



IN VIVO ANALGESIC ACTIVITY OF NOVEL HETERO FUSED THIENO PYRAMIDINE BY USING RANA TIGRINA FROGS

Kattula Rajkumar¹, M. Nagulu², P.Poli Reddy³, A.Bheemanaik⁴

¹ Assistant Professor Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda

² Professor & Principal Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda

³ Associate Professor Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda

⁴ Assistant Professor Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda

*Corresponding Author Email: polireddy0004@gmail.com

ABSTRACT

Frog is being used for a limited purpose in experimental pharmacology. From the literature survey, it is clear that frog Rana Tigrina ever since the inception of physiology laboratory in the world, frog is one of the most commonly used animal in experimental pharmacology and physiology mainly because of its availability in abundance and also because of its versatility. Frog eg: Rana tigrina has been not used by many researchers for evaluation of analgesic drugs. In our study, we used Rana tigrina which was never used for evaluation of Novel Hetero Fused Thieno Pyrimidine for their analgesic activity by using Rana tigrina frogs. The novel compounds of hetero fused thieno pyrimidines which are available in our pharmaceutical chemistry department of, Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda. Our pharmaceutical Chemistry Department synthesize library of compounds and submit it to carry out their expected activity to the pharmacology laboratory. So, by doing this project we judge usefulness of Rana tigrina to evaluate analgesic drugs. So, we are not using animals like cat, rat, mice, in experimental pharmacology to evaluate analgesic activity. But we use experimental animal frog because of its availability in abundance and because of its versatility. The maintenance is also very easy. Writhing response, which is produced by various chemicals in mammals, is difficult to understand for beginners. On the contrary, in our experiment the parameters taken were a number of blinking of eye and buccal oscillations, which can be easily counted by a beginner. Furthermore, the animal is not sacrificed during the experiment.

KEY WORDS

Novel Hetero Fused Thieno Pyrimidine, Analgesic activity, Rana tigrina frogs, Experimental pharmacology.

INTRODUCTION

PAIN: pain is a "unpleasant sensory and emotional experience associated with an actual or potential tissue damage, or described in terms of such damage it is the feeling common to such experience a toe, burning a finger, putting iodine on a cut and bumping the funny bone" An internationally recognized definition is by the International Association for the Study of Pain "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". An alternative definition is offered by McCaffrey and Beebe, "Pain is

whatever the experiencing person says it is, existing whenever the experiencing person say it does". Both of these definitions therefore highlight that a painful experience is more than just tissue damage triggering a response from the nervous system. The management of pain thus involves more than simply treating the tissue injury and some difficult roles of pain.

Classification of pain:

Acute pain:

This can be intense and short-lived in which case we call it acute pain. Acute pain may injury. When their injury heals the pain usually the produced disturbance.

Chronic pain: this is pain long action of sensation acute pain, chronic pain is interns severe pain this is a sometimes injuries series condition of period, it depends condition of pain place. **Nociceptive pain:** represents the normal response to noxious insult or injury of tissues such as skin, muscles, visceral organs, joints, tendons, or bones. Examples include: Somatic: musculoskeletal (joint pain, myofascial pain), cutaneous; often well localized Visceral: hollow organs and smooth muscle; usually referred **Neuropathic pain:** pain initiated or caused by a primary lesion or disease in the somato sensory nervous system. Sensory abnormalities range from deficits perceived as numbness to hypersensitivity (hyperalgesia or allodynia), and to parenthesis such as tingling. Examples include, but are not limited to, diabetic neuropathy, post herpetic neuralgia, spinal cord injury pain, phantom limb (post-amputation) pain, and post-stroke central pain. **Inflammatory:** a result of activation and sensitization of the nociceptive pain pathway by variety of mediators released at a site of tissue inflammation. The mediators that have been implicated as key players are pro inflammatory cytokines such IL-1-alpha, IL-1-beta, IL-6 and TNF-alpha, chemokines, reactive oxygen species, vasoactive amines, lipids, ATP, acid, and other factors released by infiltrating leukocytes, vascular endothelial cells, or tissue resident mast cells Examples include appendicitis, rheumatoid arthritis, inflammatory bowel disease, and herpes zoster.

Time course: Pain duration **Acute pain:** pain of less than 3 to 6 months duration **Chronic pain:** pain lasting for more than 3-6 months, or persisting beyond the course of an acute disease, or after tissue healing is complete.

Acute-on-chronic pain: acute pain flare superimposed on underlying chronic pain.

PAIN OF AMPHIBIANS:

Different researchers are used experimental models like frogs in used analgesic eg; pathak.s (2014) were used rana tigrina frogs in india. Pain is an aversive sensation and feeling associated with actual, or potential, tissue damage. It is widely accepted by a broad spectrum of scientists and philosophers that non-human animals can perceive pain, including pain in amphibians. Pain is a complex mental state, with a distinct perceptual quality but also associated with suffering, which is an emotional state. Because of this complexity, the presence of pain in non-human animals cannot be determined unambiguously using

observational methods, but the conclusion that animals experience pain is often inferred on the basis of likely presence of phenomenal consciousness which is deduced from comparative brain physiology as well as physical and behavioral reactions. Amphibians, particularly neurons, fulfill several physiological and behavioral criteria proposed as indicating that non-human animals may experience pain. These fulfilled criteria include a suitable nervous system and sensory receptors, opioid receptors and reduced responses to noxious stimuli when given analgesics and local anesthetics, physiological changes to noxious stimuli, displaying protective motor reactions, exhibiting avoidance learning and making trade-offs between noxious stimulus avoidance and other motivational requirements. Pain in amphibians has societal implications including their exposure to pollutants, (preparation for) cuisine (e.g. Frogs legs) and amphibians used in scientific research. Several scientists and scientific groups have expressed the belief that amphibians can feel pain, however, this remains somewhat controversial due to difference.

