Radiatus Leaf Extracts Pharmacological Studies For Anxiolytics And Analgesic Activity

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Abstract
The Malvaceae family includes the shrub Hibiscus radiatus. Known by another name, monarch rose mallow, it's a crop that grows easily in multi-cropping systems and is useful for both food and fibre, making it a perfect crop for impoverished nations. The plant is used to prepare liquid concoctions and is said to be a blood purifier and fever treatment. All of its parts are chopped and cooked. The chemical components that may be in charge of the biological activities7 include alkaloids, glycosides, phenolic compounds, steroids, sterols, tannins, saponins, flavones, and flavonoids as well as proteins, amino acids, and carbohydrates. OECD guideline No.425 of CPCSEA was adopted for acute toxicity studies. The antiulcer activity of different extracts of *Hibiscus radiatus* was investigated by employing two models i,e. pyloric ligation method and ethanol induced ulcer model by using rats, Omeprazole was taken as standard drug. The raised plus maze model and the light and dark paradigm both demonstrated anxiolytic activity, with diazepam being used as the usual medication. One may argue that there has been noticeable activity in every extract. The extract with the most notable anxiolytic action among the others is the Hibiscus radiatus aqueous extract. The acetic acid writhing model and the hot plate model were used to test the analgesic activity, and diclofenac sodium was administered as prescribed. Aqueous extract of Hibiscus radiatus has demonstrated a highly substantial analgesic efficacy among the extracts, all of which have demonstrated considerable action. According to this study, Hibiscus radiatus leaf extracts have demonstrated strong analgesic, anxiolytic properties.

INTRODUCTION

1.1:INTRODUCTION TO MEDICINAL PLANTS:

Traditional medicine is "The knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness".[1]

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments, and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. Contemporary science has acknowledged their active action, and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life[-2].

Plants serve as an important source of medicine and play a key role in world health. Medicinal plants or herbs have been known to be an important potential source of therapeutics or curative aids. The utilization of medicinal plants has attained a commanding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of disorders and diseases but also as potential material for maintaining good health and conditions. Many countries in the world, that is, twothird of the world's population rely on herbal medicine for primary health care. The reason for this is

because of their great cultural acceptability, better compatibility and adaptability with the human body system and pose lesser harmful side effects. From the records, most of the used drugs contain plant extracts.
Some of them contain chief active ingredients (bioactive components or substances) obtained from plants. Through recent research, plant derived drugs were discovered from the study of therapeutic, curative traditional cures and most especially the folk knowledge of indigenous people and some of these claims and believe of people are irreplaceable in spite of the recent advancements in science and technology. Some of the drugs believed to be obtained from plants are aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine.
Natural products derived from plants for the treatment of diseases have proved that nature stands a golden mark to show the relationship between the interrelationship between man and his environment. The researches and utilization of herbal medicine in the treatment of diseases increases every day. Before the development and civilization by the British in Nigeria, medicinal plants are believed traditionally to be value of the therapeutic agent for the treatment of diseases such as typhoid, cholera, measles, and gonorrhea.
However, the knowledge of herbal medicines for treatment of diseases is confined to mostly the practicing herbalists or plant scientists with the belief that herbal medicines will lose their potency if revealed to other people. Although some herbs may have medicinal values, sometimes the medicinal preparation inflicts certain side effects[3]. Plant components are characterized by their capability to prevent the development of certain disorders. The adverse effect of conventional and allopathic medicine has also been important factors in the sudden increased population demands and total increase in the number of the herbal drug manufactures as well as a reduction in the use of chemical drugs. Knowing the history of any science is as effective as understanding and using that science thus, the historical significance of the past, present and future to medicinal herbs will be continued to be addressed. In view of this, the present study focuses on the knowledge on medicinal uses of plants and the scientific investigation to confirm their medicinal values, and thus among such plants *Hibiscus radiatus* is one traditionally used plant, which has reported to have traditional uses and was used to cure many disorders.[4]

1.2 : PLANT PROFILE:
Hibiscus radiatus belongs to the family malvaceae. The plant is believed to be a cure for fever and is considered a blood purifier and all its parts are cut and boiled, and the liquid preparations used. In view of this, the present study focuses on the knowledge on medicinal uses of plants and the scientific investigation to confirm their medicinal values, and thus among such plants *Hibiscus radiatus*is one traditionally used plant, which has reported to have medicinal properties and was used to cure disorders[5].

1.2.1 : SCIENTIFIC CLASSIFICATION:

Botanical name **:** *Hibiscus radiatus* Cav.

Synonym **:** Hibiscus lindleyi, Canhamobraziliensis, Monarch rosemallow

Family **:** Malvaceae

Kingdom **:** Plantae

Class **:** Magnoliopsida

Order **:** Malvales

Genus **:** Hibiscus

Species **:** *Hibiscus radiatus*[6]

1.2.2 : MORPHOLOGY:-
Hibiscus radiatus Cav. also known as monarch rose mallow, is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi- cropping systems and can be used as food and fiber. The genus *Hibiscus* (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees. The plant is about 3m tall and

has a deep penetrating taproot. It has a smooth or nearly smooth, cylindrical, typically dark green to red stems. Leaves are alternate, 7 to 11cm long, green with reddish veins and long and short petioles. Leaves of young seedlings and upper leaves of older plants as simple; lower leaves are deeply 3 to 5 or even 7 – lobed and the margins are toothed.

1.2.3 : CHEMICAL CONSTITUENTS:

Alkaloids, glycosides, Phenolic compounds, steroids, sterols, saponins, flavones, flavonoids, proteins, amino acids and carbohydrates[7].

1.2.4 : MEDICINAL USES:

No reports were found on *Hibiscus radiatus,* other *Hibiscus* species are used, to treat cardiac conditions, and as a diuretic, spasmolytic, antibacterial, cholagogue, diuretic, and anthelmintic, for the treatment of high blood pressure, liver diseases and fevers. In large amounts, *hibiscus* tea acts as a mild laxative.[8]

1.2.5 : TRADITIONAL USES:

The plant is believed to be a cure for fever and is considered as blood purifier. All plants parts are cut and boiled and the liquid preparations are used.[5]

Figure No. 1.1: Photograph showing/ *Hibiscus radiatus* whole plant part

Figure No. 1.2: Photograph showing *Hibiscus radiatus flower and leaves part*

ANXIETY

1.3: INTRODUCTION:

Anxiety is characterized by psychological symptoms such as tension, fear, apprehension, lack of concentration as well as somatic symptoms such as tachycardia, tremor, sweating, and GIT distress[16]. Anxiety is an emotional state caused by the perception of real or perceived danger that threatens the security of an individual[17]. Anxiety is among the most common and mental disorders[18]. Many of people have been sustained anxiety in worldwide[19]. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety[20].

