WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 6, 676-689. Research Article ISSN 2277–7105

PHARMACOLOGICAL EVALUATION OF ETHYL ACETATE EXTRACT OF PAVONIA ZEYLANICA FOR ANALGESIC ACTIVITY

Vaddeboina Sowmya[1]*, Santhosh Illendula[2] and Kandhagatla Saisneha[3]

[1]Associate Professor, Department of Pharmacology, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana, India-508001.

[2]Associate Professor, Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana, India-508001.

[3]Assistant Professor, Department of Pharmaceutical Analysis, Teegala Ram Reddy College of Pharmacy, Meerpet, Rangareddy Dist., Telangana, India-500079.

Article Received on
30 Jan. 2024,

Revised on 20 Feb. 2024,
Accepted on 11 March 2024

DOI: 10.20959/wjpr20246-31725

***Corresponding Author**
Vaddeboina Sowmya
Associate Professor, Department of Pharmacology, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana, India-508001.

ABSTRACT

The present study was carried out to investigate the analgesic activity of *Pavonia zeylanica* using Acetic acid induce writhing, Eddy's Hot plate methods. The test and standard drugs significantly ($p<0.05$) reduced the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. The Eddy's Hot plate and acetic acid induced writhing method useful in the elucidating centrally mediated antinociceptive responses, which focused mainly on changes above the spinal cord level. All the test and standard drugs significantly ($p<0.05$) reduced the pain as compare to the control group. The results of pharmacological tests performed in the present studies suggest that the test and the standard drugs suggest the potent analgesic activity.

KEYWORDS: *Pavonia zeylanica*, Eddy's hot plate, acetic acid, test, std drug.

INTRODUCTION

Analgesia/Pain

Pain is a unpleasant sensory and emotional experience associated with tissue damage. In medicine pain relates to sensation of that hurts. If you feel pain it hurts, u feel discomfort, distress and perhaps agony, depending on the severity of it. Pain can be steady and constant,

in which case it may be an ache. It might be a throbbing pain- a pulsating pain. The pain could have a pinching sensation or a stabbing one. (Christian Nordqvist *et al.*, 2012)

Types of pain

Acute pain- this can be intense and short live, in which case we call it acute pain, it may be an indication of injury. When the injury heals the pain usually goes away.

Chronic pain- this sensation last longer than acute pain, chronic pain may be mild or intense (severe).

Classification of pain

Pain

- ✓ Nociceptive and
- ✓ Non nociceptive.

Nociceptive pain

It is divided into 2 types

1. Somatic
2. Visceral

Specific pain receptors are stimulated, these receptors sense temperature (hot/cold), vibration, stretch and chemicals released from damaged cells.

Somatic pain

A type of nociceptive pain. Pain felt on the skin, muscle, joints, bones and ligaments. The term Musculo-skeletal pain means somatic pain.

The pain receptors are sensitive to temperature, vibration and stretch (In the muscles). They are also sensitive to inflammation, as would happen if you cut yourself, sprain something that causes tissue damage. Pain as a lack of oxygen, as in ischemic muscle cramps, are a type of nociceptive pains. Somatic pain is generally sharp and well localized. If u touch it or move the affected area the pain will worsen.

Visceral pain

A type of nociceptive pain. It is felt in the internal organs and main body cavities. The cavities is divide into the thorax (Lungs and Heart), abdomen (Bowels, Spleen, Liver and

Kidneys) and the pelvis (Ovaries, bladder and Womb). The pain receptors-nociceptors sense inflammation, stretch and ischemia (Oxygen starvation).

Visceral pain is more difficult to localize than somatic pain. The sensation is more likely to be a vague deep ache. Colicky and cramping sensations are generally types of visceral pain. Visceral pain commonly refers to some type of back pain-pelvic pain generally refers to the lower back, abdominal pain to the mid-back, and thoracic pain to the upper back.