CLASSIFICATION OF ANALGESICS DRUGS:

The word *analgesic* derives from *an-* "without"), *álgos*, "pain"), and, forming Such drugs were usually known as before the 20th century. Opium poppies such as this one provides ingredients for the class of analgesics called opiates An analgesic or painkiller is any member of the group of drugs used to achieve analgesia, relief from pain. Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which temporarily affect, and in some instances completely eliminate, sensation. Analgesics include paracetamol (known in North America as acetaminophen or simply APAP), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and oxycodone. In choosing analgesics, the severity and response to other medication determines the choice of agent; the World Health Organization (WHO) pain ladder ^[1] specifies mild analgesics as its first step. Analgesic choice is also determined by the type of pain: For neuropathic pain, traditional analgesics are less effective, and there is often benefit from classes of drugs that are not normally considered analgesics, such tricyclic antidepressants They may also be classified in other ways. Sometimes they are classified by use for various classes of medical condition¹ Other times they

are sorted by the needs of special populations who would use them¹ They might be listed by availability in a geographical area, perhaps to prevent recommending a drug which is illegal in one place even if it is easily available elsewhere.

Paracetamol/ acetaminophen; Paracetamol, also known as acetaminophen or APAP, is a medication used to treat pain and fever. It is typically used for mild to moderate pain. In combination with opioid pain medication, paracetamol is used for more severe pain such as cancer pain and after surgery. It is typically used either by mouth or rectally but is also available intravenously. Effects last between two and four hours. Paracetamol is classified as a mild analgesic. Paracetamol is generally safe at recommended doses.

NSAID:

Non-steroidal anti-inflammatory drugs (usually abbreviated to NSAIDs), are a drug class that groups together drugs that provide analgesic (pain-killing) and antipyretic (fever-reducing) effects, and, in higher doses, anti-inflammatory effects. The most prominent members of this group of drugs, aspirin, ibuprofen and naproxen, are all available over the counter in most countries. As analgesics, NSAIDs are unusual in that they are non-narcotic and thus are used as a non-addictive alternative to narcotics.

COX-2 inhibitors These drugs have been derived from NSAIDs. The cyclooxygenase enzyme inhibited by NSAIDs was discovered to have at least 2 different versions: COX1 and COX2. Research suggested most of the adverse effects of NSAIDs to be mediated by blocking the COX1 (constitutive) enzyme, with the analgesic effects being mediated by the COX2 (inducible) enzyme. Thus, the COX2 inhibitors were developed to inhibit only the COX2 enzyme (traditional NSAIDs block both versions in general). These drugs (such as rofecoxib, celecoxib, and etoricoxib) are equally effective analgesics when compared with NSAIDs, but cause less gastrointestinal hemorrhage in particular.

After widespread adoption of the COX-2 inhibitors, it was discovered that most of the drugs in this class increase the risk of cardiovascular events by 40% on average. This led to the withdrawal of rofecoxib and valdecoxib, and warnings on others. Etoricoxib seems relatively safe, with the risk of thrombotic events similar to that of non-coxib NSAID diclofenac. Opioids Morphine, the archetypal opioid, and other opioids

(e.g., codeine, oxycodone, hydrocodone, dihydromorphine, pethidine) all exert a similar influence on the cerebral opioid receptor system. Buprenorphine is a partial agonist of the μ -opioid receptor, and tramadol is a serotonin norepinephrine reuptake inhibitor (SNRI) with weak μ -opioid receptor agonist properties. Tramadol is structurally closer to venlafaxine than to codeine and delivers analgesia by not only delivering "opioid-like" effects (through mild agonism of the mu receptor) but also by acting as a weak but fast-acting serotonin releasing agent and norepinephrine reuptake inhibitor. Tapentadol, with some structural similarities to tramadol, presents what is believed to be a novel drug working through two (and possibly three) different modes of action in the fashion of both a traditional opioid and as a SNRI. The effects of serotonin and norepinephrine on pain, while not completely understood, have had causal links established and drugs in the SNRI class are commonly used in conjunction with opioids (especially tapentadol and tramadol) with greater success in pain relief. Dosing of all opioids may be limited by opioid toxicity (confusion, respiratory depression, myoclonic jerks and pinpoint pupils), seizures (tramadol), but opioid-tolerant individuals usually have higher dose ceilings than patients without tolerance.

Opioids, while very effective analgesics, may have some unpleasant side-effects. Patients starting morphine may experience nausea and vomiting (generally relieved by a short course of antiemetics such as Phenergan). Pruritus (itching) may require switching to a different opioid. Constipation occurs in almost all patients on opioids, and laxatives (lactulose, macrogol-containing or co-danthramer) are typically co-prescribed. When used appropriately, opioids and other central analgesics are otherwise safe and effective, however risks such as addiction and the body's becoming used to the drug (tolerance) can occur. The effect of tolerance means that frequent use of the drug may result in its diminished effect so, when safe to do so, the dosage may need to be increased to maintain effectiveness. This may be of particular concern regarding patients suffering with chronic pain. Opioid tolerance is often addressed with "opioid rotation therapy" in which a patient is routinely switched between two or more non-cross-tolerant opioid medications in order to prevent exceeding safe

dosages in the attempt to achieve an adequate analgesic effect.

Alcohol

Describing the effects of using alcohol to treat pain is difficult. Alcohol has biological, mental, and social effects which influence the consequences of using alcohol for pain. Moderate use of alcohol can lessen certain types of pain in certain circumstances. Attempting to use alcohol to treat pain has also been observed to lead to negative outcomes including excessive drinking and alcohol use disorder.