1.3.1: CLINICAL CATEGORIES OF ANXIETY:

- Generalized anxiety disorder is an ongoing state of excessive anxiety lacking any clear reasons or focus. Essential feature of the class of anxiety is chronic worry.[21] Panic Disorder is an attack of overwhelming fear occurring in association with marked somatic Symptoms such as sweating, unexpected recurrent panic attacks, tachycardia chest pains, trembling, chocking etc. normally this condition of anxiety has a general component[22].
- Post- traumatic stress disorder elaborates anxiety triggered by insistent recall of past stressful experiences.[23]
- Social Anxiety Disorder is characterized by marked and persistent fear of performance situation when they feel, they will be the centre of attention and will do something Humiliating or embarrassing. Situation that provokers this fear may be quite specific e.g. Public speaking.[24]
- Phobia is a strong fear of specific things or situation e.g. snakes, open spaces, flying and social interaction.[25]

Introduction to GABA receptors:

The **GABA** receptors are a class of receptors that respond to the neurotransmitter gamma-aminobutyric acid **(GABA)**, the chief inhibitory neurotransmitter in the vertebrate central nervous system. There are three classes of **GABA** receptors. **GABAA** and **GABAC** receptors are ionotropic (i.e., their activation results in enhanced membrane ion conductance), **GABAB** receptor is metabotropic (i.e., their activation results in increased intracellular levels of second messenger)[26,27]. **GABAA** and **GABAC** receptors are ionotropic receptors leading to increased Cl- ion conductance, whereas **GABAB** receptors are metabotropic receptors which are coupled to G proteins and thereby indirectly alter membrane ion permeability and neuronal excitability. **GABA** receptors were widely distributed in mammalian brain and are in high concentration in cerebral cortex, hippocampus, basal ganglia, thalamus, cerebellum, and brainstem[28].

1.3.2 : PATHOPHYSIOLOGY

The amygdala, an almond-shaped mass of nuclei located deep within the medial temporal lobes of the brain, plays a pivotal role in threat processing. It is generally regarded as fundamental for the acquisition of conditioned fear and for the expression of innate and learned fear responses. Efferent neurons emerging from the amygdala activate the sympathetic nervous system, thus Driving the classic fight or
– flightresponses in end organs, such as increased heart rate and blood Pressure, and pupillary and bronchodilation[29].Functional neuro-imaging studies have demonstrated excessive arousal of the amygdala in patients suffering a range of anxiety disorder and this hyperactivity has been hypothesized as contributing to the hypervigilant monitoring of negative information reported in this disorder[30,31]. Other limbic structure, particularly the hippocampus, the medial prefrontal cortex and the locus coeruleus (which receives information directly from the sensory cortex and is largely under noradrenergic Control) are also implicated in anxiety disorder[29].At a molecular level, there is evidence of an underlying dysfunction of the neuro- inhibitory γ – aminobutyric acid (GABA) and serotonin (5-HT) neurotransmitter system. Anxiety may therefore be reduced, either by increasing the effects of GABA with anticonvulsants or by increasing serotonin with antidepressants. Both are effective strategies for anxiolysis, albeit though different pathway[32].

1.3.3 : ANTI-ANXIETY DRUGS:

These are an ill- defined group of drugs, mostly mild CNS depressants, which are aimed to control the symptoms of anxiety, produced a restful state of mind without interfering with normal mental or physical function. The anxiolytic-sedative drugs Differ markedly from antipsychotics, and more closely resemble sedative-hypnotics.
They are as follows.

1. Have no therapeutic effects to control thought disorder of schizophrenia.
2. Do not produce extra pyramidal side effects.
3. Have anticonvulsant property.
4. Produce physical dependence and carry abuse liability.
5. Do not selectively block condition avoidance in animal.[33]

: CLASSIFICATION OF ANTIANXIETY DRUGS: [33]

1. **Benzodiazepine:** Ex. Diazepam, Chlordiazepoxide , Oxazepam

2. **Azapirones**: Ex: Lorazepam, Alprazolam, Buspirone, Gepirone, Ispapirone,
3. **Sedative antihistamine**: Ex: Hydroxyzine
4. **β blocker** : Ex: propranolol

MECHANISM OF ACTION:

Benzodiazepines act preferentially on midbrain ascending reticular formation (which maintains wakefulness) and on limbic system (thought and mental functions). Muscle relaxation is produced by a primary medullary site of action and ataxia is due to action on cerebellum. BZDs act by enhancing presynaptic/postsynaptic inhibition through a specific BZD receptor which is an integral part of the GABAA receptor– Cl^- channel complex. The subunits of this complex form a pentameric transmembrane anion channel gated by the primary ligand (GABA), and modulated by secondary ligands which include BZDs. Only the α and β subunits are required for GABA action, and most likely the binding site for GABA is located on the β subunit, while the α/γ subunit interface carries the BZD binding site. The modulatory BZD receptor increases the frequency of Cl^- channel opening induced by submaximal concentrations of GABA.

The BZDs also enhance binding of GABA to GABAA receptor[34].

Buspirone is the first azapirone, a new class of antianxiety drugs, distinctly different from BZDs. Buspirone relieves mild-to-moderate generalized anxiety, but is ineffective in severe cases, in those showing panic reaction and in OCD. The therapeutic effect develops slowly: maximum benefit may be delayed up to 2 weeks. The mechanism of anxiolytic action is not clearly known, but may be dependent on its selective partial agonistic action on 5-HT1A receptors. By stimulating presynaptic 5- HT1A autoreceptors, it reduces the activity of dorsal raphe serotonergic neurones. Antagonism at certain postsynaptic 5-HT1A receptors has also been demonstrated. After chronic treatment, adaptive reduction in cortical 5-HT2 receptors may occur. Buspirone has weak dopamine D2 blocking action but no antipsychotic or extrapyramidal effects. A mild mood elevating action has been noted occasionally— may be due to facilitation of central noradrenergic system.

Hydroxyzine: An H1 antihistaminic with sedative, antiemetic, antimuscarinic and spasmolytic properties. It is claimed to have selective anxiolytic action, but accompanying sedation is quite marked; may be used in reactive anxiety or that associated with marked autonomic symptoms. Due to antihistaminic and sedative property, it is effective in pruritus and urticaria.

Beta Blockers: Many symptoms of anxiety (palpitation, rise in BP, shaking, tremor, gastrointestinal hurrying, etc.) are due to sympathetic over activity, and these symptoms reinforce anxiety. Propranolol and other nonselective β blockers help anxious patients troubled by these symptoms, by cutting the vicious cycle and provide symptomatic relief. They do not affect psychological symptoms such as worry, tension and fear, but are valuable in acutely stressful situations (examination fear, unaccustomed public appearance, etc.). They may be used for performance/situational anxiety or as adjuvant to BZDs[35].

ANALGESICS

1.5: INTRODUCTION

Analgesics (also known as painkillers) are members of the group of drugs used to relieve pain (achieve analgesia). The word *analgesic* derives from Greek *an-* ("without") and *algos* ("pain"). Analgesic drugs act in various ways on the peripheral and central nervous systems; they include paracetamol (also known as acetaminophen), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and tramadol[36].

PAIN:

Pain is the unpleasant and aversive feeling common to such experiences as stubbing a toe, burning a finger, putting iodine on a cut and bumping the "funny bone"[37]. The International Association for the study of pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".

Pain motivates us to withdraw from damaging or potentially damaging situations, protect the damaged body part while it heals, and avoid those situations in the future. It is initiated by stimulation of nociceptors in the peripheral nervous system, or by damage to or malfunction of the peripheral or central nervous systems[38].

Most pain resolves promptly once the painful stimulus is removed and the body has healed, but sometimes pain persists despite removal of the stimulus and apparent healing of the body; and sometimes pain arises in the absence of any detectable stimulus, damage or pathology. Social support, cultural values, hypnotic suggestion, excitement in sport or war, distraction, and appraisal can all significantly modulate pain's intensity and unpleasantness.

DURATION OF PAIN:

Pain is usually transitory, lasting only until the noxious stimulus is removed or the underlying damage or pathology has healed, but some painful conditions, such as rheumatoid arthritis, peripheral neuropathy, cancer and idiopathic pain, may persist for years.