Never pain or neuropathic pain

Never pain is also known as neuropathic pain. It is a type of non-nociceptive pain. It comes from within the nervous system itself. People often refer to it as pinched nerve, or trapped nerve. The pain can originate from the nerves between the tissues and the spinal cord (Peripheral nervous system) and the nerves between the spinal cord and the brain (Central nervous system, or CNS)

Neuropathic pain can be caused by nerve degeneration, as might be the case in a stroke, multiple-sclerosis, or oxygen starvation. It could be due to a trapped nerve, meaning there is pressure on the nerve. A torn or slipped disc will cause never inflammation, which will trigger neuropathic pain. Nerve infection, such as shingles can also cause neuropathic pain.

Pain that comes from the nervous system is called non-nociceptive because there are no specific pain receptors. Nociceptive in this text means responding to pain. When a nerve is injured it becomes unstable and its signaling system becomes muddled and haphazard. The brain intercepts these abnormal signals as pain. This randomness can also cause other sensations, such as numbness, pins and needles, tingling and hypersensitivity to temperature, vibration and touch. The pain can sometimes be unpredictable because of this.

Sympathetic pain

The sympathetic nervous system controls our blood flow to our skin and muscles, perspiration (Sweating), by the skin, and how quickly the peripheral nervous system works.

Sympathetic pain occurs generally after a facture or a soft tissue injury of the limbs. This pain is non-nociceptive- there are no specific pain receptors. As with neuropathic pain, the nerve is injured, becomes unstable and fires off random, chaotic, abnormal signals to the brain, which interprets as pain.

Generally with this kind of pain the skin and the area around the injury become extremely sensitive. The pain often becomes so intense that the sufferers aren't use the effected arm or leg. Lake of limb use after a time can cause other problems such as muscle vesting osteoporosis, and stiffness in the joints.

Etiology of pain

Acute pain can last a moment rarely does it become chronic pain. Chronic pain persists for long periods. It is resistant to most medical treatment and cause severe problems. However the types and causes of pain are nerve pain, chest pain, burn pain, pinched nerve pain, foot pain, knee pain, lower leg pain, pelvic pain, elbow pain etc.

Pathophysiology of pain

In the past it was thought that a sensory input, such as a pinprick, would simply cause a pain "signal" to be send directly to the brain via in single nerve. Although still not completely understood today, the science of pain reveals a much more complex process, and theories are still continuing to evolved.

New receptors, pathways, hypothesis are being investigated everyday. In addition to identifying new pathways, genetic variations have been discovered at the receptor level that can further complicate treatment. It is important to have a basic knowledge of the physiology to treat pain effectively.

Plant: *Pavonia Zeylanica*
Family: Malvaceae
Uses: Inflammation, Analgesic, Hepatoprotective, anti-pyretic, Anthelminthic, Dysentry, Haemorrhage

Figure 1: *Pavonia zeylanica.*

Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defence against predation by microorganisms, insects and herbivores. Further, some of which may

involve in plant odour (Terpenoids), pigmentation (Tannins and Quinines) and Flavour (capsaicin).

However, several of these molecules possess medicinal properties. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anticarcinogenic, antibacterial, and antiviral activities. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing, and in recent years, there has been a worldwide trend towards the use of the natural phytochemical present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables. Pavonia zeylanica is a plant that has shown potential as a source of chemotherapeutic compounds. Phytochemical studies have revealed that the plant is rich in flavonoids and other water soluble polyphenolic compounds (Surabhi shrivastava et al., 2011).

MATERIALS AND METHODS

Materials

Table 1: Materials for analgesic activity.