Medical cannabis

Medical cannabis or medical marijuana, can refer to the use of cannabis and its cannabinoids to treat disease or improve symptoms. There is limited evidence suggesting cannabis can be used to treat chronic pain and muscle spasms.

Combinations Analgesics are frequently used in combination, such as the paracetamol and codeine preparations found in many non-prescription pain relievers. They can also be found in combination with vasoconstrictor drugs such as pseudoephedrine for sinus-related preparations, or with antihistamine drugs for allergy sufferers.

While the use of paracetamol, aspirin, ibuprofen, naproxen, and other NSAIDs concurrently with weak to mid-range opiates (up to about the hydrocodone level) has been said to show beneficial synergistic effects by combatting pain at multiple sites of action, several combination analgesic products have been shown to have few efficacy benefits when compared to similar doses of their individual components. Moreover, these combination analgesics can often result in significant adverse events, including accidental overdoses, most often due to confusion that arises from the multiple (and often non-acting) components of these combinations.

Alternative medicine

Many people use alternative medicine treatments including drugs for pain relief. There is some evidence that some treatments using alternative medicine can relieve some types of pain more effectively than placebo. The available research concludes that more research would be necessary to better understand the use of alternative medicine.

Psychotropic agent's other psychotropic analgesic agents include ketamine), clonidine and other α_2 -

adrenoreceptor agonists, and mexiletine and other local anaesthetic analogues.

Other drugs

Drugs that have been introduced for uses other than analgesics are also used in pain management. Both first-generation (such as amitriptyline) and newer antidepressants (such as duloxetine) are used alongside NSAIDs and opioids for pain involving nerve damage and similar problems. Other agents directly potentiate the effects of analgesics, such as using hydroxyzine, promethazine, carisoprodol, or tripeleminamine to increase the pain-killing ability of a given dose of opioid analgesic.

Adjuvant analgesics, also called atypical analgesics, include nefopam, orphenadrine, pregabalin, gabapentin, cyclobenzaprine, scopolamine, and other drugs possessing anticonvulsant, anticholinergic, and/or antispasmodic properties, as well as many other drugs with CNS actions. These drugs are used along with analgesics to modulate and/or modify the action of opioids when used against pain, especially of neuropathic origin.

Dextromethorphan has been noted to slow the development of tolerance to opioids and exert additional analgesia by acting upon the NMDA receptors; some analgesics such as methadone and ketobemidone and perhaps piritramide have intrinsic NMDA action.

High-alcohol liquor, two forms of which found in the US Pharmacopoeia up until 1916 and in common use by physicians well into the 1930s, has been used in the past as an agent for dulling pain, due to the CNS depressant effects of ethyl alcohol, a notable example being the American Civil War. However, the ability of alcohol to relieve severe pain is likely inferior to many analgesics used today (e.g., morphine, codeine). As such, in general, the idea of alcohol for analgesia is considered a primitive practice in virtually all industrialized countries today. The use of adjuvant analgesics is an important and growing part of the pain-control field and new discoveries are made practically every year. Many of these drugs combat the side-effects of opioid analgesics, an added bonus. For example, antihistamines including orphenadrine combat the release of histamine caused by many opioids. Stimulants such as methylphenidate, caffeine, ephedrine, dextroamphetamine, methamphetamine, and cocaine work against heavy sedation and may

elevate mood in distressed patients as do the antidepressants. The use of medicinal cannabis remains a debated issue. In patients with chronic or neuropathic pain, various other substances may have analgesic properties. Tricyclic antidepressants, especially clomipramine and amitriptyline, have been shown to improve pain in what appears to be a central manner. Nefopam is used in Europe for pain relief with concurrent opioids. The exact mechanism of carbamazepine, gabapentin, and pregabalin is similarly unclear, but these anticonvulsants are used to treat neuropathic pain with differing degrees of success. Anticonvulsants are most commonly used neuropathic pain as their mechanism of different drugs.

DRUG PROFILE:

NOVEL HETERO FUSED THIENO PYRAMIDINES:

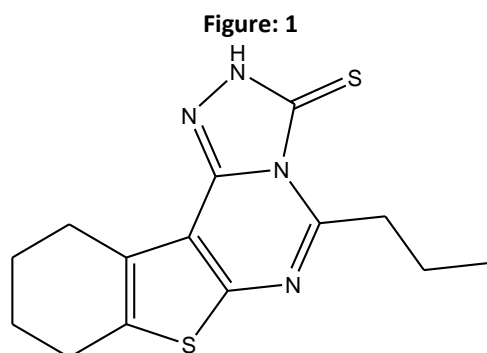
Pyrimidine derivatives are very important heterocyclic compound especially in life sciences, medicinal chemistry and in pesticidal chemistry and continue to attract great interest due to a wide variety of interesting biological activities such as anti-inflammatory, anticancer, antiviral, and antimicrobial activities. Fused pyrimidinones were reported to exhibit wide spectrum of activities like bronchodilatory, antihistaminic, anticancer among the pyrimidinones, thienopyrimidines have been evaluated pharmacologically and used as antimicrobial, analgesic, anti-inflammatory and antiviral agents.^{1,2,4} triazoles possess important pharmacological activities like anti-inflammatory, antifungal and cytotoxic activities.

Fused triazoles are proved to have diverse applications as antibacterial and anticancer agents. Recent literature reveals that when one heterocyclic system is coupled with another, a molecule with enhanced biological activity is produced. Click chemistry is a modular approach that uses only the most practical and reliable chemical transformations. Which describes the chemistry tailored to generate substances quickly and reliably by joining small units together. Its applications are increasingly found in all aspects of drug discovery, ranging from lead finding through combinatorial chemistry and target template in situ chemistry, to proteomics and DNA research, using bio-conjugation reactions.

