards the central nervous system.

5. Entry into Dorsal Horn: The impulses enter the dorsal horn of the spinal cord upon reaching the central nervous system.

6. Synaptic Transmission: Chemical synapses transmit the impulses from primary afferent neurons to second-order neurons within the dorsal horn.

7. Ascending Tracts: The signals are then conveyed along ascending tracts towards higher brain centers.

8. Brain Processing: Impulses are relayed to specific areas of the brain, including the somatosensory cortex and amygdala, where they are processed into a psychophysical experience.

9. Integration of Responses: The brain incorporates protective motor reflexes and emotional responses into the experience.Ongoing Research: The details of this process remain incompletely understood, making it a subject of ongoing neuroscientific research.

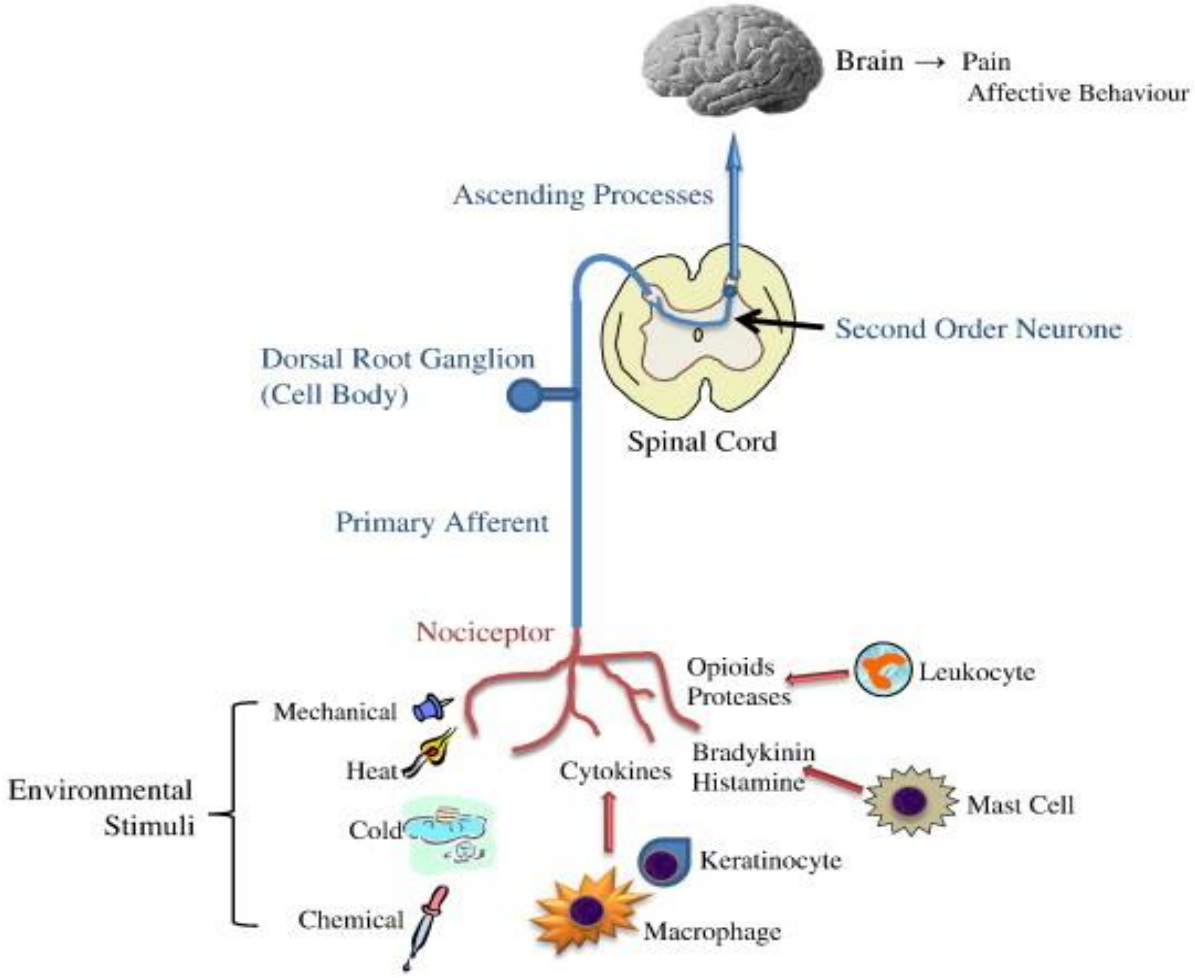


Image 1: pain pathway(30)

Types of analgesic: There are two major classes of analgesic NSAIDs and opioid analgesic . Opioids are narcotics or morphine like analgesics and NSAIDs (non-steroidal anti-inflammatory drugs) are non-narcotic or aspirin like analgesic.(6)

NSAIDs (non-steroidal anti-inflammatory drugs):All drugs grouped under this category consists of analgesic, antipyretic and anti-inflammatory effect in different measures. In comparison to morphine these are weaker analgesics and do not produce dependency when consumed. Based on the mechanism of action these are further classified into the following classes(6)

Opiates act on specific receptors in humans and animals to induce highly selective alterations in responses to strong and aversive stimuli. Microinjection techniques in various species, from rodents to primates, have identified key brain regions where opiates exert potent antinociceptive actions.(8)

These regions include the periaqueductal gray (PAG) with μ receptor involvement, rostral ventral medulla (μ/δ receptors), substantia nigra (μ receptor), and spinal dorsal horn (μ/δ/K receptors). Mechanistic studies have revealed distinct mechanisms at these sites. In the PAG, opiates likely indirectly activate bulbospinal pathways, influence rostral projections, and alter afferent input.In the spinal cord, opiates act presynaptic ally on primary afferents and post-synaptically to hyperpolarize projection neurons.(8)

Peripheral μ and K receptors can modulate the sensitized state of small afferent terminals in inflamed tissue, exerting anti-hyperalgesic effects.Systemic administration of opiates engages a wide range of central and peripheral systems to produce powerful analgesic effects.[8]

1.6.TYPES OF PAIN RECEPTORS (9)

Pain receptors, also known as nociceptors, are specialized sensory receptors that play a crucial role in the perception of pain and the body's response to harmful or potentially damaging stimuli. Previous research has helped elucidate several types of pain receptors, each of which responds to specific types of noxious stimuli. Here are some types of pain receptors based on previous research:

1. Thermal Nociceptors: These nociceptors are sensitive to temperature changes and respond to extreme heat (thermal heat) and extreme cold (thermal cold). They are responsible for the perception of thermal pain and are critical for detecting burns and frostbite.

2. Mechanical Nociceptors: These nociceptors are activated by mechanical forces, such as pressure, stretching, or cutting of tissues. They play a vital role in detecting physical injuries, including cuts, bruises, and tissue damage.

3. Chemical Nociceptors: Chemical nociceptors are activated by specific chemical substances released during inflammation, injury, or tissue damage. These substances include prostaglandins, histamines, bradykinin, and various other inflammatory mediators.

4. Polymodal Nociceptors: These nociceptors are versatile and can respond to multiple types of noxious stimuli. They are often activated by a combination of mechanical, thermal, and chemical stimuli. Polymodal nociceptors are involved in the perception of a wide range of pain sensations.

5.Visceral Nociceptors: These nociceptors are found in the internal organs (viscera) of the body and are responsible for detecting pain arising from organ-related issues, such as inflammation, stretching, or ischemia (lack of blood flow).