S. No.	Materials
1	Acetic acid
2	Ibuprofen
3	Tremadol

Scheme of work

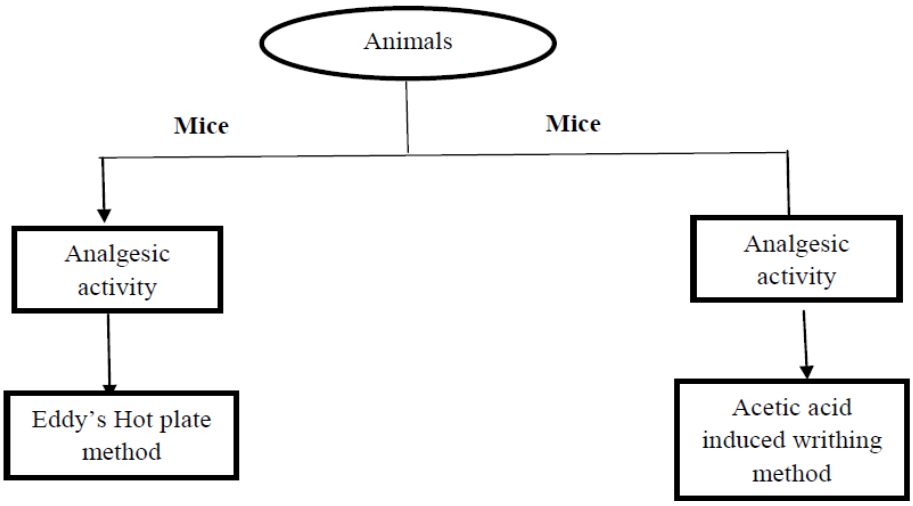


Figure 2: Scheme of work.

Experimental animals

Adult mice of either sex weighing about 20-25g were used for the study. Mice were housed properly and fed with free access to water. Room temperature was controlled at 25±2℃ with 12-hour light, 12-hour dark cycle. All the experiments were carried out according to the guidelines recommended by the Committee For The Control and Supervision Of Experiments on Animals (CCSEA), Government of India.

Experimental design

The experimental group consists of 24 mice divided equally into four groups, each consisting of 6 animals.

Group 1- Normal control.

Animals were treated with water after 12 hours of fasting

Group 2-Standard

Animals were treated with standard drug Ibuprofen/ Tremadol 10mg/kg administered thorough oral route.

Group 3- *Pavonia zeylanica* Dose-1

Animals were treated with *Pavonia zeylanica* (150 mg/kg) after 12 hours of fasting, administered orally.

Group 4- *Pavonia zeylanica* Dose-2

Animals were treated with *Pavonia zeylanica* (300 mg/kg) after 12 hours of fasting, administered orally.

METHODS

Analgesic activity

Animals: Healthy adult male Albino mice (25-35g) were used. The mice were housed in polypropylene cages and maintained under suitable nutritional and environmental (12hr light: 12hr dark cycle) throughout the experiment. The animals were fed with water and standard mice pellets.

Figure 3: Mice.

METHODOLOGY

Hot plate latency test

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses.

The animals were each placed on a hot plate maintained at 55^0C, 30 min after administration of test compounds, standard and saline. The time taken for the rats to respond to the thermal stimulus (Usually by jumping) was noted as the latency (In second).

The test drugs, control and std were administered orally to the animals after 12 hrs of fasting. The animals were each placed on a hot plate maintained at 55^0C, 30 min after administration of test compounds, std and saline. The time taken for the rats to respond to the thermal stimulus (Usually by jumping) was noted as the latency (In second).

The mean of the latency for each group was determined. The effects of the test drugs, std and saline were also determined after 0, 15, 30, 60,120 min of administration to mice.

Figure 4: Analgesiometer.

Table 2: Groups of Eddy's hot plate method.

Group(n=6)	Treatment
1	Water (Control)
2	Std drug
3	*Pavonia zeylanica* Dose-1 (150mg/kg)
4	*Pavonia zeylanica* Dose-2 (300mg/kg)

Acetic acid induced writhing method

Acetic acid induced writhing method was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response. Swiss albino mice weighing between 15-35g were used for evaluation of analgesic activity; in each group six albino mice were kept.

A solution of acetic acid (1% v/v) in distilled water was prepared. A solution of Ibuprofen (dose-100mg/kg/10ml) was prepared in normal saline water.

Wistar albino mice of either sex were divided into four different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 30 minutes writhing was induced by intraperitonial injection of 1% acetic acid in volume of 0.1 ml/10g body weight. The writhing episodes were recorded for 20 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted.

The percentage protection of writhing method is calculated by the following formula

Wc-Wt/Wc*100

Where, Wc= No. of writhing's of control group

Wt= No. of writhing's of test group

Figure 5: Mice producing writhing's.

Table 3: Groups of acetic acid induced writhing method.