Pain that lasts a long time is called *chronic*, and pain that resolves quickly is called *acute*. Traditionally, the distinction between *acute* and *chronic* pain has relied upon an arbitrary interval of time from onset; the two most commonly used markers being 3 months and 6 months since the onset of pain, though some theorists and researchers have placed the transition from acute to chronic pain at 12 months. Others apply the term *acute* to pain that lasts less than 30 days, „*chronic*" to pain of more than six months duration, and sub acute to pain that lasts from one to six months. A popular alternative definition of *chronic pain* as involving no arbitrarily fixed durations is "pain that extends beyond the expected period of healing."

SYSTEM AND REGION:

Pain can be classed according to its location in the body, as in headache, low back pain and pelvic pain or according to the body system involved, i.e.

- Myofascial (emanating from skeletal muscles or the fibrous sheath surrounding them)
- Causalgic ("burning" pain in the skin of the arms or sometimes legs; thought to be the product of peripheral nerve damage)
- Neurologic (caused by damage to or malfunction of any part of the nervous system)
- Rheumatic (emanating from the joints and surrounding tissue)
- Vascular (pain from blood vessels).

1.5.1 : ETIOLOGY OF PAIN:

The crudest example of classification by etiology simply distinguishes "**somatogenic**" pain (arising from a perturbation of the body) from "**psychogenic**" pain (arising from a perturbation of the mind. When a thorough physical examination, imaging, and laboratory tests fail to detect the cause of pain, it is assumed to be the product of psychic conflict or psychopathology). Portenoy divided *somatogenic* pain into "nociceptive" (caused by activation of nociceptors) and "neuropathic" (caused by damage to or malfunction of the nervous system).

Nociceptive pain is divided into "superficial" and "deep" - and deep pain is further divided into "deep somatic" and "visceral". *Superficial pain* (or "superficial somatic" or "cutaneous" pain) is caused by injury to the skin or superficial tissues and is usually a sharp, well-defined, clearly localized pain. Examples of injuries that produce superficial pain include minor wounds and minor (first degree) burns. *Deep somatic pain* originates in ligaments, tendons, bones, blood vessels, fasciae and muscles, and is a dull, aching, poorly-localized pain; examples include sprains, broken bones and myofascial pain. *Visceral pain* originates in the viscera (organs) and is usually more aching or cramping than somatic pain. Visceral pain may be well- localized, but is often extremely difficult to locate, and several visceral regions produce "referred" pain when injured, where the sensation is located in an area completely unrelated to the site of injury. Nociceptive pain may also be classified according to the type of noxious stimulation. The most common categories are "thermal" (heat or cold), "mechanical" (crushing, tearing, etc.) and "chemical".

1.5.2 : MECHANISM OF PAIN PERCEPTION AND NOCICEPTIVE AFFERENT NEURONS: [39]

Trigeminal and dorsal root ganglia (DRG) contain nociceptor cell bodies which gives rise to myelinated Aδ and unmyelinated C fibers. Under normal conditions pain is associated with electrical activity in small diameter primary afferent fibers 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. Since they are normally activated only by stimuli of noxious intensity, sufficient to cause some degree of tissue damage. Stimuli sufficient to excite these small afferent fibres also evoke a painful sensation. Non-myelinated C fibres have low conduction velocities (<1m/s). Aδ fibres conduct more rapidly but respond to similar peripheral stimuli.

Aδ and C fibers transduce and propagate noxious stimuli to the dorsal horn of the spinal cord from where these stimuli are transmitted to the brain. At the level of the spinal cord and at supraspinal sites, various neurotransmitters come into play, which together with environmental and cognitive factors, contribute to the eventual sensation of pain.

With many pathological conditions, tissue injury is the immediate cause of the pain, and this result in the local release of a variety of chemical agents, which are assumed to act on the nerve terminal, either activating them directly or enhancing their sensitivity to other forms of stimulation.

Nociceptors are receptors that respond to painful stimuli and are thin nerve fibers in the skin, muscle, and other body tissues, that, when stimulated, carry pain signals to the spinal cord and brain. Normally, nociceptors only respond to strong stimuli such as a pinch. However, when tissues become injured or inflamed, as with a sunburn or infection, they release chemicals that make nociceptors much more sensitive and cause them to transmit pain signals in response to even gentle stimuli such as breeze or a caress. This condition is called allodynia, a state in which pain is produced by innocuous stimuli.

1.5.3 : MANAGEMENT OF PAIN:

Analgesics refer to the class of drugs that includes most painkillers, such as aspirin, acetaminophen, and ibuprofen. Nonprescription or over-the-counter pain relievers are generally used for treating mild to moderate pain. Different types of drugs used to relieve pain, include:

a) Opioid / narcotic / morphine like analgesics.

OPIOID ANALGESICS:[36]

Classification of opioids:-

1. Natural opium alkaloids: Morphine, Codeine
2. Semi synthetic opiates: Diacelymorphine (Heroin), Pholcodeine Hydromorphine, oxymorphine, hydrocodone, oxycodone
3. Synthetic Opioids: Pethidine (Meperidine), Fantanyl, Methadone,

Dextropropxyphene, Tramadol

Opioid agonists produce analgesia by binding to specific G protein-coupled receptors, located primarily in brain and spinal cord regions involved in the transmission and modulation of pain. Three major classes of opioid receptors (μ, δ and κ) when activated, reduces intracellular cAMP formation and opens K^+ channels (mainly through μ and δ receptors) or

suppresses voltage gated N type Ca^{2+} channels (mainly κ receptor). These actions result in neuronal hyperpolarization and reduced availability of intracellular Ca^{2+} and therefore decreased neurotransmitter release by CNS and myenteric neurons.

b) Non-opioid / Non narcotic / Aspirin like / antipyretic and/or anti- inflammatory analgesics.
Non steroidal anti-inflammatory agents (NSAIDs) are used primarily to treat inflammation, mild to moderate pain, and fever. Specific uses include the treatment of headaches, arthritis, sports injuries, and menstrual cramps.

CLASSIFICATION OF NSAID"s:[36]

A. Non selective COX inhibitors (Traditional NSAID"s):

1. Salicylates : Aspirin

2. Propionic acid derivatives: Ibuprofen, Naproxen, Ketoprofen

3. Anthranilic acid derivatives: Mephenamic acid

4. Aryl-acetic acid derivatives: Diclofenac, Acelofenac

5. Oxicam derivatives: Piroxicam, tenoxicam

6. Pyrrolo-pyrrole derivatives: Ketorolac

7. Indole derivatives: Indomethacin, Pyrazolone derivatives: Phenylbutazone, Oxyphenbutazone

B. Preferential COX-2 inhibitors: Nimesulide, Meloxicam, Nabumetone

C. Selective COX-2 inhibitors: Celecoxib, Etoricoxib, Parecoxib

D. Analgesic- antipyretics with poor anti-inflammatory action:

1. Paraaminophenol derivative: Paracetamol (Acetaminophen)

2. Pyrazolone derivatives: Metamizol (Dipyrone), Propiphenazone

3. Benzoxazocine derivative: Nefopam

Cyclooxygenase (COX) enzymes:[39]
COX catalyses the conversion of arachidonic acid (AA) to prostaglandins (PGs), prostacyclins, and thromboxanes. It is well reported that the traditional nonselective NSAIDs provide their effects through the inhibition of these COX enzymes. COX exists as isomers Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) and catalyse the same reactions. These two enzymes share 60% homology in amino acid sequence, however the conformation for the substrate binding sites and catalytic regions are slightly different. COX-2 has a larger and more flexible substrate channel than COX-1 has, and COX-2 has a large space at the site where inhibitors bind. This structural difference between COX-1 and COX-2 lead to the development of COX-2 selective inhibitors.