6. Silent Nociceptors: These nociceptors are normally "silent" or inactive but can become sensitized and responsive to pain signals under certain conditions, such as chronic inflammation or injury. Research has indicated their role in chronic pain conditions.

7. Specialized Nociceptors: Some nociceptors are specialized to respond to specific types of pain, such as pruriceptors (itch receptors), which respond to itch-inducing substances, and proprioceptors, which detect pain related to joint and muscle movement.

8. Molecularly Defined Nociceptors: Advances in molecular research have identified specific receptors and channels associated with nociceptors. For example, TRPV1 (Transient Receptor Potential Vanilloid 1) receptors are associated with the perception of heat and certain chemical irritants.

9. Nociceptors in Different Body Regions: Nociceptors are distributed throughout the body, and research has identified variations in their properties and responses in different regions, such as cutaneous (skin), somatic (muscles and bones), and visceral (organs).

Understanding the different types of pain receptors and their characteristics is essential for developing targeted pain management strategies and treatments. Research in this field continues to advance our knowledge of how pain is sensed and processed in the body.

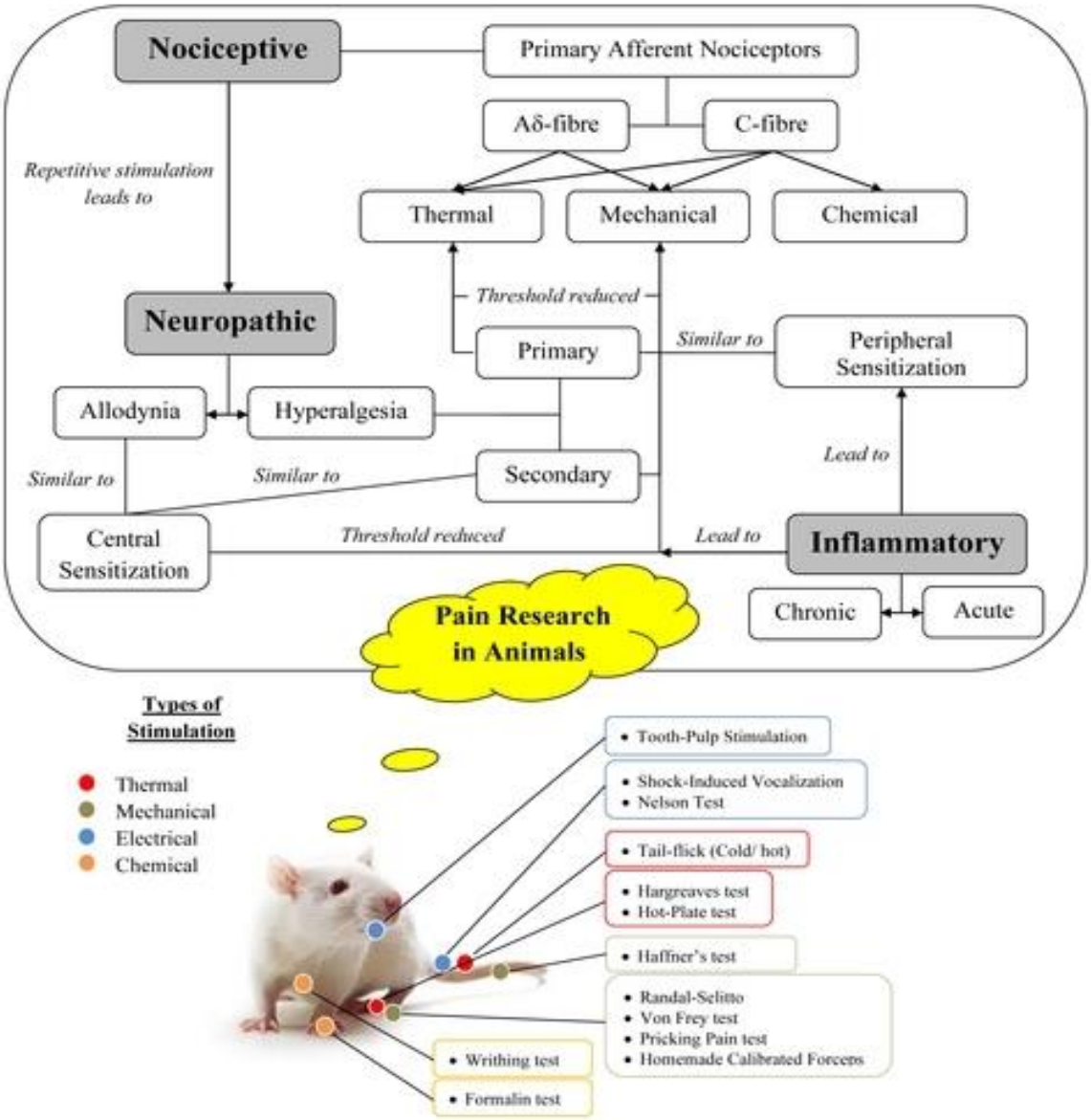


IMAGE 2 : Different stimulations and transmission of pain(31)

1.7. SCREENING METHODS FOR ANALGESIC ACTIVITY:[8,35]

There are several screening methods for assessing analgesic activity in both preclinical and clinical settings. Here are different types of screening methods for analgesic activity, along with brief descriptions of each:

1. Hot Plate Test:

In this test, animals (usually rodents) are placed on a heated surface, and their reaction time to the heat stimulus (licking, jumping, or paw withdrawal) is measured. Analgesic substances can increase the reaction time, indicating reduced sensitivity to pain.

2. Tail Flick Test:

This test involves exposing the tail of an animal to a radiant heat source. The time it takes for the animal to flick its tail away from the heat is measured. Analgesics that prolong this reaction time are considered to have pain-relieving effects.

3. Formalin Test:

The formalin test assesses both acute and chronic pain responses. A small amount of formalin is injected into the paw of an animal, and the time spent licking or biting the paw is recorded. This test helps evaluate the analgesic effects on inflammatory and neuropathic pain.

4. Writhing Test:

The acetic acid-induced writhing test, mentioned earlier, is an example of this method. It involves the injection of irritants like acetic acid into the peritoneal cavity, causing abdominal contractions or writhing. Analgesics can reduce the number of writhing responses.

5. Pressure Pain Threshold Measurement (Algometry):

In humans, pressure algometry is used to measure the pain threshold by applying pressure to a specific body part with a pressure algometer. Analgesic efficacy can be assessed by an increase in the pressure required to induce pain.

6. Electrophysiological Methods:

Electrophysiological techniques, such as nerve conduction studies and evoked potentials, can be used to measure the effect of analgesics on nerve function. These methods are more common in clinical trials and research involving neuropathic pain.

7. Von Frey Filament Test:

This test is used to assess mechanical allodynia, a condition where non-painful stimuli become painful. Filaments with varying levels of force are applied to the skin, and the animal's response is recorded. Analgesics can alleviate this heightened sensitivity.

8. Clinical Pain Assessment Scales: In clinical trials involving humans, various pain assessment scales are used, such as the Visual Analog Scale (VAS) and Numeric Rating Scale (NRS). Participants rate their pain intensity before and after analgesic treatment, allowing for subjective evaluation.

9. Radiographic Imaging:

In some cases, radiographic imaging techniques like magnetic resonance imaging (MRI) or functional MRI (fMRI) can be used to visualize changes in brain activity associated with pain perception and analgesic effects.