Group(n=6)	Treatment
1	Water(Control)
2	Std drug
3	*Pavonia zeylanica* Dose-1 (150mg/kg)
4	*Pavonia zeylanica* Dose-2 (300mg/kg)

RESULTS AND DISCUSSION

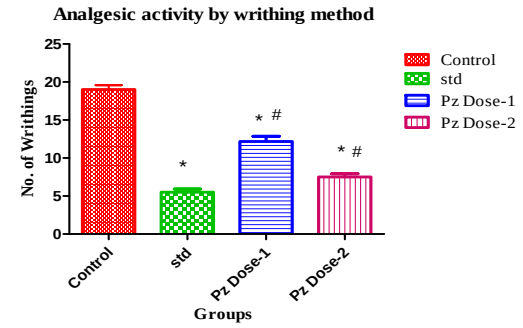
Statistical analysis

The experimental results were expressed as the Mean + SEM. Data were assessed by the method of analysis of ANOVA followed by Dunnet's multiple comparison test.

Table 4: Writhing response of drugs with time in analgesic activity.

S. No	Groups	No. of writhing's	% Protection
1	Control	19±0.58	-
2	Standard	5.5±0.43*	71%
3	*Pavonia zeylanica* Dose-1	12.17±0.70*	33%
4	*Pavonia zeylanica* Dose-2	7.5±0.42*	60%

The results obtained as percentage protection against writhing are shown in table -1. The results showed that standard drug, ethyl acetate extract of PZ (150 and 300 mg/kg), p.o suppressed the acetic acid-induced writhing response significantly in a dose dependent manner. The results were found to be highly significant in comparison to the control.



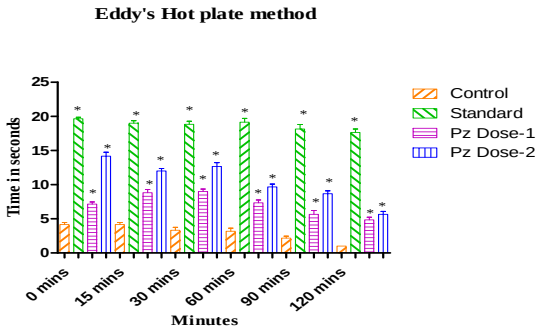
Values are Mean±SEM, N=6, P<0.05, P<0.0001. *P<0.05 Compared with control, #P<0.05 compared with standard

Figure 6: Graph representing the analgesic activity by writhing method.

Table 5: Analgesic response of drugs with time in analgesic activity.

S. No	Group	0 min	15 min	30 min	60 min	90 min	120 min
1	Control	4.17±0.3	4.16±0.3	3.33±0.42	3.16±0.47	2.16±0.3	1
2	Standard	19.67±0.21*	19±0.36*	18.83±0.47*	19.17±0.54*	18.17±0.65*	17.67±0.49*
3	*Pavonia zeylanica* Dose-1	7.17±0.3*	8.8±0.47*	9±0.36*	7.33±0.42*	5.67±0.56*	4.83±0.40*
4	*Pavonia zeylanica* Dose-2	14.17±0.60*	12±0.36*	12.67±0.56*	9.66±0.42*	8.67±0.42*	5.66±0.42*

The results for eddy's hot plate method are shown in table-2. After drug administration, reaction time increased significantly for the standard and test groups when compared to the control. Standard drug (Tramadol 5mg/kg, i.p), test drug (ethyl acetate extract of *Pavonia zeylanica* 150 & 300mg/kg p.o) produced a dose-dependent increase in the reaction time at various time intervals of observation. The results were found to be highly significant in comparison to the control.



Values are Mean ± SEM, N=6, P<0.05. *P<0.05 compared with control

Figure 7: Graph representing the analgesic activity by eddy's hot plate method.

DISCUSSION

The ethyl acetate extract of pavonia zeylanica was evaluated for analgesic activity by using Eddy's hot plate method and Acetic acid induced writhing method.

The hot plate test is useful in elucidating anti-nociceptive responses, which focuses mainly on changes above the spinal cord level. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic. Descending noradrenergic and serotonergic system. The analgesic effect produced by the test compounds may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key players in inflammation and pain (Pasero *et al.,* 1999, Millan *et al.,* 2002). In this study test compounds showed maximum significant activity.

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. In general, acetic acid causes pain by liberating endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins and substance P, endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. The method has also been associated with prostanoids in general that is, increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products.

