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Pharmacological Investigation of Plumbago Root Extract in Neuropathic Pain

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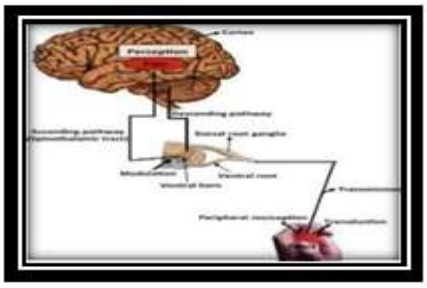
ABSTRACT: This study was conducted with the root of plant Plumbago indica L. (Family: Plumbaginaceae) to investigate the Pharmacological standard in experimental model of pain and biological estimation for pain mediators.The present study describes the role of plumbago plant roots extract in neuropathic pain (NP), pathophysiology of NP, phytoconstituents and mechanism of action plumbago as well as treatment strategy for NP. In the present study, CCI Induced Neuropathic pain parameters like mechanical stimulation, thermal hyperalgesia and cold allodynia were found to be reduced by plumbago extract it showed analgesic effect in neuropathic pain, analgesic activity against carrageenan induced inflammatory pain indicated by decrease in the number of paw licking. By using hot plate, tail fick and acetic acid induced writhing was reduced by plumbago extract and thus it shows analgesic effect.

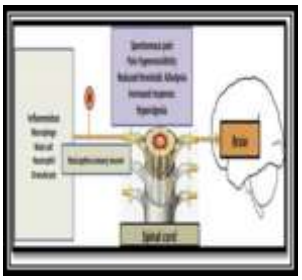
Keywords: Pharmacological, Plumbago zeylanica Linn, Plumbago Extract, Analgesic, Anti-inflammation, carrageenan.

I. INTRODUCTION:

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of damage (Swieboda et al., 2013). Pain is an immediate firing occuring in reflexive action when stimulus induces a reaction from pain receptor. Stimuli for temperature and touch have lower firing than of pain receptor and nociceptor. Pain receptor reacts due to stimuli such as cuts, scraps, heat, chemical substances that cause tissue damage and lack of blood circulation in specific area and the production of prostaglandins cause an irritation and signalling body about unpleasant feeling (Hamsarekha et al., 2019). Pain medication are useful in 20% to70% of cases. Chronic pain is most common cause of long term disability. Pain managed is estimated 90% individual with acute or cancer pain. Pain may experience anxiety, fear, anger or depression. The pain management agency for health care policy research (AHCPR) published first clinical practice guidelines (CPG) in 1992 for multiple health care disciplines (eg. surgery, anesthesiology, nursing) and pain management. American pain society is International association for study of pain (IASP) (Kaur et al., 2012).

Pain Pathway:





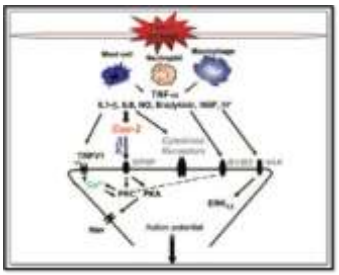


Fig 1: Pain Pathway **Fig 2**: Inflammatory Pain **Fig 3**:Pathophysiology ofNeuropathologyPain

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DRUG OR PLANT PROFILE

Plumbagin

Name	Plumbagin
Family	Plumbaginaceae
Order	Caryophyllales
Genus	Plumbago
Species	Zeylanica
Plant parts	Leaves, flower, roots
Geographical distribution	It grows abundantly in India in south India Such as Kodaikanal, Munnar ,Mysore, Bangalore etc
Chemical constituents	Chitanone,zeylanone,isozylanone,sitosterol,stigmasterol,compesterol, dihydro flavonol 3-chloroplumbagin 3,3-biplumbagin binapthoquinone.
Uses	Digestive stimulant, dysentery, Itchy skin problems, expectorant, laxative ,muscular pain

II. MATERIALS AND METHODS:

Plant materials

The root barks of Plumbago zeylanica were collected from TAMPCOL farm, Chennai and got authentication from the botany department of Captain Srinivasa Murthi Drug Research Institute, Dept. of AYUSH, Chennai, India.