10. Inflammatory Models:

Animal models of inflammation, such as the carrageenan-induced paw edema test, can be employed to assess the anti-inflammatory and analgesic properties of substances by measuring the reduction in paw swelling and pain-related behaviors.

These screening methods help researchers and clinicians evaluate the analgesic potential of substances and assess their efficacy in managing pain, whether in preclinical animal studies or clinical trials involving human subjects. The choice of method depends on the specific type of pain being studied and the goals of the research.

2.PLANT PROFILE

Plant name: Euploca ovalifolia

Geographical source: The native range of this species is Türkiye to Egypt, Dry Tropical Africa, Madagascar, Arabian Peninsula, Indian Subcontinent to Indo-China, Lesser Sunda Islands, Solomon Islands. It is an annual or subshrub and grows primarily in the seasonally dry tropical biome.[9]

The plant Heliotropium ovalifolium has been traditionally used in India for its medicinal properties. Traditional medicine has been practiced widely in many countries, including India, and it often relies on the knowledge of diverse plant species.(36)

Heliotropium ovalifolium is widely distributed, not only in its native range in Africa but also in regions such as Arabia, Canary Islands, Pakistan, India, Indo-China, and Australia. This broad distribution makes it accessible for study and potential use in various regions.(36)

Nomenclatute:

Scientific name: Euploca ovalifolia

Common name: Grey leaf heliotrope

Synonym: Heliotropium ovalifolium

Taxonomical classification:

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Boraginales

Family: boraginaceae

Genus: Euploca

Species: ovalifolia

General description [9,10]:

Most branched procumbent or erect perennial herb up to 0.5 m height, branches terete, scabrous when older.

Leaves alternate, petiolate, lamina ovate or elliptic to obovate, 0.5–2.5 × 0.5–1.5 cm, entire, obtuse-mucronate, covered with densely adpressed hairs, margin slightly revolute.(Image 4)

Flowers on binate or trinate extra axillary 3–8 cm scorpoid racemes, 1.5–2 mm across, ebracteate, subsessile as shown in [image 3]

The calyx is bell-shaped, consisting of five sepals that are deeply divided at the base, measuring 1.5–2 mm in length. The exterior of the sepals is densely covered with fine hairs, while the inner side has a short pubescent texture. The sepals have acute tips, with the outer one being wider than the others and growing larger as the fruit develops.

There are five petals, with a 2 mm long tube that is shortly pubescent inside near the throat. The petal lobes are white and somewhat pointed.

There are five stamens attached to the petals, measuring 0.5 mm in length. Their anthers are cream white and loosely connected at their tips.

The ovary is four-celled and ovoid, measuring 1.2 mm in length. There is no distinct style, but the stigma is discoid and encircles the base of a 0.5 mm long conical stigmatic head, which has five hairs at the top.

The fruits are subglobose, with a diameter of 2 mm, and the nutlets separate when mature. The seeds have a convex, pubescent dorsal face, and the lateral face features pits measuring 2 × 1 mm. [9]

Image 3: flowers of plant euploca ovalifolia

Image 4: branched lateral view of plant euploca ovalifolia

CHEMICAL CONSTITUENTS:

Heliotropium species are rich in pyrrolizidine alkaloid. Pyrrolizidine alkaloids are for as esters that are either mono or dibasic acids known as necic acids.(12)

Heliotropium ovalifolium is found to possess Helifoline and Retronecine as major alkaloids and both are hepatotoxic pyrrolizidine alkaloids.(11)

In the realm of traditional folk medicine, the Heliotropium ovalifolium plant has a history of being employed to treat a variety of ailments, including inflammation, poison bites, skin issues, and nervous disorders. To gain a deeper understanding of its potential benefits, researchers conducted an analysis of the plant's roots and aerial parts using Gas Chromatography/Mass Spectrometry (GC/MS), which unveiled a diverse array of constituents.(25)

In the root extract, a predominant presence of hexadecanoic acid, methyl ester (24.50%), methyl stearate (15.61%), oxiraneoctanoic acid, 3-octyl, methyl ester (12.29%), 9-octadecenoic acid (Z)-, methyl ester (10.17%), and 9,12-Octadecadienoic acid (Z, Z)-, methyl ester (7.29%) was observed. On the other hand, the aerial parts extract primarily featured eight alkane compounds, constituting a substantial portion (62.89%) of the extract. Notably, heneicosane emerged as a prominent bioactive molecule, representing 34.14% of the aerial parts extract.[11,12,13]

This study underscores the potential industrial and pharmaceutical applications of the fatty acids found in H. ovalifolium, shedding light on their significance for future exploration and product development. Ibrahim, M.A. and Hatil Hashim, E.K., Gas Chromatography/Mass Spectrometry (GC/MS) analysis of Heliotropium ovalifolium Forssk. root and aerial part extracts.(25)

The alkaloidal portion extracted from Heliotropium ovalifolium produced two compounds: retronecine and a novel pyrrolizidine alkaloid named helifoline. Helifoline's chemical structure was determined as 1α-angelyloxymethyl-8α-pyrrolizidine-2β,7β-diol, based on spectroscopic analysis and its conversion into the necine base, which appears to be indistinguishable from croalbinecine. (11)

Three alkaloids namely supinine,7-angelylheliotridine and an alkaloid which is found as heliotridine monoester type with dehydrated viridiflic acid.(12)

A new naphthalene derivative 4,7,8-trimethoxy naphthalene 2-carboxylic acid and 6-hydroxy-5,7-dimethoxy naphthalene-2-carbaldehyde were isolated from the aerial parts of the plant Heliotropium ovalifolium Forssk.(13)

The dichloromethane extract of heliotropium ovalifolium aerial parts shows Heliotopamide a new alkaloid with a novel oxopyrrolidine-3-carboxamide central moiety as a major product. (14)

TRADITIONAL USES: [15]
Euploca ovalifolia has been traditinally used for its analgesic property and also for other properties like wound healing effet
Heliotropium ovalifolium is used against syphilis in at least 3 different African countries and is reported to have analgesic properties.
In Tanzania the dried plant is mixed with butter and the mixture is smeared thickly over painful places during fever. In both Ethiopia and Tanzania it is applied to scorpion stings.
In Senegal and Kenya the plant is grazed by all livestock and in Australia by camels.
In Kenya, the leaves are chewed as a substitute for tobacco.

Ethnopharmacological Uses(36)

The plant has a history of ethnopharmacological use for treating syphilitic ulcers, as documented in ancient literature. This historical usage suggests its potential medicinal value.

Anti-Inflammatory Potential: The study aims to investigate the bioactivities of Heliotropium ovalifolium, particularly related to its anti-inflammatory properties. Inflammation is implicated in various diseases, and plants with anti-inflammatory effects can be valuable in medical research and treatment.