Considering all these potential biological activities of thieno pyrimidines and triazoles which were reported to be more potent and less toxic, it has been felt worthwhile to take up the present investigation in an

effort to incorporate triazole ring system into thieno pyrimidine derivatives with methylene bridge to synthesize novel O-alkynyl triazolo thienopyrimidines as antimicrobial agents our previous work, some polycyclic heterocyclic derivatives were studied as 5 α -reductase inhibitors, antiviral and anti-tumor agents. Some of these compounds also showed aromatase and quinone reductase-2 inhibitors, anti-inflammatory, analgesic and antipyretic and anti-arthritic and immunosuppressive activities Pyrimidines and fused pyrimidines, being integral parts of DNA and RNA, play an essential role in several biological processes and also have considerable chemical and pharmacological importance as antibiotics, antibacterial, cardiovascular as well as agrochemical and veterinary products. Heterocyclic compounds play an important role in designing new classes of structural entities of medicinal importance with potentially new mechanisms of action. These heterocyclic compounds are well known to possess diverse pharmacological properties, viz. antimicrobial, analgesic, anti-inflammatory, anticancer, anticonvulsant and anti-malarial activities. On the other hand, we have reported that some of our new substituted heterocyclic compounds exhibited anti parkinsonian antitumor and anti-inflammatory activities. In addition, during the last few years, condensed thienopyrimidine derivatives have received considerable attention. Many of these derivatives were found to possess a variety of pronounced activities such as anti-inflammatory and analgesic antimicrobial, anti-avian influenza virus (H5N1), anti-herpes simplex virus type-1 (HSV-1) and hepatitis-A virus (HAV), serotonin 5-HT₆ receptor antagonist, antiarrhythmic agent properties. Pyrimidine derivatives have been previously reported as platelet aggregation inhibitors, antagonists, anti-conceptive and anti-parkinsonism agents. Heterocyclic compounds have also exhibited anthelmintic, anti-HIV activity and hypoglycemic activities. In view of these observations and as continuation of our previous works on heterocyclic chemistry, we report herein the synthesis of some new heterocyclic containing pyrazolo thienopyrimidine moieties and the study of their anti-inflammatory, analgesic activities.

STRUCTURE:



IUPAC NAME:

4-PROPYL --7,8,9,10, TETRA HYDRO--2H-6-THIA--1,2,3A,5-TETRAAZA -- CYCLOPENTA [C] FLUORENE – 3 THIONE.

M.F: **C₁₄H₁₆N₄S₂**

M.W: **304.43**

Physical properties:

STATE: solid

ODOUR: odorless

TASTE: taste less

COLOUR: light brown.

Solubility Profile:

| SOLVENT | SOLUBILITY |
|-----------------------------|------------|
| WATER | -- |
| ETHANOL | + |
| ACETONE | + |
| DMSO (Di methyl sulphoxide) | + |

CATEGORY: Analgesic

USES: analgesic.

LITERATURE REVIEW:

In this study, a series of novel 2-Methyl-4-(1-methyl-1H-[1,2,3] triazol-4-yl-methoxy)-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d] pyrimidine derivatives (IVa-e) were synthesized by treating 2-Methyl/Phenyl-4-prop-2-ynyloxy-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d] pyrimidine (IIIa/b) with alkyl azides in the presence of copper sulphate and dimethylsulphoxide. By treating 2-Methyl-5,6,7,8, -tetrahydrobenzo[b]thieno [2,3-d] pyrimidine-4-one(II) and its derivatives with propargyl bromide in the presence of potassium carbonate in acetone results in the formation of O-substituted compound-III and its derivatives. The compound-II and its derivatives were synthesized by treating 2-aminothiophene -3-carboxylic acid ethyl ester (I) with alkyl/aryl nitrile in the presence of dry HCl and 1,4-

dioxane. The newly synthesized compounds (IVa-e) were purified, separated, and characterized by TLC, IR, and Mass spectra. These representative analogues were screened for invitro antimicrobial activity. The compounds exhibited significant antibacterial and antifungal activities. pyrimidine derivatives this drug was good analgesic activity pharmaceutical chemistry purpose this drug never used in pharmacologically in frog's method.

A new series of fused triazolo- and tetrazolopyrimidine derivatives 2–14 were synthesized and their anti-inflammatory and ulcerogenic activities were evaluated. The pharmacological screening showed that many of these obtained compounds have good anti-inflammatory activities, comparable to the reference drug. The toxicity of the compounds was also assayed via the determination of their LD50 values. The structures of newly synthesized compounds were confirmed by IR, 1H-NMR, MS spectral data and elemental analysis.

Different species of frogs had been used by many researchers for evaluation of analgesic drugs e.g., *Rana pipiens* and African claw frog. In our study, we used *Rana tigrina*, which was never used for evaluation of analgesic activity of drugs. So, by doing this project, we judged usefulness of *R. tigrina* to evaluate analgesic drugs.

Methods: Animals used were *R. tigrina* of either sex weighing 100-150 g. Glass flask with porous platform was used for observation of frog. All groups were treated with 4% NaCl solution S.C. on abdomen). Characteristic parameter i.e., number of eye blinkings (this parameter was observed during the pilot study after 4% NaCl S.C. injection on lower third of frogs abdominal wall) were observed before and after drug administration. Each observation was for 5 mins. Centrally and peripherally acting drugs effect was tested on the number of blinks and buccal oscillations.