COX-1:
- Responsible for physiological production of prostaglandins.
- COX-1 is constitutive enzyme in most cells- its activity is not changed once the cell is fully grown.COX-1 is described as a "house keeping enzyme" that regulates normal cellular processes, such as secretion of mucus for protection of gastric mucosa, homeostasis, platelet-aggregation and maintenance of renal function.
- The COX-1 enzyme of the stomach produces certain chemical messengers (called prostaglandins) that ensures the natural mucus lining which protects the inner stomach.

COX-2:
- It causes the elevated production of prostaglandins that occurs at the sites of diseases and inflammation.
- COX-2 is normally present in insignificant amounts, is inducible by cytokines, growth factors and other stimuli during the inflammatory response.
- COX-2 is constitutively expressed in some tissues, such as the brain, kidney and bones. It leads to inflammatory and other pathological changes. Its expression at other sites is increased during states of inflammation.

Selective COX-2 inhibitors are newly developed drugs for treating inflammation that selectively block the COX-2 enzyme. Blocking this enzyme impedes the production of the chemical messengers (prostaglandins) that cause the pain and swelling of arthritis inflammation.
Common anti-inflammatory drugs like aspirin block the function of the COX1 enzyme along with another enzyme, COX-2. When the COX-1 enzyme is blocked, inflammation is reduced, but the protective mucus lining of the stomach is also reduced, which can cause stomach upset, ulceration, and bleeding from the stomach and intestines. COX-2 selective inhibitory action provides the benefits of reducing inflammation without irritating the stomach.

Adverse effects of NSAID's:[36]
1. Gastrointestinal: Gastric irritation, erosions, peptic ulceration, gastric bleeding/perforation, esophagitis,
2. Renal: Na^+ and water retention, chronic renal failure, interstitial nephritis, papillary necrosis (rare).
3. Hepatic: Raised transaminase, hepatic failure (rare).
4. CNS: Headache, mental confusion, behavioural disturbances, seizure precipitation.
5. Haematological: Bleeding, thrombocytopenia, haemolytic anaemia and agranulocytosis.
6. Others: Asthma exacerbation, nasal polyposis, skin rashes, pruritis, angioedema.

OBJECTIVES
The objectives of the present study is as follows

1. **Collection and authentication of *Hibiscus radiatus***
Hibiscus radiatus plant was collected from the local areas of Shivamogga district and it has been authenticated by Ms. Soukya. N Botanist, Department of Botany, SRNMC, Shivamogga.
2. **Drying and powdering of leaves parts of *Hibiscus radiatus***
Leaves of *Hibiscus radiatus* were cut in to small piece and were shade dried and powdered.
3. **Extraction of the plant using different solvents.**
Powdered leaves of the *Hibiscus radiatus* were subjected to extraction using different solvents i.e., aqueous, methanol and ethyl acetate.
4. **Phytochemical investigation of extracts.**
Different extracts of *Hibiscus radiatus* were investigated for the presence of Phytochemicals by using standard procedures.
5. **Evaluation of Toxicological studies.**
Toxicity studies were carried out as per OECD guidelines No 425 in albino mice using the different extract to find out the LD50 of the extracts.
6. **Pharmacological screening of different extract of *Hibiscus radiatus.***
Different extract of *Hibiscus radiatus* were investigated for its anxiolytic and analgesic activities.

METHODOLOGY
4.1 **: Preparation of *Hibiscus radiatus***
4.1.1 **: Collection of Authentication of *Hibiscus radiatus***
The leaves of *Hibiscus radiatus* was collected from in and around the Shivamogga
District of Karnataka and was authenticated by the botanist, Ms. Soukya. N, Department of Botany, SRNMC, Shivamogga.
4.1.2 **: Drying and powdering of leaves parts of *Hibiscus radiatus***
The leaves parts of *Hibiscus radiatus* were shade dried and reduced to a coarse Powder in a pulvirizer (Sunbeam, Munger, India) using mesh no. 3 and passed through a Sieve No. 40 to obtain about 1kg of powder.
4.1.3 : Extraction of the plant using different solvents.
Various extracts of the plant material were prepared by maceration and Soxhlet extraction method. The powdered material of *Hibiscus radiatus* was extracted with different solvents (aqueous, methanol, ethyl acetate) in a Soxhlet extractor. The extract was concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and finally desiccator dried. The extract so obtained was labeled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered aerial part of the plant. These extracts are then used for the activities.
4.2 : Phytochemical investigation of extracts[40,41]
The extracts so obtained from each of the solvents were subjected to the following qualitative tests to detect the major chemical constituents.

1. **Test for carbohydrates**

a. **Molisch's test:** To the test solution, few drops of Molisch's reagent and 2ml. of concentration sulphuric acid were added slowly through the sides of the test tube. A purple ring formed at the junction of the two liquids indicates the presence of Carbohydrates.

b. **Barfoed's test:** To the test solution, Barfoed's reagent was added, boiled on water bath, brick red precipitate was formed.

c. **Benedict test:** To the best solution, Benedict's reagent was added and boiled on water bath, reddish brown precipitate was formed.

2. **Test for tannins:**

a. **Ferric chloride test:** Test solution with few drops of ferric chloride solution gives dark red color.

b. **Gelatin test:** Test solution when treated with gelatin solution white precipitate.

3. **Test for saponins**

a. **Foam test:** Saponins when mixed with water and shaken, shows the formation of froth, which was stable at least for 15 minutes.

b. **Haemolysis test:** 2ml. each of 18% sodium chloride solution is taken in two test tubes test tubes. To one test tube 2ml of distilled water was added and to the other 2ml. of the test sample was added. A few drops of blood were added to both the test tubes, mixed and observed for haemolysis under microscope.

4. **Test for flavonoids:**

a. **Ferric chloride test:** Test solution with few drops of ferric chloride solution shows Intense green color.

b. **Shinoda test:** The solution with few fragments of magnesium ribbon and concentrated hydrochloric acid shows pink to magenta red color.

c. **Zink-Hydrochloric acid reduction test:** Test solution with zinc dust and few drops of hydrochloric acid shows magenta red color.

d. **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which becomes colorless on addition of few drops of dilute acid.

e. **Lead acetate solution test:** Test solution with few drops of lead acetate (10%) solution gives yellow precipitate.

6. **Test for glycosides**

a. **Baljet test:** The test solutions when with solution picrate give yellow to orange Color.

b. **Keller- Killiani test:** The test solution was tested with few drops of ferric chloride solution and mixed. When concentrated sulphuricacid acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green.

c. **Raymond's test:** The test solution when treated with dinitrobenzene in hot methanolic alkali gives violet color.

d. **Bromine water test:** The solution when dissolved in bromine water gives yellow precipitate.

e. **Legal's test:** The solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red color.

7. **Test for alkaloids**

a. **Mayers's test:** When Mayer's reagent (potassium mercuric iodide) was added to the test solution, it gives cream colored precipitate.

b. **Wagner's test:** The acidic test solution with Wagner's reagent (iodine in potassium iodide) gives brown colored precipitate.

c. **Dragendroff's test:** When Dragendroff's reagent (solution of potassium bismuthiodide) was added to the test solution, it gives orange brown colored precipitate.

d. **Hager's test:** When Hager's reagent (saturated picric acid solution) was added to the test solution, it gives yellow colored precipitate.