Cytokine Regulation: The research specifically focuses on the regulation of key cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). These cytokines play central roles in inflammatory disorders such as rheumatoid arthritis and ulcerative colitis. Targeting them is a well-established therapeutic strategy.(36)

Therapeutic Potential: The study aims to prove the efficacy of isolated compounds from Heliotropium ovalifolium in inhibiting TNF-α and IL-6, suggesting the potential for developing these compounds into therapeutic agents for inflammatory conditions.(36)

Contribution to Drug Discovery: The identification of two new compounds from the plant indicates the potential for drug discovery. These compounds could serve as lead molecules for further structure-activity relationship (SAR) studies, with the goal of developing them into drug candidates. (36)

3.LITERATURE REVIEW

1. Stucky, C.L., Gold, M.S. and Zhang, X., 2001 ,[(1)]Pain stands as a significant global healthcare and socioeconomic challenge, exerting a profound impact on patients' overall health, quality of life, daily functioning, and societal productivity. It also places substantial financial burdens on healthcare systems and society as a whole. Pain is categorized based on its underlying pathophysiological mechanisms into two main types: (i) nociceptive and (ii) non-nociceptive neuropathic pain. Nociceptive pain emerges from the activation and stimulation of nociceptors and pain pathways, often triggered by inflammation, chemical agents, or physical events. Nociceptive pain further subdivides into somatic and visceral pain. On the other hand, neuropathic pain develops due to damage, injury, dysfunction, or disease affecting the somatosensory nervous system. Neuropathic pain is then classified into central and peripheral forms. Presently, primary approaches for pain relief include opioid analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), and non-anti-inflammatory antipyretic analgesics. However, these conventional treatment options for pain management still possess limitations in terms of efficacy and undesirable side effects. In contrast, herbal remedies and multi-component, multi-target, multi-pathway polypharmacological therapeutics have gained significant attention for pain treatment due to their noteworthy analgesic effects coupled with a reduced risk of adverse effects and toxicity.

2. Dubin, A.E. and Patapoutian, A., 2010,[(2)] Pain is a complex experience triggered by tissue damage, encompassing sensory, emotional, and cognitive elements. Despite being unpleasant, pain serves a vital purpose, as the inability to perceive pain can lead to infections, self-harm, and reduced lifespans in certain conditions. Nociception and pain perception are activated by extreme physical or chemical stimuli, detected by specialized sensory neurons called nociceptors. Pain varies in qualities and timing depending on the stimulus, with "first pain" being sharp and "second pain" encompassing diverse sensations and emotional components.Pain perception is highly individual and subjective, influenced by factors like stress and anticipation, making it complex to understand and manage.

3. Banoth, Santhosh Kumar, Krishna G. Mohan, Sandhya M. Rani, and Fatima Saba, (2015),[(20)] The study aimed to scientifically assess the analgesic potential of the methanolic extract from the entire Coldenia procumbens Linn. plant. Various in vivo analgesic tests, including the Hot-plate method, Tail-flick method, Tail-immersion method, and Acetic acid-induced Writhing test, were conducted on mice to evaluate the extract's effectiveness in managing pain. The results indicated that the methanolic extract of C. procumbens demonstrated consistent analgesic effects across these diverse pain models, whether the stimuli were thermal or chemical in nature. Particularly, the extract exhibited significant central analgesic activity at a dose of 200 mg/kg in centrally acting analgesic tests. Additionally, the extract, especially at doses of 200 mg/kg and 400 mg/kg, displayed noteworthy peripheral analgesic effects in the acetic acid-induced abdominal constriction test, likely linked to the modulation of prostaglandin levels. These findings underscore the potential of Coldenia procumbens methanolic extract as an analgesic agent in both central and peripheral pain management.

4. Bharathajothi, C. and Bhaaskaran, C.T., 2015,[(11)]The study investigated the analgesic and antipyretic activities of aqueous and chloroform extracts from Heliotropium indicum. In the analgesic evaluation using the hot plate method, both extracts displayed highly significant activity, surpassing the standard control acetyl salicylic acid. The most remarkable effects were observed at 50 mg/kg dosage. The extracts also exhibited antipyretic properties by reducing yeast-induced fever, with the chloroform extract showing a more pronounced effect than the aqueous extract when administered at 50 and 75 mg/kg. Phytochemical analysis revealed the presence of flavonoids, tannin, alkaloids, and saponin in the plant extracts. The observed actions suggest potential mechanisms involving the inhibition of prostaglandin synthesis, supporting the traditional uses of Heliotropium indicum in Siddha Medicine for pain relief, inflammation, scorpion stings, wound healing, and fever management.

5. Bhattacharya, A., Agrawal, D., Sahu, P.K., Kumar, S., Mishra, S.S. and Patnaik, S., 2014,[(13)] This study investigated the analgesic properties of Moringa oleifera (EMO) through various experimental models. EMO demonstrated significant analgesic activity in both central and peripheral models in a dose-dependent manner. Previous research on extracts from

different parts of the Moringa oleifera plant suggested the presence of compounds such as flavonoids, tannins, saponins, and alkaloids, all of which were claimed to possess analgesic properties. The proposed mechanisms of action included prostaglandin inhibition and the modulation of central pain receptors. Specifically, the study utilized the acetic acid writhing method to assess peripheral analgesic effects, indicating a potential inhibition of nociceptive mediators. The hot plate method, which assesses centrally mediated pain responses, demonstrated that EMO increased latency to discomfort, possibly through the activation of endogenous peptides in the periaqueductal gray matter. The phytochemical components in EMO, including flavonoids and tannins, may also contribute to its analgesic effects. Overall, this research suggests that EMO possesses both central and peripheral analgesic properties, highlighting its potential as a natural pain-relief agent. Further studies are needed to confirm these mechanisms.

6. Jaiswal, S.R. and Sontakke, S.D., 2012,[(14)]Recent interest has emerged in exploring the analgesic and anti-inflammatory properties of statins, which have long been employed for the treatment of dyslipidemia. This study delved into their potential as pain relievers and inflammation reducers. The tail flick method revealed that all four drug-treated groups exhibited a significant increase in pain threshold, with tramadol showing the most pronounced effect. Notably, aspirin's primary mode of action is peripheral, which might explain why its analgesic potency in this model was higher than that of simvastatin, which exhibited comparable activity.

In the acetic acid-induced writhing model, where analgesics with less than 70% analgesia are considered minimally effective, both simvastatin and atorvastatin fell short of this threshold, while aspirin surpassed it with over 80% analgesia. This model assesses peripherally acting analgesics, highlighting aspirin's effectiveness in this context. While statins' selective analgesic action hasn't been extensively evaluated in commonly used models, they have demonstrated efficacy in inflammatory nociception. Though the study didn't delve into the precise mechanisms of statins' nociception inhibition, they have been linked to reducing the release of various mediators, including bradykinin, TNF-α, IL-1β, CXCL, and PGE2. However, their clinical utility as analgesics may be more relevant in settings with inflammation.

In models of acute and chronic inflammation, simvastatin and atorvastatin displayed anti-inflammatory activity comparable to aspirin. These results align with previous studies indicating that statins can inhibit the release of inflammatory mediators, such as histamine, serotonin, kinins, and prostaglandins, thus curbing inflammation.

While statins exhibited significant analgesic and anti-inflammatory activities, they were less potent than aspirin, suggesting that their routine use solely for these effects may not be justified. However, they could serve as valuable alternatives in conditions characterized by chronic inflammation, especially when coexisting with dyslipidemia. Overall, statins may hold promise in addressing inflammatory nociception, although their primary role as hypolipidemic agents and safety concerns should be considered.

7. Yaksh, T.L., 1997,(7) Opiates exert highly selective analgesic effects in humans and animals by targeting specific receptors. Research involving microinjection techniques across various species has identified key brain regions, including the periaqueductal gray (PAG), rostral ventral medulla, substantia nigra, and spinal dorsal horn, where opiates produce potent antinociceptive actions. These effects are mediated by receptor-specific actions, primarily involving μ and δ receptors.Mechanistic investigations have uncovered diverse processes underlying opiate-induced analgesia. In the PAG, opiates indirectly activate bulbospinal pathways, influence rostral projections, and modify afferent input into the brainstem core. In the spinal cord, they act both presynaptic ally on primary afferents and post-synaptically to hyperpolarize projection neurons.