This is method is analgesic activity is find easy to evaluation of drugs. find eye blinking, and buccal oscillations in drugs central acting drugs are peripherally acting drugs were used in *rana tigrina* frog. this method for find observation 4% sodium chloride is major point of irritating induced pain subcutaneous route *Rana tigrina* frogs eye blinking injects drugs and after 30min., 4% sodium chloride was injected every drug teste subcutaneously is a pilot process of 5min.observation in this eye blinkings and buccal

oscillations. pathak. s, were singnificants of drugs in frogs method is very difficult, and easy and new thought of literature researcher is very attraction of this method.

Tramadol is a centrally acting weak μ opioid agonist that has few of the adverse side effects common to other opioids. Little work has been done to establish an effective analgesic dose of tramadol specific for surgical laparotomy and visceral manipulation in mice. We used general appearance parameters to score positive indicators of pain including posture, coat condition, activity, breathing, and interactions with other mice, activity events (that is, the number of times each mouse stretched up in a 3-min period) used as an indicator of decreased pain, von Frey fibers, and plasma levels of corticosterone to determine whether tramadol at 20, 40, or 80 mg/kg prevented postoperative pain in male and female C57BL/6 mice. A ventral midline laparotomy with typhlectomy was used as a model of postoperative pain. In male mice, none of the markers differed between groups that received tramadol (regardless of dose) and the saline-treated controls. However, general appearance scores and plasma corticosterone levels were lower in female mice that received 80 mg/kg tramadol compared with saline. In summary, for severe postoperative pain after laparotomy and aseptic typhlectomy, tramadol was ineffective in male C57BL/6 mice at all doses tested. Although 80 mg/kg ameliorated postoperative pain in female C57BL/6 mice, this dose is very close to the threshold reported to cause toxic side effects, such as tremors and seizures. Therefore, we do not recommend the use of tramadol as a sole analgesic in this mouse model of postoperative pain.

The present study was conducted to determine the histopathological a biochemical change in liver due to injection of tramadol in albino mice (*Mus musculus*). For this purpose, forty albino mice (25-30g) were divided into four groups, each group carried ten mice (three experimental and control group). Experimental groups (B, C and D) were injected tramadol intramuscularly equal to 12.5 mg, 25mg and 50 mg/kg body weight/day respectively for fourteen days. Biochemical analysis indicated that the levels of serum aminotransferase (ALT, AST) significantly ($P < 0.05$) increased than the control group. Similarly, creatinine and blood urea nitrogen (BUN) were also increased significantly ($P < 0.05$) in the experimental groups than

control. The histopathological studies indicated the necrosis, vacuolization, central vein dilation, hemorrhage, cytolysis and complete cell membrane degeneration in hepatocytes in the treated groups. Therefore, it is recommended that tramadol should be taken only with the prescription of doctor and self-medication of this medicine may be hazardous.

Numerous species of amphibians are frequently utilized as animal models in biomedical research. Despite their relatively common occurrence as laboratory animals, the regulatory guidelines that institutional animal care and use committees (IACUCs) must employ provide little in the way of written standards for ectothermic animals. Yet, as vertebrates, laboratory amphibians are covered by the National Research Council Guide for the Care and Use of Laboratory Animals and the Public Health Service (PHS) Policy for federally funded research. This article focuses on three issues that are relevant to IACUC oversight of the use of amphibians in research: (1) recommended educational requirements of investigators and animal care staff engaged in research with amphibians, (2) zoonoses and other issues of occupational health importance, and (3) indicators of stress and disease. Addressing these issues should enable investigators, IACUCs, and animal care staff to meet the regulatory expectations of the PHS and accrediting bodies such as the Association for Assessment and Accreditation of Laboratory Animal Care International.

Frogs guidelines followed by their maintaining animals care and also main experimental process. the frogs are used in particular separate cages and other guidelines followed the ethics and methodology of frogs in a all should maintaining in the my project work. novel hetero fused thieno pyrimidine drug is rana tigrina frogs for never used in analgesic purpose so, in this method for drugs replacement of analgesic activity observation. the mainly standard drugs and test drugs analgesic activity find out in test. pathak. s is a modified method is done but I am taking in a new drug of NHFTP compares of standard drugs, and ethanol activity of also find, totally experiment is based on "test drug analgesic activity" is how much work in pharmacologically study rana tigrina frogs.

AIM OF WORK:

The aim of present work is investigation of "In vivo analgesic activity of Novel Hetero Fused Thieno Pyrimidine" by using Rana tigrina frogs.

OBJECTIVES:

1. To investigate analgesic activity of Novel Hetero Fused Thieno Pyrimidine drug.
2. To compare test substance Novel Hetero Fused Thieno Pyrimidine with the Buprenorphine, Tramadol hydrochloride, Piroxicam, Diclofenac sodium.
3. Hetero Fused Thieno Pyrimidine may exhibit analgesic activity based on its structural activity relationship. So, it will be investigated in frogs and it was not standardized in our experimental pharmacology.
4. To compare the Novel Hetero Fused Thieno Pyrimidine activity for both the centrally and peripherally analgesic actions.
5. Our study the method used for evaluating analgesic activity of drugs by using Rana tigrina frogs (Indian bull frogs).

DRUGS SELECTION:

Centrally acting drugs are follows:

1. Tramadol hydro chloride,
2. Buprenorphine,
3. Distilled water.

Peripherally acting drugs:

1. Diclofenac sodium,
2. Piroxicam,
3. Distilled water.

Test drugs:

1. Novel hetero fused thieno pyrimidine,
2. Ethanol.
3. Sodium chloride (4%).

Apparatus and requirements:

Glass beakers, electronic weigh balance, bulbs of 10 w, porous platform, glass flasks, stop watch, glass rod, 2ml glass syringes, cotton, conical flasks etc.