4.3 : Pharmacological activities:

Animals:

Healthy young adult mala and non-pregnant female albino rats (200-250g) of either sex and non- pregnant female albino mice (20-25gm) were used for the acute toxicity and pharmacological studies (antiulcer, anxiolytic and analgesic activity) using aqueous, methanol and ethyl acetate extracts of leave parts of *Hibiscus radiatus*. The animal was procured from Central Animal House, National College of Pharmacy, Shivamogga, and Karnataka. After randomization in to various groups, animals were acclimatized for period of 10 days under Standard husbandry conditions. Room temperature $27^0 \pm 2^0$C; Relative humidity 65±10%;12hours – light/dark cycle All the
animals were fed with rodent pellet diet(Gold mohr, Lipton India Ltd.,) and water was allowed ad-libitum under strict hygienic condition.

Ethical clearance (Clearance number: NCP/IAEC/) for performing experiments on animals was obtained from institutional animal Ethics committee (IAEC).

Statistical analysis:

All the values were expressed as mean ± S.E.M. Statistical analysis was carried out by performing One- way ANOVA followed by pair wise comparisons of Turkey's HSD (honestly significant difference) Test. A probability level of $P<0.05$

was considered moderately significant, P<0.01 is considered as Significant and P<0.001 is considered as highly significant.

4.3.1 **: Acute toxicity study:**[42] Acute oral toxicity study for the *Hibiscus radiatus*
extracts was carried out using OECD Guideline-425 (modified, adopted March 23, 2006), the sequential test uses a maximum of five Animals. A test dose of 2000 or exceptionally 5000 mg/kg may be used in situation where experiment has information indication that the test material is likely to be nontoxic. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD50 confidence intervals. The test also allows the observation of sign of toxicity and can also be used to identify chemicals that are likely to have low toxicity. As suggested, after acclimatization of animals for 4-5 days, study was carried out as follows.
Healthy, young adult female albino mice (18-25gm) were used for this study. Food but not water, was withheld for 3-4 hours and further 1-2 hours after administration of sample under study. One animal was received test drug (plant extract) by oral route. Since this first test animal survived, four other animals were dosed (orally) at subsequent days, so that a total of five animals were tested. The acute toxicity studies of AEHR, MEHR and EEHR extracts was carried out according to above prescribed methods. At 2000mg/kg test sample did not produce any observable toxic Effects during entire duration of study. So, there was no mortality of the mice was found at 2000mg/kg b. w. hence, we have selected $1/10^{th}$ LD50 (200mg/kg) of the dose selected for further pharmacological screening

4.3.3. Anti-anxiety activity:
Antianxiety activity was evaluated by using in-vivo using total leaves extract of *Hibiscus radiatus* Modes used to evaluate the antianxiety activity are:

A. ELEVATED PLUS MAZE TEST:[45]
The plus maze apparatus consisting of two open arms (16×5) and two closed arms (16×5×12) having an open roof, with the plus maze elevated (25cm) from the floor, was used to observed anxiolytic behavior in animals. All the animals in the different groups were administered the normal water, extracts and standard drug orally using a tuberculin syringe fitted with oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus maze apparatus 45min after the administration of the dose. Each mouse was placed at the centre of the elevated plus maze with its head facing the open arm. During this 5min experiment the behavior of the mouse was recorded as:

- Preference of the mouse for its first entry into open arms.
- The number of the entries into the open arms or closed arms.
- Average time spent by the mouse in the open arms. (Average time=Total duration in the arms/number of entries)

During the entries experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals. Similar observation were recorded for the standard group II (Diazepam 2mg/kg) as well as the control group I (vehicle 1ml).

Rats were divided into five groups of six animals each.
Group-I : Control group treated with distilled water
Group –II: Received reference standard Diazepam (2mg/kg b.w.i.p.)
Group –III: Received aqueous extract of *Hibiscus radiatus.*
Group –IV: Received methanol extract of *Hibiscus radiatus.*
Group –V: Received ethyl acetate extract of *Hibiscus radiatus.*

4.3.4. Screening of analgesic activity:
Analgesic activity was evaluated by using in-vivo using total leaves extract of *Hibiscus radiatus* models used to evaluate the analgesic activity are:

A. ACETIC ACID INDUCED WRITHING[46]**:**
Albino mice of either sex weighing between 20-25gm were selected. Drug treatment will be done accordingly. First test drugs and vehicle will be administered. 30 min later intraperitoneal injection of acetic acid 0.7% v/v (0.1 ml/10 g) will be given to Swiss Albino mice overnight fasting. For Diclofenac sodium it will be 15 min. Animals will be then observed individually for counting the number of writhing made by them in 15 minutes from 5 minutes after the i.p acetic acid injection[10].

Mice were divided into 5 groups of 6 animals each. Group I: (Control) will be receives only vehicle.
Group II: Received Diclofenac sodium. (50mg/kg)
Group III: Received aqueous leaf extract of *Hibiscus radiatus.*
Group IV: Received methanolic leaf extract of *Hibiscus radiatus.*
Group V: Received ethyl acetate leaf extract of *Hibiscus radiatus*.

B. HOT PLATE METHOD[47]**:**
Albino mice of either sex weighing between 20-25gms were selected. Hot plate consists of an electrically heated surface, the temperature of which is maintained at 55°C to 56°C. The rats will be placed on hot plate after oral administration of drugs and the reaction time between placing the animal on hot plate and licking of fore/hind limb (Paw response) or the

jump response will be recorded by a stop watch. The average normal reaction time being 5sec. A cut off time 30 sec will be followed to avoid any thermal injury to the paws. The latency will be recorded before and after 20, 60, 90 and 120 minutes following oral administration of vehicle or drugs[11].

Mice were divided into 5 groups of 6 animals each.
Group I: (Control) will be receives only vehicle.
Group II: Will be receives Diclofenac sodium (50mg/kg)
Group III: Will be receives aqueous leaf extract of *Hibiscus radiatus.*
Group IV: Will be receives methanol leaf extract of *Hibiscus radiatus.*
Group V: Will be receives **e**thyl acetate leaf extract of *Hibiscus radiatus*.

Group	Dose (mg/k g b.w)	No. of entries		Time spent in (sec.)	
		Open Arm	Closed arm	Open arm	Closed arm
Control	-	2.11±0.37	11.00±0.54	68.17±0.53	182.18±0.31
Standar d	2	11.00±0.57** *	6.17±0.49***	151.18±0.54***	94.33±0.49***

Statistical analysis: All the value was expressed as mean ± S.E.M. The statistical analysis was carried out using way ANOVA followed by Turkey's comparison test. **A probability level of P<0.05 was considered moderately** Significant, **P<0.001 is** Considered as highly significant.

Result
II. Anti-anxiety activity:
All test samples of *Hibiscus radiatus* were evaluated for their anti-anxiety effect employing elevated plus maze method and light-dark model in albino mice of either sex weighing 25-30g.
The chloroform and ethyl acetate extract extract has been shown significant anti- anxiety activity when compared to control in Elevated plus maze model (Table No: 5.5) and light-dark model (Table No: 5.6)

Table 5.5: Table showing the effect of petroleum ether, chloroform, ethyl acetate leaf extract of *Hibiscus radiatus* on EPM Model.

Aqueous extract	200	8.17±0.41**	7.00±0.36**	134.18±0.30**	96.18±0.47**
Methano l extract	200	7.34±0.33*	7.18±0.41*	130.0±0.57*	117.57±0.768*
Ethyl Acetat e extract	200	5.34±0.33*	10.17±0.47*	110.51±0.41*	127.84±0.71*

NOTE: Date was analysed using one way ANOVA followed by Turkey's pairwise
Comparison **values are expressed as mean ±S.E.M. n=6, ***P<0.001 is considered as** highly significant.