Moreover, peripheral μ and K receptors can modulate the sensitivity of small afferent terminals in inflamed tissues, resulting in anti-hyperalgesic effects. Overall, opiates, when administered systemically, engage a complex network of central and peripheral systems, providing a comprehensive understanding of the powerful analgesic effects of this drug class.

8. Wani, T.A., Kumar, D., Prasad, R., Verma, P.K., Sardar, K.K., Tandan, S.K. and Kumar, D., 2012,[(26)]The present study investigated the potential pain-relieving properties of SRE (unspecified plant extract). Various experimental models were employed to assess its antinociceptive activity, distinguishing between chemical- and thermal-induced pain and examining its impact on both inflammatory and non-inflammatory pain.For central pain relief mechanisms, the hot plate and tail-flick tests in mice were used. SRE demonstrated a central analgesic effect, prolonging

response times in these tests. To evaluate peripheral analgesic activity, the acetic acid-induced writhing test was conducted, which showed significant pain reduction with SRE.The formalin-induced paw licking test further confirmed SRE's antinociceptive activity, involving both acute neurogenic and inflammatory pain phases. SRE significantly reduced paw licking time in both phases, suggesting central action.

In a model of mechanical hyper nociception induced by carrageenan in rats, SRE exhibited effectiveness similar to the standard drug etoricoxib, indicating its interference with various pathways in inflammatory pain signaling. Postoperative pain models in rats revealed that SRE could reverse mechanical, cold, and radiant heat hyperalgesia in a dose-dependent manner.

These findings suggest that SRE possesses antinociceptive properties at both central and peripheral levels, making it a promising candidate for treating chronic pain. Further research is needed to elucidate the underlying mechanisms, but SRE holds potential as a safe and effective herbal remedy for conditions with inadequate treatment options, such as chronic pain, aligning with its traditional medicinal use.

9. Yimer, T., Birru, E.M., Adugna, M., Geta, M. and Emiru, Y.K., 2020,[28]The passage discusses the potential of Echinops kebericho, a traditional medicinal plant used in Ethiopian folk medicine, as an analgesic and anti-inflammatory remedy. Despite its widespread traditional use, there is a lack of scientific evidence regarding its effectiveness in experimental animal models. To assess its analgesic properties, researchers employed the acetic acid-induced writhing test, known for its sensitivity in detecting antinociceptive effects. The test involves intraperitoneal injection of acetic acid, triggering the release of inflammatory mediators and causing visceral pain characterized by muscle constriction and body elongation. This model is indicative of visceral pain associated with increased prostaglandin levels. The study found that Echinops kebericho extract displayed dose-dependent peripheral analgesic activity, significantly reducing writhing responses at doses of 100, 200, and 400 mg/kg. The highest dose exhibited the most substantial reduction (57.84%) compared to the control group. These results suggest that Echinops kebericho may indeed possess analgesic properties, supporting its traditional use for pain management. Further research is needed to explore its anti-inflammatory effects and underlying mechanisms. The study underscores the potential of traditional medicinal plants in the development of effective and safe pain-relief treatments.

10. , P.K., Sahu, J. and Chandel, S.S., 2016,[8] Screening methods for assessing analgesic activity vary in complexity and application. The Hot Plate and Tail Flick tests gauge analgesic effects in animals by measuring their response to heat stimuli, while the Formalin test assesses acute and chronic pain responses. The Writhing Test, using irritants like acetic acid, records abdominal contractions and evaluates analgesics' efficacy. Pressure Pain Threshold Measurement, or algometry, measures the force required to induce pain in humans. Electrophysiological methods, like nerve conduction studies, are used for neuropathic pain research. Von Frey Filament Testing detects mechanical allodynia, and clinical pain assessment scales rely on subjective reporting in human trials. Radiographic imaging can visualize changes in brain activity related to pain and analgesic effects. Inflammatory models, such as the carrageenan-induced paw edema test, assess anti-inflammatory and analgesic properties. These diverse methods help researchers evaluate analgesics' potential across a range of pain types, from acute to chronic, and in both preclinical and clinical settings, enabling the development of effective pain management strategies.

11. Hayfaa, A.A.S., Sahar, A.M.A.S. and Awatif, M.A.S., 2013,[29]
In an acute toxicity study, the LD50 (lethal dose for 50% of the subjects) of alkaloids extracted from M. fragrans seeds was determined to be 5.1 g/kg. According to toxicity classifications by Pascoe and Loomis and Hayes, this LD50 corresponds to ratings of slightly toxic and practically nontoxic, respectively. These findings are consistent with previous studies on extracts from M. fragrans, including the acetone-soluble component of the n-hexane extract and a 50% ethanolic extract, both of which caused no deaths and had no impact on behavior up to doses of 3 and 4 g/kg, respectively.

Regarding analgesic activity, the study employed the acetic acid-induced writhing model to assess the impact of alkaloids extracted from M. fragrans seeds on visceral pain. This model is commonly used to evaluate analgesics, particularly nonsteroidal anti-inflammatory drugs (NSAIDs). The results indicated that these alkaloids produced a modest but statistically significant reduction in writhing behavior, primarily in female mice.

The acetic acid-induced writhing model induces pain by releasing endogenous mediators like interleukin-1β, interleukin-8, prostaglandins, and lipooxygenase products. NSAIDs exert their analgesic effects by inhibiting prostaglandin synthesis through the cyclooxygenase pathway. Extracts from Myristicaceae plants have been observed to inhibit phospholipase A2, reducing the availability of arachidonic acid precursor for prostaglandin synthesis. Additionally, some researchers have suggested that plant extracts may suppress the release of interleukin-1β and interleukin-8 by resident peritoneal cells or inhibit prostaglandins and bradykinin, contributing to their antinociceptive activity.

4.AIM AND OBJECTIVE

The current study aims to evaluate the analgesic activity of methanolic extract of Euploca ovalifolia forssk in swiss albino mice.

4.1.PLAN OF WORK

OBJECTIVES:

1. Collection and authentication of plant, using soxhlation method to obtain the methanolic extract of the plant.

1. The purpose of the present research is to study the phytochemical constituent present in plant EUPLOCA OVALIFOLIA FORSSK.

2. To determine the acute oral toxic dose of methanolic extract of Euploca ovalifolia in mice, following the OECD guideline 425.

3. To determine the analgesic activity of methanolic extract of the whole plant Euploca ovalifolia in mice and comparing the result with that of control and standard groups result

5.MATERIALS AND METHODS:

5.1. COLLECTION OF PLANT: The whole plant of Euploca ovalifolia (Heliotropium ovalifolium) was collected at Sarapaka section, Manuguru range and division, Bhadradri Kothagudem district, Telangana state ,India. The plant was identified and authenticated by Assistant professor Dr.A.Vijaya Bhasker Reddy, Department of Botany ,Osmania university, Hyderabad. The given voucher specimen number OUAS-141 is identified on the basis of macroscopic studies of the sample(Twig) followed by detailed security of literature.