NHFTP Test drug preparation:

Novel hetero Fused Thieno Pyrimidine /Ethanol 99.9% (50mg/50ml) stock solution prepared (200mg/kg body weight gives inject subcutaneous route 0.1ml, 0.2ml equal to 100 µg, 200 µg.)

Preparation of 4% NaCl solution: Take beaker in 100ml, 4gm of NaCl drug weight and dissolved 100 ml of distilled water.

Animal requirements:

All the rana tigrina frogs (100 – 150 g) requirements were conducted according to the IAEC. Approved by animals are placed in a separate glass flasks 2-liter water is produced each frog. Frog kept in a porous plat form arranged by the animal house of SRTIPS college, NALGONDA.

Were divided in centrally acting drugs: 4 group each one 6 animals used and peripherally acting drugs: 4 groups each one group 6 animals used, determination of 4% NaCl purpose 4 groups used. And estimation of ethanol concentration purpose 4 groups used in each one group 6 animals used. Animals are procured in a local supplier. the care according to guidelines given by taylor (2009).

Feeding condition:

Mainly rana tigrina frogs feed is a house holds cockroaches and mosquitoes. To attract in each cage 10 w bulbs in a kept. Current power supply must. sometimes generator used to observation all animal's safety requirements compulsory maintain is most important.

EXPERIMENTAL PARAMETERS:

1. Eye blinks before 4% NaCl injection, after 4% NaCl injection subcutaneously, pilot study of 5minutes. Counted the observations.
2. Buccal oscillations before 4% NaCl injection, after 4% NaCl injection subcutaneously, pilot study of 5minutes. Counted the observations.

METHOD:

Frogs of either sex weighing 100-150 g were procured from local supplier. The care of frogs was taken according to guideline given by Taylor (2009). Each frog was kept in a separate cage with 2 L of water. Water was replaced every day. To attract insects toward the cage, bulb of 10 w was kept glowing above each cage. Apart from this frog were also fed with household cockroaches. Drugs were purchased from the hospital pharmacy in local area.

Commonly used irritants for producing pain in animal's sodium chloride. In our study, we used sodium chloride solution to induce pain in frogs. 4% NaCl solution was used to produce writhing rapidly in Rana tigrina frogs.

After injection of 4% sodium chloride solution (0.5 ml) to we saw characteristic responses to this irritant stimulus. These responses were an increase in change in number of eye blinking and buccal oscillations. 4% solution showed maximum number of blinking and buccal oscillations. After this the centrally and peripherally acting drugs were given to see their effects on sodium chloride induced changes in blinking and buccal oscillations.

1. Effect of centrally acting drugs:

For this purpose, frogs were divided into four groups (each containing 6 animals):

- Control group: received 2 ml Distilled water followed by 4% NaCl subcutaneously
- Test Group 1: received Buprenorphine followed by 4% NaCl subcutaneously
- Test Group 2: received Tramadol hydrochloride followed by 4% NaCl subcutaneously
- Test Group 3: received Novel hetero fused thieno pyrimidine followed by 4% NaCl subcutaneously.

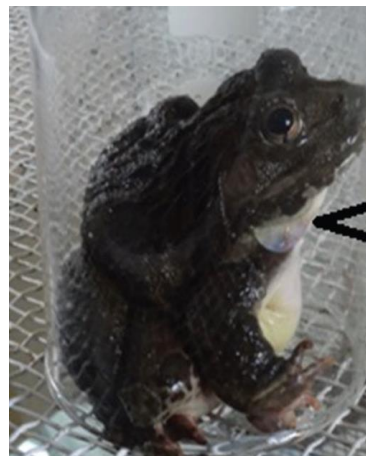
2. Effect of peripherally acting drug:

For this purpose, frogs were divided into four groups each containing 6 animals):

- Control group: received 2 ml distilled water followed by 4% NaCl subcutaneously.
- Test Group 1: received Piroxicam followed by 4% NaCl subcutaneously.
- Test Group 2: received Diclofenac sodium followed by 4% NaCl subcutaneously
- Test Group 3: received Novel hetero fused thieno pyrimidine followed by 4% NaCl subcutaneously.

Test groups received drugs by subcutaneous route in dorsal lymph sac. Each drug was given 30minuts before subcutaneous injection of 4% NaCl on lower third of frogs abdominal wall. For observation glass beaker and porous platform was used. Each frog was observed for 5mins after subcutaneous injection of 4% sodium chloride solution.

Figure:1



➤ Arrow mark buccal oscillations obseravation.

Figure: 2



➤ Observation eye blinks in an eye is closed and opened.

Statistical analysis:

Statistical analysis was done using graph prism pad software. One way ANOVA test was done followed by Dunnett's multiple comparison test. $P < 0.05$ was considered to be significant.

RESULTS:

Determination of effective concentration of NaCl solution

In our study 4% NaCl showed maximum number in both parameters like, Eye blinks and Buccal oscillation.

Effect of drugs: Centrally acting standard drugs Buprenorphine decreased rise in number of links and buccal oscillations significantly. Tramadol hydrochloride drug is not significantly decreased eye blinks and buccal oscillations,

Test drug of Novel hetero fused thienopyrimidine was highly significant. Small dose 0.1ml, 0.2ml is equal to

100µg, 200µg of test substance was decreased. rise in number of blinks and buccal oscillations significantly. Peripherally acting standard drugs diclofenac sodium, piroxicam not significantly decreased rise in number of

blinks and buccal oscillations comparatively the test drug of novel hetero fused thieno pyrimidine.