Figure No.5.7: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on time spent in open arm and closed arm in EPM

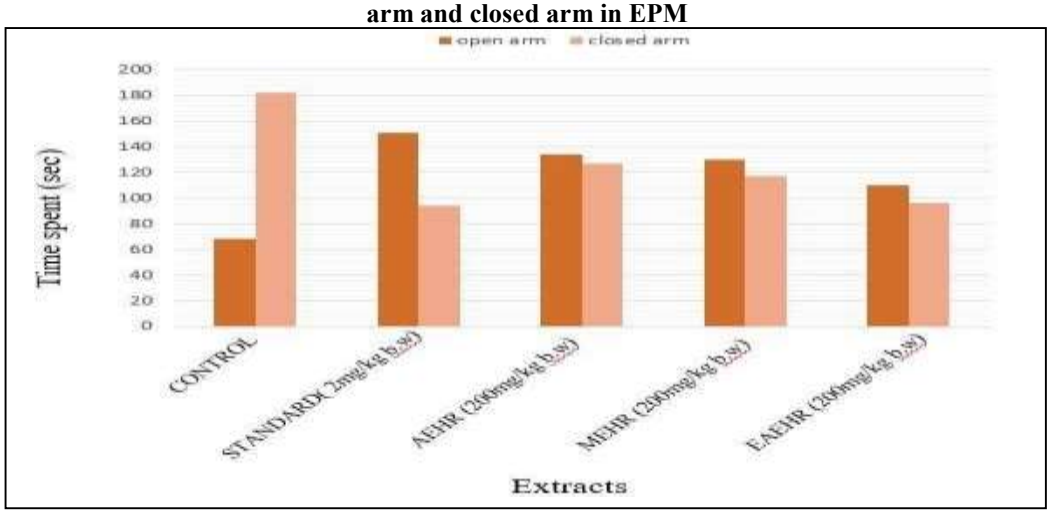


Figure No.5.8: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on number of entries in open arm and closed arm in EPM

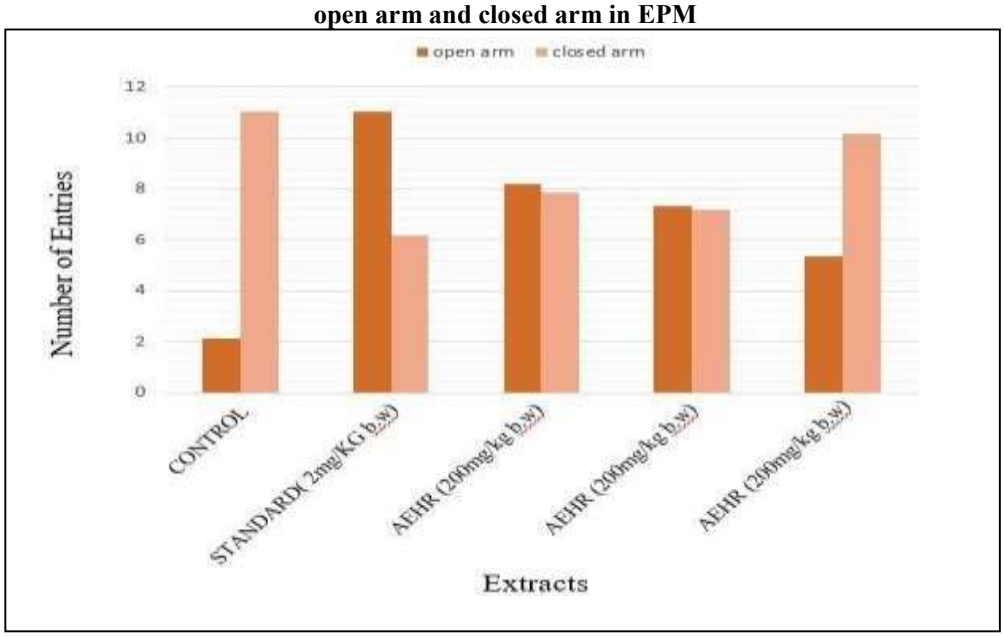


Table 5.6: Table showing the effect of aqueous, methanol and ethyl acetate Leaf extract of *Hibiscus radiatus* on Light-dark Model.

GROUPS	Dose (mg/kg.b.w)	No. of Entries		Time Spent in (sec)	
		Light Chambe r	Dark Chamber	Light Chamber	Dark Chamber
Control (Saline)	-	2.11±0.37	11.00±0.54	68.17±0.53	182.18±0.31
Standard (Diazepam)	2	11.00±0.57***	6.17±0.49***	151.18±0.54***	94.33±0.49***
Aqueou s extract	200	9.18±0.42**	8.00±0.31**	139.12±0.33**	99.12±0.47**
Methano l extract	200	8.43±0.22*	8.21±0.3*	132.0±0.48*	120.5±0.68*
Ethyl acetate extract	200	6.45±0.11*	11.21±0.25*	115.52±0.32*	129.48±0.81*

NOTE: Date was analysed using one way ANOVA followed by Turkey's pairwise Comparison **values are expressed as mean ±S.E.M. n=6, ***P<0.001 is considered as** highly significant.

Figure No.5.9: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on time spent in light and dark chamber in light and dark model.

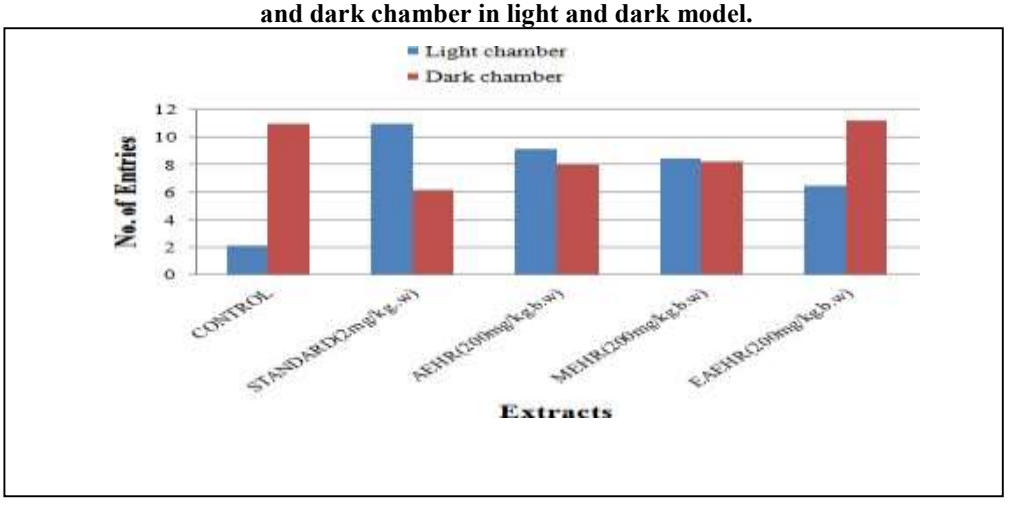
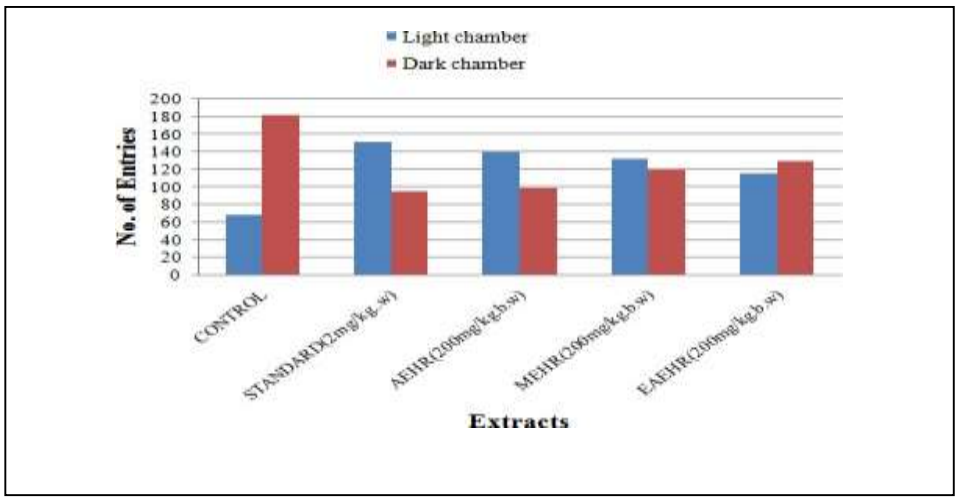


Figure No.5.10: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on number of entries in light and dark chamber in light and dark model.