Extract preparation

The collected plants were shade dried for a period 10 days and the root barks were separated. The dried barks were coarsely powdered using a pulverizer. The powder was stored in an airtight container until further use. The coarsely powdered plant drugs were subjected to successive soxhlet extraction with solvents of 85% of Methanol / 15% of Water and stored in the refrigerator. The extract was concentrated in-vaccuo and percentage of yields was calculated.

EXPERIMENTAL WORK AND RESULTS:

Experimental Protocol for neuropathic pain model

- Group I: Control

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- Group II: Sham control
- Group III : Chronic constriction injury (CCI)
- Group IV: Plumbago extract treatment (10mg/kg)
- Group V: Plumbago extract treatment (20mg/kg)
- Group VI: STD Gabapentine

Experimental Protocol for Inflammatory Pain models

- Group I: Control (Normal Saline)
- Group II: Carrageenan Induced
- Group III: Std Dexamethasone
- Group IV: Plumbago extract treatment (10mg/kg)
- Group V: Plumbago extract treatment (20mg/kg) \

Experimental Protocol for Nociceptive Pain models Hot Plate model

- Group I: Control (Normal Pain)
- Group II: Std diclofenac
- Group III : Plumbago extract treatment (10mg/kg)
- Group IV : Plumbago extract treatment (20 mg/kg)

Tail flick model

- Group I: Control (Normal Pain)
- Group II : Std diclofenac
- Group III: Plumbago extract treatment (10mg/kg)
- Group IV : Plumbago extract treatment (20mg/kg)

Acetic acid Induced model

- GroupI:Control
- GroupII:Stddiclofenac
- Group III : Plumbago extracttreatment(10mg/kg)
- Group IV : Plumbago extracttreatment(20mg/kg)

Induction of Neuropathic pain

Induction by chronic constriction nerve injury (CCI):

Peripheral neuropathic pain was induced by Chronic constriction nerve injury (CCI) using silk 4-0 sutured instead of chromic gut suture .the rats were deeply anesthetized with sodium thiopental anaesthesia(40mg/kg I.P).the hair on lower back and thigh of rats was shaved and skin was sterilized with alchol. The skin of lateral surface of left thigh was incised and cut made (3-4 mm) directly through biceps below femoris muscle expose sciatic nerve. Once expose sciatic nerve was ligated with silk 4-0 thread at ligate with silk 4-0 thread at four site with a 1mm gap. The ligature were loosely tied with short flick of ipsilateral hind limb was observed. The muscle and skin were closed in two layers with use of thread and topical antibics (soframycin) was applied. (Khangura et al., 2017).

Figure 3: CCI Induced Surgery (Hervera et al.,2010)

Induction of Inflammatory Pain

Induction by subplantar injection of caragennan

Peripheral Inflammatory pain was Induced by carrageenan 1%w/v solution inject 0.1ml to plantar region in to left paw of rat using 29G needle. Placed rats immediately in to boxes. Pain like behaviour (licking, grooming, Flicking) was observed for 1 hr.

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Figure 4: Induction of Inflammatory Pain (Archer et al., 2015)

Evaluation of Pain Parameters for Neuropathic Pain Models

Paw cold allodynia (acetone drop test):

Allodynia was assessed by spraying 100µl of a acetone on to the surface of the rat paw. The response of rat to acetone was noted for 20 sec and was graded on a 4 point scale (0-no response,1-quick withdrawl ,2-flick or stamp of the paw ,3-prolong withdrawl ,4- repeated flicking. acetone was applied three times to the hind paw with a gap of 5 min between the acetone application and individual score noted at 20sec interval were added to obtain a single score over a cummulative period of 1 min. The minimum score was 0 and maximum possible score was 9 (Jaggi and Singh.,2011**).**

Pain Parameters For Neuropathic Pain Models

Figure 5: Hargreaves Test (Hargreaves et al.,1988)

Figure 6: Pin Prick Test (Meng ting Zhang et al.,2017)

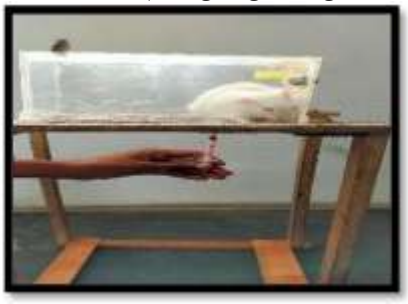

Figure 7: Acetone Drop Test (Yoon etal.,1994)