Table 1: Different groups of animals used for determination of effective concentration of sodium chloride

| | | | |
|---------|---------|-----------|---|
| Group 1 | Control | 6 animals | 2ml distilledwater By s.c route |
| Group 2 | Test | 6 animals | 4% Nacl solution By s.c route(0.5ml) |

Table 2: Determination of effective 4% Nacl concentration solution (number of eye blinks):

| Groups (n=6) | 2ml s.c injection | no.f eye blinks before 4% Nacl (5minutes) | no. of eye blinks after 4% Nacl (5 minutes) | % increases of blinks |
|--------------|-------------------|---|---|-----------------------|
| Control | Distilled water | 1.73±0.30 | 3.73+0.60 | 115.7 |
| Test | 4% Nacl | 2.80±0.47 | 25+0.2.50 | 1055*** |

n=number of animals. ***p<0.001

Table 3: Determination of effective 4% Nacl concentration solution (number of buccal oscillations):

| Groups (n=6) | 2ml s.c | no.of B.O before 4% Nacl (1minute) | no. of B.O after 4% Nacl (1minute) | %increases of B.O |
|--------------|-----------------|------------------------------------|------------------------------------|-------------------|
| Control | distilled water | 50± 2.50 | 55+0.50 | 10.01 |
| Test | 4% Nacl | 55±3.0 | 114+2.71 | 111.44**** |

****P < 0.0001

Table 4: Effect of centrally acting drugs and Test drug on eye blinking:

| Group (n=6) | Number of blinks | % of inhibition blinks |
|---------------------------------|------------------|------------------------|
| Distilled water | 25±0.25 | 0 |
| Buprenorphine (5mcg) | 10±0.79 | 52.09**** |
| Tramadol hydrochloride (0.01ml) | 17.50±2.25 | 25*** |
| NHFTP(0.2ml) | 8.09±0.5 | 93.02**** |

P<0.001 *P<0.0001

Table 5: Effect of centrally acting drugs and Test drug on buccal oscillations:

| Group (n=6) | Number of B.O | % of inhibition B.O |
|---------------------------------|---------------|---------------------|
| Distilled water | 120±06 | 0 |
| Buprenoprine (5mcg) | 60±1.50 | 52.49**** |
| Tramadol hydrochloride (0.01ml) | 89±0.01 | 23.05 |
| NHFTP(0.2ml) | 23±0.5 | 93.89**** |

P <0.001 *P<0.0001

Table 6: Effect of peripherally acting drugs and Test drug on eye blinking:

| Group (n=6) | Number of E.B | %of inhibition |
|----------------------------|---------------|----------------|
| Distilled water | 25 ±0.25 | 0 |
| Piroxicam (0.50mg) | 18.55±2.0 | 16.36 |
| Diclofenac sodium (1.35mg) | 19.56±1.05 | 14.56 |
| NHFHP(0.1ml) | 11±2.6 | 58.84**** |

Table 7: Effect of peripherally acting drugs and Test Drug on buccal oscillations:

| | | |
|----------------------------|------------|-----------|
| Distilled water | 25 ±0.25 | 0 |
| Piroxicam (0.50mg) | 18.55±2.0 | 16.36 |
| Diclofenac sodium (1.35mg) | 19.56±1.05 | 14.56 |
| NHFHP(0.1ml) | 12±2.6 | 61.07**** |

DISCUSSION:

Many researchers did experiments on amphibians for detecting analgesic activity of drugs. Pezalla et.al in his experiment on *Rana pipiens* showed how to assess the nociceptive threshold in frogs using the acetic acid test. In this test, the frog is exposed to various concentrations of acetic acid until it shows response. This process may damage tissue of the thigh. Suckow et.al show hypothermia induced analgesia where they used tourniquet of test leg and ice water for producing hypothermia. Such procedure can damage the tissue and animal will suffer a lot. Swan and Pathak et.al has reported a modified method for evaluating analgesic activity of drugs using *Rana tigrina* frog. This method was followed in our study and this method may not cause any damage to the tissues and furthermore the animal is not sacrificed during this experiment in our experiment the parameters taken were blinking of eye and buccal oscillations, which can be easily counted by a beginner. We gave 0.5 ml of 4 % NaCl solution subcutaneously to the frog. The quantity and concentration of sodium chloride solution is very low and it will not harm the frog anyway. Hence this method was selected according literature survey and this study may be helpful to give an alternative animal model by using frog.

Opioid and non – opioids are the main groups of drugs used for the treatment of pain. Opioids act on the central nervous system (block synaptic transmission of impulse signaling pain) for producing analgesia. Non-opioids act on peripheral nervous system (block impulse generation at pain receptor) for producing analgesia. Many animals and methods are used for

evaluating analgesic activity of a test substance e.g. rats, mice, dogs, and monkeys. Frog is also used as alternative to mammalian pain models. For this purpose, many species of frogs were used by many researchers e.g. *Rana pipiens* was used by Stevens in his studies on pain. African claw frog was used by Coble et al. In our study, we used *Rana tigrina* (Indian bull frog), which was never used for evaluation of analgesic activity of drugs in our pharmacology laboratory. Hence, this study was done to evaluate reproducibility, advantages and disadvantages with using *R. tigrina* as animal's model for evaluation of analgesic drugs.

In our study, a Non – opioid novel hetero fused thieno pyrimidine acts on peripheral nervous system (block impulse generation at pain receptor) for producing analgesia. This test substance was not used previously to test its analgesic activity. We were selected 100ug and 300 ug of NHFTP for testing its analgesic activity in frogs. This substance was also tested on, whether it acts on central nervous system and may produce analgesia activity. Interestingly this substance NHFTP was produced analgesic activity significantly. THP acts on central nervous system and peripheral nervous system and produced analgesia. Centrally and peripherally acting drug effect was tested on the number of eye blinking and buccal oscillations. Peripherally acting drug NHFTP inhibit rise in number of eye blinkings and buccal oscillations as comparatively with that of standard both centrally acting and peripherally acting drugs. With lower dose it produced the analgesic activity and whereas higher

dose (after increasing the dose) it was produced significant analgesia effect.