II. Analgesic activity:

All test samples of *Hibiscus radiatus* were evaluated for their analgesic effect employing hot plate method and acetic acid writhing model in albino mice of either sex weighing 25-30g.

The aqueous and methanol extract has been shown significant analgesic activity when compared to control in hot plate (Table No: 5.7) and acetic acid writhing model (Table No: 5.8)

Table 5.7: Table showing the effect of aqueous, methanol and ethyl acetate Leaf extract of *Hibiscus radiatus* on acetic acid writhing model.

Groups	Dose(mg/kg)	Number of writhes in 5-15(min)	Percentage inhibition
Control (Saline)	-	42.66±8.32	-
Standard (Diclofena c)	50	14.83±2.85***	65.23
AEHR	200	22.50±4.51**	47.25
MEHR	200	25.00±6.12*	41.39
EAEHR	200	30.32±3.8*	28.92

NOTE: Date was analysed using one way ANOVA followed by Turkey's pairwise Comparison **values are expressed as mean ±S.E.M. n=6, ***P<0.001 is considered as** highly significant.

Figure No.5.11: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on acetic acid induced writhes.

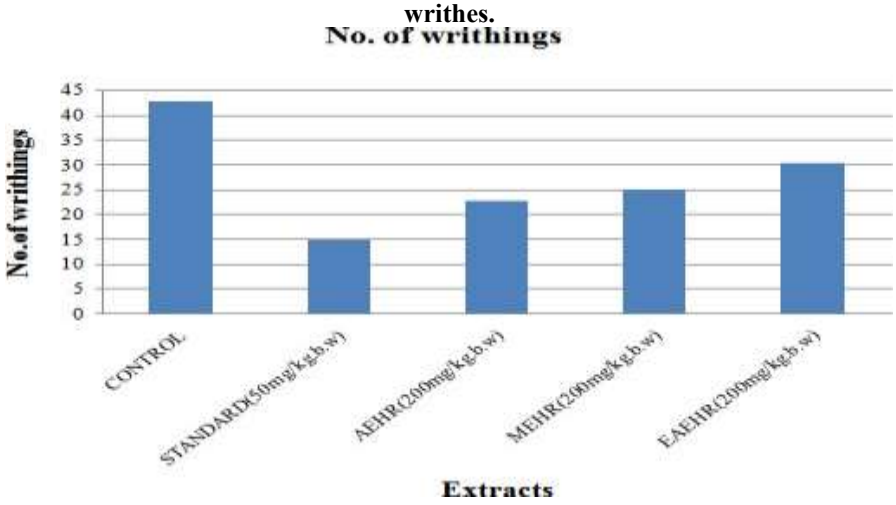


Figure No.5.12: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on acetic acid induced writhes.

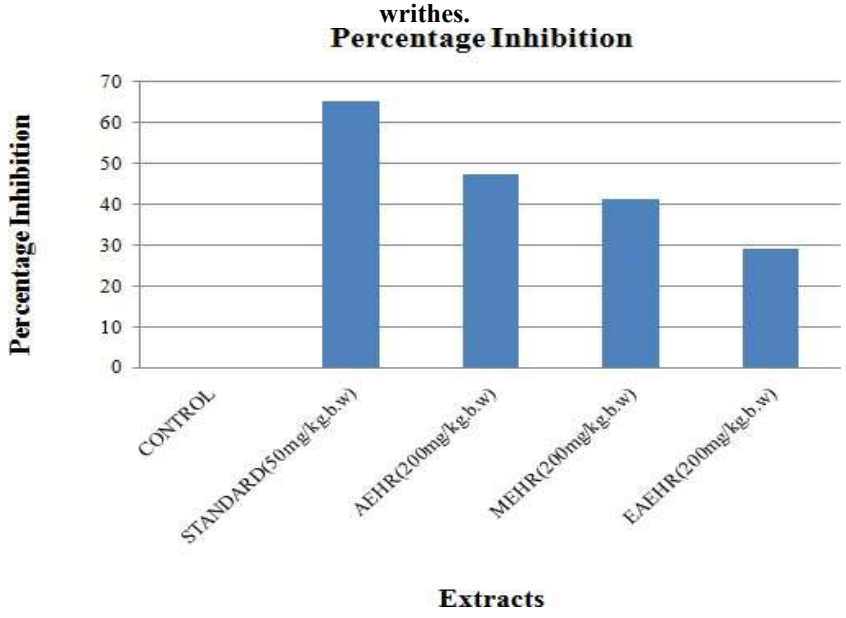
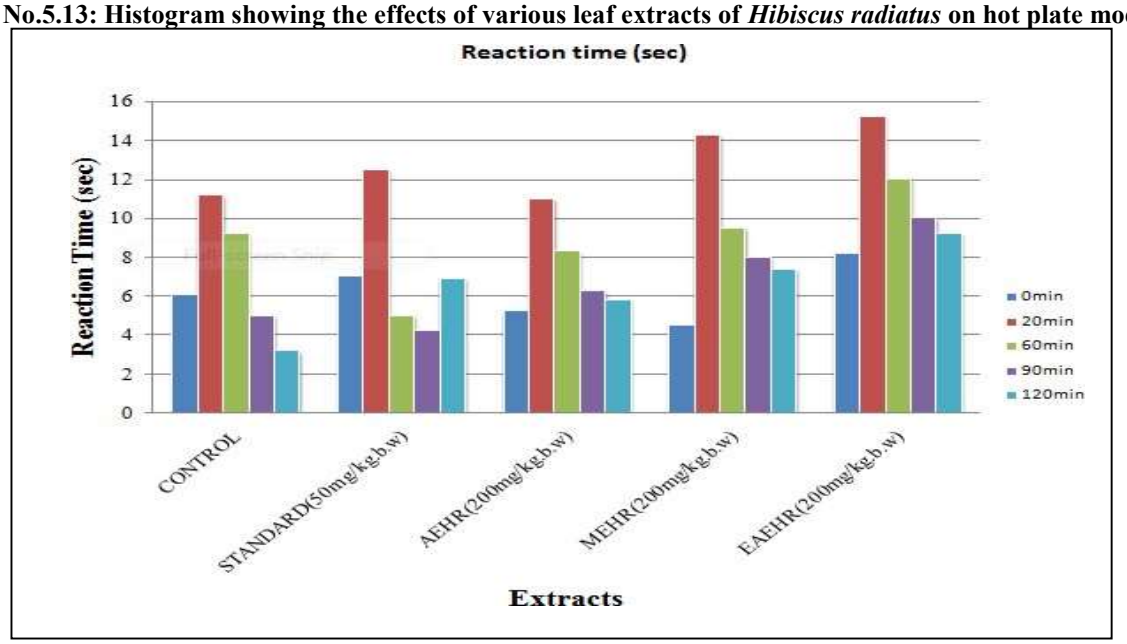


Table 5.7: Table showing the effect of aqueous, methanol and ethyl acetate leaf extract of *Hibiscus radiatus* on hot plate model.