5.2. PREPARATION OF EXTRACT: The whole was air dried under shade at room temperature and crushed into a coarse powdered form. This is again grinded to make a fine powder. Methanolic extract was prepared using soxhalaton method. The solvent recovery and preparation of concentrated extracted was done using evaporation method. The crude extract was stored in an air tight container at 10◦c in refrigerator for further studies . [16,17,33]

Image 3: whole plant kept for shadow drying

Image 4: Dried plant made into fine powder

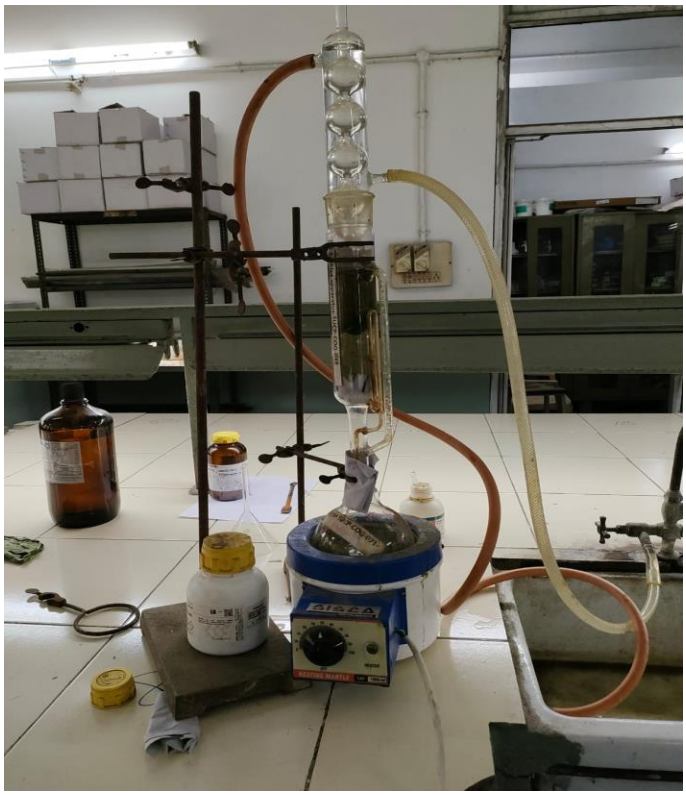


Image 5 : Soxhalation

Image 6 : powdered plant and methanolic extract

5.3. PHARMACOLOGICAL STUDIES:

Toxicity studies: The toxicity studies have been performed according to OECD guidelines 425 in mice.The median lethal dose[LD_{50}] of methanolic extract of Euploca ovalifolia was found to be 2000mg/kg. The selection of doses were done by taking $1/10^{th}$ and $1/5^{th}$ dose of LD_{50}

5.4. ANIMALS: Swiss mice weighing 25 -30g were used in this investigation .The animals were purchased from Sainath agency,Musheerabad,Hyderabad.

5.5.EXPERIMENTAL PROTOCOL:

GROUP 1:It is a control group in which animals are without treatment.

GROUP 2 :This group animals were treated with the methanolic extract of Euploca ovalifolia in dose of 200mg/kg.

GROUP 3: This group animals were treated with the methanolic extract of Euploca ovalifolia in dose of 400mg/kg.

GROUP 4:This group animals were induced with diclofenac sodium 10mg/kg.

Note: All these injections are given 30mins before induction of acetic acid.

TABLE 1 : GROUP AND TREATMENT

S.No	Name of the group and treatment	DOSES
1	Group 1-Control group	1ml saline water
2	Group 2-Methanolic extract of euploca ovalifolia	200mg/kg
3	Group 3-methanolic extract of Euploca ovalifolia	400mg/kg
4	Group 4-Diclofenac sodium	10mg/kg

Image 7 : weighing of animals

Image 8 : injecting drug in animals

Image 9: injection of standard drug in mice

5.6. ACETIC ACID INDUCED WRITHING TEST:

This is a common method used in pharmacological and physiological studies to assess the analgesic or anti-inflammatory effects of substances. The reduction in writhes suggests a potential pain-relieving effect of the treatment, and the percent reduction quantifies this effect. Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenyl quinone, acetic acid in mice. Analgesic activity is determined from decrease in frequency of writhing. (18)

A writhe is indicated by stretching of abdomen with simultaneous stretching of hind limb or limbs.(19)

PROCEDURE (19,20)
1.Experimental method(19)
•Writhes were induced by intraperitoneal injection of 1% acetic acid at a dose of 10 ml per kilogram of body weight.
•The writhes were then counted 5 minutes after the acetic acid injection.
•The counting continued over a 20-minute period.
2.Data Collection:
• collect data on the number of writhes in each group under study.
3.Comparison with Control:
•Comparison of number of writhes in each group with a control group.
4.Percent Reduction Calculation:
•The formula to calculate the percent reduction of writhes is as follows:
% inhibition =(Ncontrol-Ntest)/Ncontrol×100

Where, N is the number of writhes.

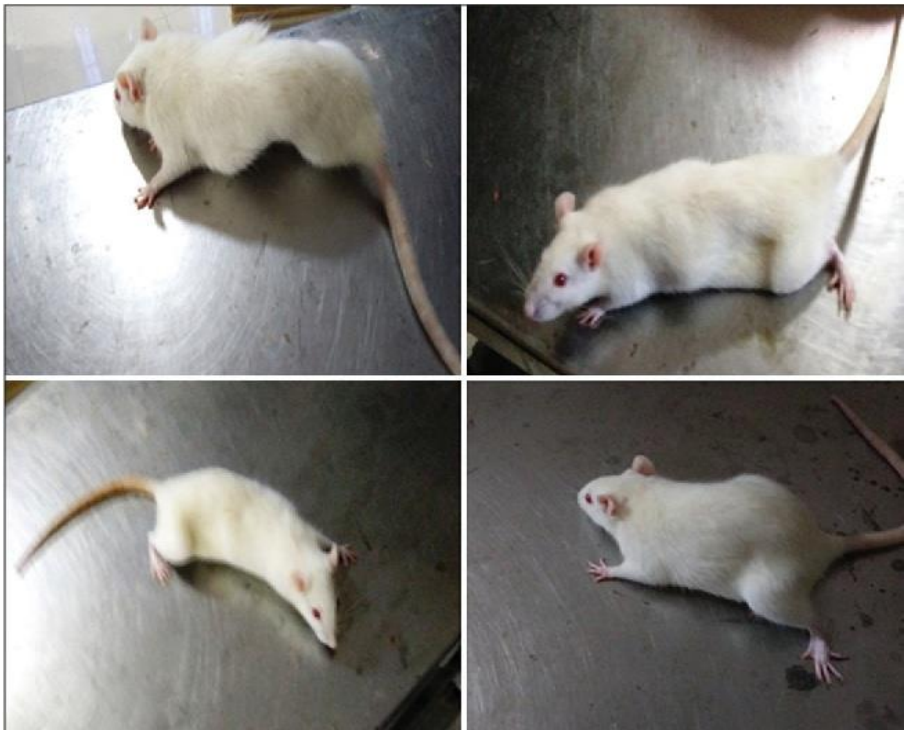


Image 10 : writhing of mice of different groups

5.7. ANALGESIC ACTIVITY: Acetic-acid induced writhing method:

The table 1 illustrate the time of onset of writhes in minutes and number of writhes in 10 minutes which is compared with the number of writhes occurred in control group.

TABLE 2

Effect of different doses of Euploca ovalifolia and on onset of writhing and number of writhes in acetic acid induced writhing model of analgesia in mice.