CONCLUSION:

Attempt to utilize frog as a modified method for evaluation of analgesic drugs and test substance THP using *Rana tigrina*. It was observed that very easily a beginner can carry out analgesic activity of opioids and non opioids for analgesic activity in experimental pharmacology laboratory comparatively other animal models.

Ethical approval: The study was approved by the Institutional Animal Ethics Committee (IAEC).

REFERENCES:

1. McCaffery, M., Beebe, A., 1989. Pain: clinical manual for nursing practice. C.V. Mosby, St. Louis.
2. Turk, D.C., Dworkin, R.H., 2004. What should be the core outcomes in chronic pain clinical trials? *Arthritis Res Ther*, *Arthritis Res Ther* 6, 151-4.
3. Holden, A.V., Winlow, W., Group., N.Neurobiolo, 1984. The Neurobiology of pain. Manchester University Press.
4. Taxonomy., I.Associatio, Bogduk, N., Merskey, H., 1994. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms, 2ndnd ed. IASP Press, Seattle.
5. Pathak S et al. *Int J Basic Clin Pharmacol*. 2014 Jun;3(3):496-500, *International Journal of Basic & Clinical Pharmacology* | May-June 2014 | Vol 3 | Issue 3 Page 498
6. Clark JS, Bentley E, Smith LJ. Evaluation of topical nalbuphine or oral tramadol as analgesics for corneal pain in dogs: a pilot study. *Vet Ophthalmol*. 2011;14(6):358-64.
7. Manning BH, Merin NM, Meng ID, Amaral DG. Reduction in opioid-and cannabinoid-induced antinociception in rhesus monkeys after bilateral lesions of the amygdaloid complex. *J Neurosci*. 2001;21(20):8238-46.
8. Stevens CW, Rothe KS. Supraspinal administration of opioids with selectivity for mu-delta- and kappa-opioid receptors produces analgesia in amphibians. *Eur J Pharmacol*. 1997;331(1):15-21.
9. Stevens CW, MacIver DN, Newman LC. Testing and comparison of non-opioid analgesics in amphibians. *Contemp Top Lab Anim Sci*. 2001;40(4):23-7.
10. Coble DJ, Taylor DK, Mook DM. Analgesic effects of meloxicam, morphine sulfate, flunixin meglumine, and xylazine hydrochloride in African-clawed frogs (*Xenopus laevis*). *J Am Assoc Lab Anim Sci*. 2011;50(3):355-60.
11. ANZCCART. The University of Adelaide Australia. Available from: http://www.adelaide.edu.au/ANZCCART/publications/A13_FrogsandToadsFactSheet.pdf. [Last accessed on 2013 Sep 1].
12. Fukawa K, Kawano O, Hibi M, Misaki N, Ohba S, Hatanaka Y. A method for evaluating analgesic agents in rats. *J Pharmacol Methods*. 1980;4(3):251-9.
13. Pezalla PD, Stevens CW. Behavioral effects of morphine, levorphanol, dextrophan and naloxone in the frog *Rana pipiens*. *Pharmacol Biochem Behav*. 1984;21(2):213-7.
14. Suckow MA, Terril LA, Grigdesby CF, March PA. Evaluation of hypothermia-induced analgesia and influence of opioid ntagonists in leopard frogs (*Rana pipiens*). *Pharmacol Biochem Behav*. 1999;63(1):39-43. doi: 10.5455/2319-2003.ijbcp20140616 drugs using *Rana tigrina* frog. *Int J Basic Clin Pharmacol* 2014; 3:496-500.
15. Stevens CW. Relative analgesic potency of mu, delta and kappa opioids after spinal administration in amphibians. *J Pharmacol Exp Ther*. 1996;276(2):440-8.
16. Taxonomy., I.Associatio, Bogduk, N., Merskey, H., 1994. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms, 2ndnd ed. IASP Press, Seattle.
17. McCaffery, M., Beebe, A., 1989. Pain: clinical manual for nursing practice. C.V. Mosby, St. Louis.Bahashwan, S.A.; Al-Harbi, N.O.; Fayed, A.A.; Amr, A.E.; Shadid, K.A.; Alalawi, A.M.; Bassati, I.M.S. Synthesis and pharmacological evaluation of novel triazolo [4,3 b] pyrazolo[3,4-c] pyridazine derivatives. *Int. J. Biol. Macromol*. 2012, 51, 7–17.
18. Al-Harbi, N.O.; Bahashwan, S.A.; Fayed, A.A.; Aboonq, M.S.; Amr, A.E. Anti-parkinsonism, hypoglycemic and anti-microbial activities of some new poly ring heterocyclic candidates. *Int. J. Biol. Macromol*. 2013, 57, 165–173.
19. Alanazi, A.M.; Al-Omar, M.A.; Abdulla, M.M.; Amr, A.E. Anti-arthritis and immune-suppressive.
20. Vijesh, A.M.; Isloor, A.M.; Shetty, P.; Sundershan, S.; Fun, H.K. New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. *Eur. J. Med. Chem*. 1962, 10, 410–41
21. Kolb H.C and Sharpless K.B; *Drug Discovery Today*., (2003), 8, 1128-1137.



***Corresponding Author:**

Kattula Rajkumar

Email: polireddy0004@gmail.com