Groups	Dose (mg/kg)	Reaction Time (sec)				
		0min	20min	60min	90min	120min
Control (Normal saline)	-	6.1±0.14	7.0±0.45	5.23±0.9	4.48±0.6	8.2±0.9
Standard (Diclofenac sodium)	50	11.2±0.5***	12.5±0.88***	11±0.2***	14.25±0.8***	15.23±0.68***
AEHR	200	9.2±0.36**	5.0±0.9**	8.3±0.25**	9.5±0.98**	12.0±0.23**
MEHR	200	5.0±0.7*	4.2±0.6*	6.26±0.28*	8.00±0.97*	10±0.89*
EAEHR	200	3.2±0.87*	6.9±0.7*	5.8±0.28*	7.4±0.42*	9.22±0.69*

NOTE: Date was analysed using one way ANOVA followed by Turkey's pairwise Comparison **values are expressed as mean ±S.E.M. n=6, ***P<0.001 is considered as** highly significant.

Figure No.5.13: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on hot plate model.



Discussion

I. Anti-anxiety activity

In the present study the test samples of leaf extract of *Hibiscus radiatus* belongs to the family Malvaceae were tested for anti-anxiety activity. Several reports are available on many plant species belonging to the presently studied family Malvaceae with anti-anxiety activity. In the present study anti-anxiety activity was evaluated by elevated plus maze model.

Elevated plus maze animals whenever subjected to unknown environment exhibits a particular form of behavioural inhibition, termed as anxiety[55]. The neurobiology of anxiety disorders is not fully known[53]. Low level of GABA in CNS is most frequently associated with anxiety disorders[54] .In addition to GABA; 5-HT plays an important role in the development and the persistence of anxiety disorders[55]. Anxiety disorders can also be due to free radical induced damage to Neurotransmitter system[56]. It involves spontaneous or natural aversive stimuli, i.e., height unprotected opening, and novelty[57]. In EPM, mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by fear of open spaces.

Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics[58].

In our study, we observed that AEHR and MEHR induced significant increases in the both the number of entries and time spent in the open arms. The number of entries and the time spent in the closed arms were reduced in the extract-treated group as compared to the control group.

Photochemical tests of *Hibiscus radiatus* revealed the presence of flavonoids, tannins, and saponins (Table No.5). It is possible that the mechanism of anxiolytic action of *Hibiscus radiatus* may be due to the binding of any of these phytochemicals to the GABAA-BZD complex.

In the present study the aqueous, methanol and ethyl acetate leaf extracts exhibited significant ($P < 0.0001$ & $P < 0.001$ respectively) anti-anxiety activity. Among these test samples aqueous and methanol extracts exhibited more significant antianxiety action when compare to control.

Role of flavonoids:

Flavonoids can act on ionotropic receptors for the inhibitory neurotransmitter GABA in many ways. They can act as positive, negative, and neutralizing allosteric modulators as well as agents that modulate other allosteric agonists. They appear to act at a variety of modulatory sites on GABAA receptors. Many investigators have noted structural similarities between certain flavonoids and Benzodiazepines, such as Diazepam, that are the most widely studied positive modulators of GABAA receptors[59]. However, further studies are necessary to find the exact mechanism of Anti-anxiety effect and to isolate the active compound(s) responsible for this pharmacological activity.

II. Analgesic activity:

In this present study the test samples of leaves extracts of *Hibiscus radiatus* belonging to the family Malvaceae were tested for analgesic activity. Several reports are available on many plant species belongs to the presently studied family Malvaceae with antiulcer activity.

The acetic acid-induced writhing model is a model involving chemical stimulus, where in acetic acid is administered intraperitoneously, which stimulates nociceptors and results in abdominal constrictions, known as writhes. It is widely used for the evaluation of peripheral by acting analgesic. In this model, pain is generated indirectly via endogenous mediators like bradykinin, serotonin, histamine and prostaglandins, all acting by stimulating peripheral nociceptive neurons. These fibers are sensitive to both narcotics such as morphine and non-steroid anti- inflammatory drugs (NSAIDs) so even centrally acting analgesics show analgesic activity through this model. Intraperitoneal injection of acetic acid can produce peritoneal inflammation (acute peritonitis) which causes the response characterized by contraction of the

abdominal muscle accompanied by an extension of the forelimbs and elongation of the body. This writhing response is considered as a visceral inflammatory pain model. This method has been associated with the increased levels of prostaglandins in the peritoneal fluids[60].

In acetic acid-induced abdominal writhes, arachidonic acid is released via cyclooxygenase pathway, and prostaglandin biosynthesis plays a role in the nociceptive mechanism. Among the prostanoids, mainly prostacyclin (PGI2) has been held responsible for the causation of pain after acetic acid administration[60].

It has been suggested that acetic acid stimulates the vanilloid receptor (VR1) and bradykinin B2 receptor in the pathway comprising sensory afferent C-fibers[60].

The analgesic effect of the extracts may therefore be due to either its action on
visceral receptors sensitive to acetic acid or due to their ability to interfere with the synthesis or release of those endogenous substances or desensitization of the nerve fibers involved in the pain transmission pathway.

To evaluate for a possible central antinociceptive effect of *Hibiscus radiatus*, the hot-plate test was selected. It has several advantages, particularly the sensitivity to analgesics and limited tissue damage. The ability of the extracts to prolong the reaction latency to thermally induced pain (Hot plate test) in mice further suggests central analgesic activity. Thermal nociceptive tests are sensitive to opioid μ receptors.

From the results obtained by both the models it can be concluded that the extract may be showing analgesic activity by both peripheral and central mechanism.

In the present study the aqueous, methanol and ethyl acetate leaf extracts exhibited significant ($P < 0.0001$ & $P < 0.001$ respectively) analgesic activity. Among these test samples aqueous and methanol extracts exhibited more significant analgesic action when compare to control.

Flavonoids, alkaloids and saponins are reported to have analgesic effect. Flavonoids are reported to have enzyme inhibitory activity, thus by inhibiting the cyclooxygenase enzyme, they may be preventing the production of prostaglandins, resulting in analgesic activity. Thus the analgesic effect of the extracts may be due to the presence of flavonoids, tannins, alkaloids and saponins either singly or in combinations which were found to be present in the extracts during phytochemical tests.

Conclusion

Anxiolytic activity:

Anxiolytic activity was carried out by using elevated plus maze. Diazepam was taken as standard reference drug. Aqueous and methanol extract of leaves have been shown a significant activity by increase in time spent in open arm by elevated plus maze test when compared to control. It can be concluded that active constituent is present in the leaves extract that is responsible for anti-anxiety activity. The overall study concluded that, the plant *Hibiscus radiatus* could be considered as anxiolytic agent.

However, further, studies are necessary to find the exact mechanism of anxiolytic effect and to isolate the active compound(s) responsible for this pharmacological activity.

Analgesic activity

Analgesic activity of various leaves extracts of *Hibiscus radiatus* was carried out by using two models namely, acetic acid writhing and hot plate model. In the present study all the test samples (aqueous, methanol and ethyl acetate) of leaves extracts exhibited significant analgesic activity. Among these test samples aqueous and methanol exhibited more analgesic activity ($P < 0.001$) when compared to control. It can be concluded that the active constituents are responsible for analgesic activity might be present in the leaves extracts. However, further, studies are necessary to find the exact mechanism of analgesic effect and to isolate the active compounds responsible for this pharmacological activity.

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