NAME OF GROUP	DOSE	NO OF WRITHES MEAN+SD	PERCENTAGE ANALGESIA
GROUP 1 :CONTROL GROUP	nil	58.00+2.13	
GROUP 2:EUPLOCA OVALIFOLIA	200mg/kg	22.18+1.9	54.7
GROUP 3:EUPLOCA OVALIFOLIA	400mg/kg	12.83+1.83	78.61
GROUP 4:DICLOFENAC SODIUM	10mg/kg	12.46+1.60	81.6

These readings are taken by using ONE WAY ANNOVA test followed by Dunnetts test and the level of significance of methanolic extract of Euloca ovalifolia was found as $p<0.001$.

5.8.Tail flick method:(18,39,40,41,42)
The tail flick test is a commonly used method in laboratory research to assess pain sensitivity or nociception in mice. It measures the response of a mouse to a noxious thermal stimulus applied to its tail. This test is based on the concept that when a mouse experiences a painful stimulus, it will reflexively flick or withdraw its tail to avoid or minimize the pain.
Procedure:

In the tail flick method, pain sensitivity was induced by applying radiant heat to the tail of the mice, specifically targeting a location approximately 5 centimeters away from the tip of the tail. An Analgesiometer was employed for this purpose.

During the testing procedure, the mice were gently secured within a loosely wrapped towel to ensure minimal stress and proper exposure of the tail.
The reaction time was meticulously measured, commencing from the moment the tail was exposed to the light beam until the tail's withdrawal response was observed.

The flicking response is noted for 0mins,15mins,30mins,45mins and 60mins, after administration of dose 1 hour before examining it for tail flick method.

To prevent potential tissue damage, a predetermined cut-off time of 10-12 seconds was enforced as a safety measure during the experiment. This time limit ensured that the thermal stimulus did not persist excessively, thereby protecting the mice from harm while still allowing for the assessment of their pain sensitivity.

The changes in latency time was calculated using the formula $T-T_0$
Here T_0 is the latency at time zero.

Table 2: Assessment of analgesic activity of methanolic extract of Euploca ovalifolia using tail flick method

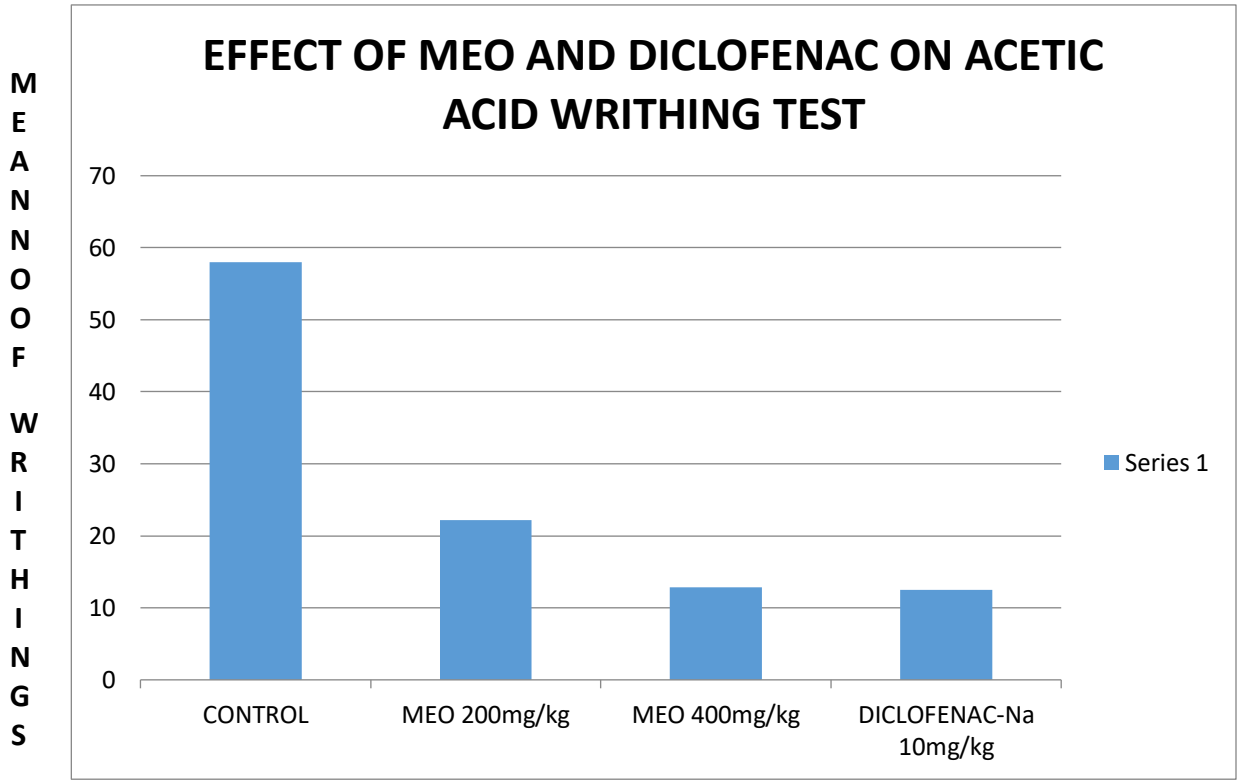
s.no	Group name	Doses mg/kg	Basal reaction time (sec)	15mins	30mins	45mins	60mins
1	Control group	Normal saline 1ml	3.2+0.22	3.4+0.32	3.2 + 0.22	3.0 + 0.33	3.7+ 0.31
2	Methanolic extract of Euploca ovalifolia	200mg/kg	3.8+0.22	5.8+0,64	5.4+0.33	5.5+0.24	5.3+0.27
3	Methanolic extract of Euploca ovalifolia	400mg/kg	4.1+0.26	5.4+0.34	4.1+0.24	3.9+0.32	4.5+0.26
4	Diclofenac sodium	10mg/kg	3.2+0.34	16.3 +1.34	19.3+0.32	16.0+0.26	16.4+0.23

These results are expressed as Mean+ SEM from six observation ; using ONE WAY ANNOVA followed by Dunnett's test

6.RESULT AND DISCUSSION:

Acetic acid induced writhing test:

The methanolic whole plant extract of *Euploca ovalifolia forssk* showed significant ($P < 0.01$) analgesic activity at 200, 400 mg/kg in the acetic acid-induced writhing test in comparison with control. The results of the acetic acid-induced writhing test along with the percentage inhibition of writhes are given in [Table 1]. Dose-dependent increase in the percentage inhibition of writhes was noted with methanolic Euploca ovalifolia (MEO) 200, 400 mg/kg showing 54.71% and 78.61%, respectively. The MEO at 400 mg/kg showed 78.61% inhibition of writhes comparable with the standard diclofenac, which showed 81.61%. [figure 1]
Figure 1:



TREATMENT

Effect of Methanolic Extract of Euploca ovalifolia on Analgesic Activity

In the acetic acid-induced writhing test, the methanolic whole plant extract of Euploca ovalifolia forssk demonstrated significant analgesic activity ($p < 0.01$) at doses of 200 mg/kg and 400 mg/kg when compared to the control group. The results of this test, including the percentage inhibition of writhes, are summarized in Table 1.

At a dosage of 200 mg/kg, the methanolic Euploca ovalifolia (MEO) exhibited a notable 54.71% inhibition of writhes.

When administered at 400 mg/kg, MEO displayed an even more substantial analgesic effect, with a remarkable 78.61% inhibition of writhes.

Notably, the highest dose of MEO (400 mg/kg) achieved an inhibition of writhes that was comparable to the standard diclofenac, which exhibited a 81.61% inhibition rate (see Figure 1).