The significant increase in pain threshold produced by tests and standard in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems. The analgesic effect produced by the tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain.

The selective cox-2 inhibitor has high effective than the conventional NSAIDs and has low GI and high cardiovascular side effects than to the conventional NSAID^{s}. Etoricoxib is a cox-2 inhibitor with a high degree of selectivity of its target. It provides an alternative to other selective and traditional NSAIDs in tarting patients with arthritis and other painful conditions.

CONCLUSION

The present experimental study protocol showed that ethyl acetate extract of *Pavonia zeylanica* elicited significant analgesic activity in Eddy's hot plate method and acetic induced writhing model. In both model they exhibited analgesic effect in a dose dependent manner which can be comparable with that of diclofenac and tramadol respectively. On preliminary phytochemical screening the ethyl acetate extract of *Pavonia zeylanica* was found to contain flavonoids, phenolic compounds & tannins, saponins, triterpenoids, alkaloids, and steroids compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception. Hence, the presence of flavonoids may be contributory to the analgesic activity of ethyl acetate extract of *Pavonia zeylanica*. Further studies may reveal the exact mechanisms of action responsible for the analgesic activity of ethyl acetate extract of *Pavonia zeylanica*. It was also concluded that the extract showed analgesic activity peripherally and centrally. The plant may have the phytoconstituents which inhibit cyclooxygenase enzyme for reducing analgesia peripherally or act on central opioid receptors (μ receptors) for reducing analgesia centrally. Standard drug diclofenac sodium act on cyclooxygenase pathway of prostaglandins synthesis and tramadol act on central opioid receptors mechanism.

ACKNOWLEDGEMENT

The authors are grateful to the management of Nalanda College of Pharmacy, Nalgonda for providing the facilities to carry out the present research work.

Conflicts of interest

None to declare for all authors.

Funding

No outside financing was used for research study.

REFERENCES

1. Jo Ann Gurenlian, R, Inflammation: The Relationship Between Oral Health and Systemic Disease, 2006.
2. *David S. Pearlman*, MD. Pathophysiology of Inflammatory Response, The Journal of Allergy and Clinical Immunology, 1999; 104, 4: 132-s137.
3. Kevin L. Zacharoff. MD. Pathophysiology of Pain. The Director of Medical Affairs.

4. Shalini Dalal, MD, and Donna S. Zhukovsky, MD, FACP. *pathophysiology* of *fever*, 2006.
5. Bencsics A, Elenkov IJ, Vizi EJ., Alpha 2-, alpha 2A-, alpha 2B/2C-adrenoceptor subtype antagonists prevent lipopolysaccharide-induced fever response in rabbits, 1995; 705: 302–306.
6. Luheshi GN, Stefferl A, Turnbull A., Febrile response to tissue inflammation involves both peripheral and brain IL-1 and TNF-alpha in the rat. Am J Physiol, 1997; 272: 862–868.
7. Roth J, Conn CA, Kluger MJ. Kinetics of systemic and intrahypothalamic IL-6 and tumor necrosis factor during endotoxin fever in the guinea pig. Am J Physiol, 1993; 265: 653–658.
8. Li S, Ballou LR, Morham SGCyclooxygenase-2 mediates the febrile response of mice to interleukin-1 beta. Brain Res, 2001; 910: 163–173.
9. Vizi ES., Receptor-mediated local fine-tuning by noradrenergic innervation of neuroendocrine and immune systems. Ann N Y Acad Sci, 1988; 851: 388–396.
10. Jones SR. Fever in the elderly. In: Mackowiak PA, ed, Fever: Basic Mechanisms and Management. New York: Raven Press, 1991; 233–242.
11. Roberts NJ Jr. Impact of temperature elevation on immunologic defenses. Rev Infect Dis, 1991; 13: 462–472.
12. Surabhi Shrivastava, Sampath Kumar and Leelavathi. S. Pharmacogenetic and preliminary phytochemical investigation on leaf extracts of *Pavonia zeylanica* Cav. Int. J. Drug Dev. & Res., April-June, 2011; 3(2): 192-197.
13. Shailaja Ande, Anjum, Khadar Shaik, Nisa Firdouse, Ravindra chary Dharmoji, Sowmya Ragini Yelwarthi. Pharmacological evaluation of oxazine sulfonamide derivatives for anti-inflammatory, analgesic and antipyretic activities. International Journal of Pharmacology and Toxicology, 2016; 4(2): 111-114.
14. K. Lalrinzuali, M. Vabeiryureilai, and Ganesh Chandra Jagetia. Investigation of the Anti-Inflammatory and Analgesic Activities of Ethanol Extract of Stem Bark of *Sonapatha Oroxylum indicum* In Vivo. International Journal of Inflammation, 2016, Article ID 8247014, 8 pages.
15. Avijit Chatterjee, Biswarup Sen, Sumanta Das, Tapan Kumar Chatterjee Anti-inflammatory and Analgesic Activity of Methanolic Extract of Medicinal Plant *Rhodiola rosea*. Rhizomes International Journal of Pharma Research & Review, 2015; 4(2): 1-8. ISSN: 2278-6074