These findings indicate a dose-dependent increase in the percentage inhibition of writhes when using the methanolic extract of Euploca ovalifolia. The results also highlight the potent analgesic activity of MEO, especially at the 400 mg/kg dose, which approached the efficacy of the established analgesic standard, diclofenac.

Tail flick method analgesic activity:

In the tail flick method, the administration of different doses of euploca exhibited a remarkable analgesic effect, with a highly significant increase in flicking latency observed at the dose of 200mg/kg ($p < 0.001$) when compared to the control group as represented under figure 2 . Specifically, at the 15-minute mark, the flicking latency in the 200mg/kg dose group demonstrated a significant enhancement ($p < 0.001$) relative to the control group. These findings underscore the potential analgesic properties of euploca at this particular dosage and time point.

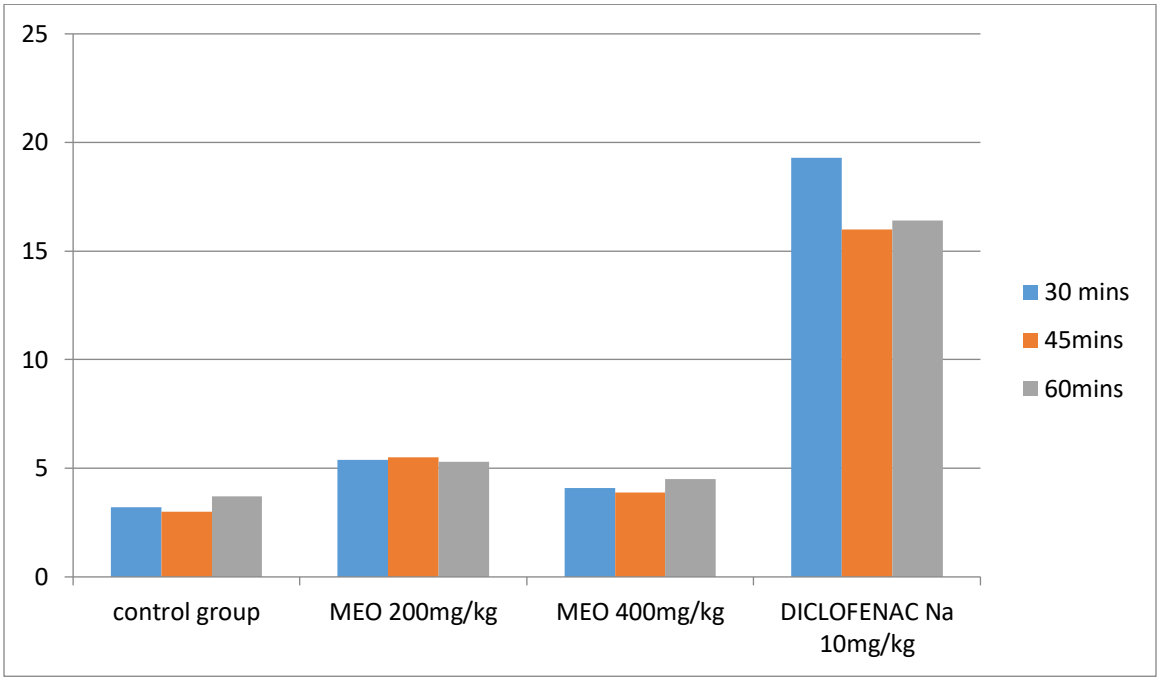


Figure 2 :
Diagrammatic representation of tail flick using methanolic extract of Euploca ovalifolia and its comparision with the normal group and group treated with diclofenac sodium:

DISCUSSION:

In this study, we have found compelling evidence supporting the analgesic properties of the methanolic extract derived from Euploca ovalifolia. The observed analgesic effects of this extract show a clear and consistent pattern, where the degree of pain relief increases in a dose-dependent manner. This means that as the dosage of the extract administered increases, the pain-relieving effects become more pronounced.

This scientific validation lends credence to the traditional knowledge and folklore claims associated with Euploca ovalifolia, which have suggested its effectiveness as a pain-relieving remedy. The study's findings provide empirical support for the traditional use of this plant in alleviating pain, reinforcing the idea that traditional remedies often have a basis in scientific reality.

However, while this research is a significant step in establishing the efficacy of Euploca ovalifolia as an analgesic agent, there is still more work to be done. To fully harness the therapeutic potential of this plant, it is essential to isolate and identify the specific active compounds responsible for its analgesic effects. This will allow for a more precise understanding of how Euploca ovalifolia exerts its pain-relieving properties. Furthermore, investigating the mechanism of action behind its anti-nociceptive activity is crucial. Understanding how Euploca ovalifolia interacts with the body to reduce pain sensations can help develop more targeted and effective pain management strategies. In summary, this study provides robust scientific evidence supporting the analgesic properties of the methanolic extract of Euploca ovalifolia. However, further research is needed to isolate active compounds and uncover the mechanisms underlying its pain-relieving effects, potentially paving the way for the development of novel analgesic treatments.

The results of the tail flick method utilized to assess the analgesic effects of euploca reveal intriguing findings. When different doses of euploca were administered, a significant analgesic effect was observed, particularly at the dose of 200mg/kg. This observation is supported by the highly significant increase in flicking latency, which serves as a key indicator of analgesia. The p-value of less than 0.001 emphasizes the robustness of this effect, signifying that it is not likely due to chance.

Furthermore, when comparing the 200mg/kg dose group to the control group, the results remained consistent. At the 15-minute time point, there was a remarkable and statistically significant improvement in flicking latency in the 200mg/kg dose group, once again with a p-value of less than 0.001. This specific time point is crucial as it highlights the rapid onset of euploca's analgesic properties at this dosage.

These findings suggest that euploca has substantial potential as an analgesic agent, particularly when administered at 200mg/kg. The rapid onset of its effects at the 15-minute mark underscores its suitability for pain management. Further research is warranted to explore the mechanisms underlying euploca's analgesic properties and to determine its safety profile at this dosage for potential therapeutic applications. Overall, this study contributes valuable insights into the analgesic effects of euploca, opening doors for future investigations and potentially novel pain relief strategies.

CONCLUSION:

This research demonstrates that the methanolic extract of Euploca ovalifolia exhibits analgesic effects in a manner that correlates with the dosage administered, providing scientific support for the traditional knowledge associated with this plant. Nevertheless, additional investigations are required to identify the specific active compounds responsible for this observed effect and to elucidate the potential mechanism of action responsible for its anti-nociceptive properties.

In conclusion, the tail flick method employed to evaluate the analgesic effects of euploca yielded noteworthy results. The administration of euploca at various doses demonstrated a significant analgesic effect, with the most striking outcome observed at the 200mg/kg dosage. This effect was underscored by a highly significant increase in flicking latency, with a p-value of less than 0.001, indicating its statistical robustness.

Moreover, the 200mg/kg dose group exhibited a rapid and significant improvement in flicking latency at the 15-minute mark compared to the control group, further accentuating the potency of euploca as an analgesic agent at this dosage and time point.

These findings collectively suggest that euploca holds promise as a potential analgesic treatment, particularly when administered at 200mg/kg, offering a rapid onset of pain relief. Nevertheless, further investigations are necessary to elucidate the underlying mechanisms of euploca's analgesic properties and to evaluate its safety profile at this dosage for potential therapeutic applications. This study provides valuable insights into the analgesic potential of euploca and lays the foundation for future research endeavors aimed at enhancing pain management strategies.

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