16. D Hepcy Kalarani, A Dinakar, N Senthilkumar Antidiabetic, Analgesic and Anti-Inflammatory activity of Aqueous extracts of Stem and Leaves of *Alangium salvifolium* and *Pavonia zeylanica*. Int. J. Drug Dev. & Res., October-December, 2012; 4(4): 298-306.
17. M Chinna eswaraiah, Sudhakar kommu, Santhosh Illendula, In-vitro anthelmintic activity of Tamilnadia uliginosa fruits, Research Journal of Pharmacy & Technology, 2019; 12(1): 321-323.
18. Hepcy Kalarani D, Dinakar A, Senthilkumar N Analgesic and anti-inflammatory activity of ethanolic extracts of *alangium salvifolium* and *pavonia zeylanica*. Int. J. Pharm. Sci. Rev. Res, 2012; 17(1): 16, 77-80 ISSN 0976 – 044X.
19. Anitha Kumari1, K.G. Lalitha1, T. Venkatachalam2, P. Kalaiselvi2, M.G. Sethuraman3. Anti-inflammatory and Antinociceptive activity of *Pavonia zeylanica Linn*. Asian J. Res. Pharm. Sci, 2011; 1(4): 113-116.
20. AHM Zulfiker, M Mahbubur Rahman, M Kamal Hossain, K Hamid, MEH Mazumder, M Sohel Rana In vivo analgesic activity of ethanolic extracts of two medicinal plants - *Scoparia dulcis L*. and *Ficus racemosa Linn*. Biology and Medicine, 2010; 2(2): 42-48.
21. Amberkar mohan babu vittal rao, Tara shanbhag, Meena kumari K., K. L. Bairy and Smita shenoy. Evaluation of anti-inflammatory and analgesic activities of alcoholic extract of *kaempferia galanga* in rats. Indian J Physiol Pharmacol, 2011; 55(1): 13–24.
22. Wibool Ridtitid, Peerati Ruangsang, Wantana Reanmongkol and Malinee Wongnawa. Studies of the anti-inflammatory and antipyretic activities of the methanolic extract of *Piper sarmentosum Roxb*. leaves in rats. Songklanakarin J. Sci. Technol, 2007; 29: 6.
23. S. Venkata raman, Sini baby, R. Meera, P. Devi Anti Inflammatory, Analgesic and Antipyretic Activity of *Pergularia daemia forsk*. International Journal of Pharmaceutical & Biological Archives, 2010; 1(4): 371-375.
24. Dhirender Kaushik, Ajay Kumar, Pawan Kaushik, and A.C.Rana. Analgesic and Anti-Inflammatory Activity of *Pinus roxburghii Sarg*.
25. Vaddeboina Sowmya, Papagatla Poli Reddy, Vaddeboina Jyotsna, K.N.V.Rao "Evaluation of anti-nociceptive activity of crocus sativus", IJARMPS, 2023; 8, 2: 1-9. March-April.
26. V.Sowmya, Syed Arbaz Ahmed, Syed Sameer, P.Polireddy, K.Rajeswar Dutt "Extraction, Isolation and Purification of various components of Cassia Uniflora and it's invitro studies", IJPSR, 2020; 11, 11: 246-253.