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Evaluation of Nociceptive Pain Models
Nociceptive Pain Models

Figure 10: **Tail flick apparatus (**Patel et al.,2016)

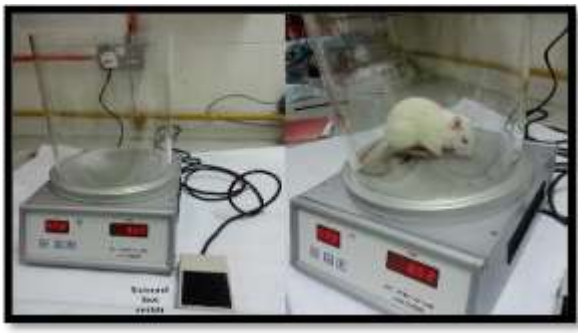

Figure 11: Hot plate apparatus (Wong et al.,2015)

Figure 12: Acetic acid Induced writhing (Satyam et al.,2018)

RESULT:

UV Spectral analysis of calibration curve

- The %purity of procurement of plumbago extract powder & std plumbagin was found to be 100%.
- The UV spectrum of standard Plumbagin and Plumbago extract powder showed the λ_{max} at 406nm & 425 nm respectively and the calibration curve showed that both drug follow Beer Lambert law.

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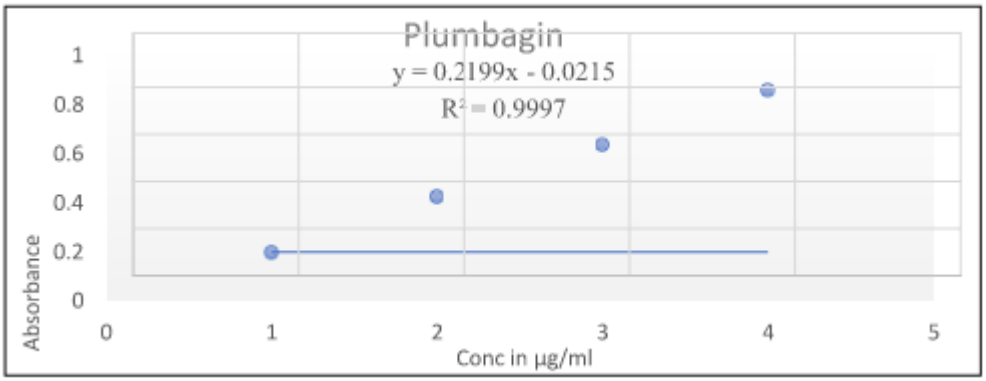

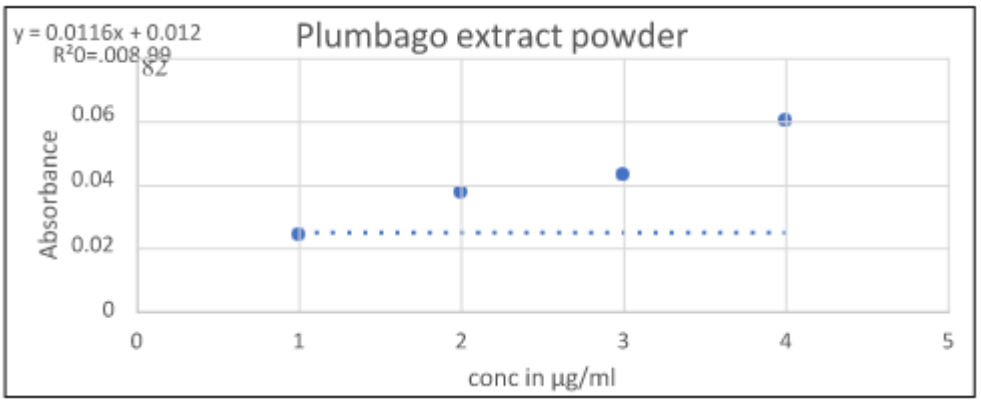

Pharmacological Evaluation:

- **Effect of Plumbago extract CCI Induced neuropathic pain parameters**
- **Effect on Thermal Hyperalgesia evaluated by radiant heat test**

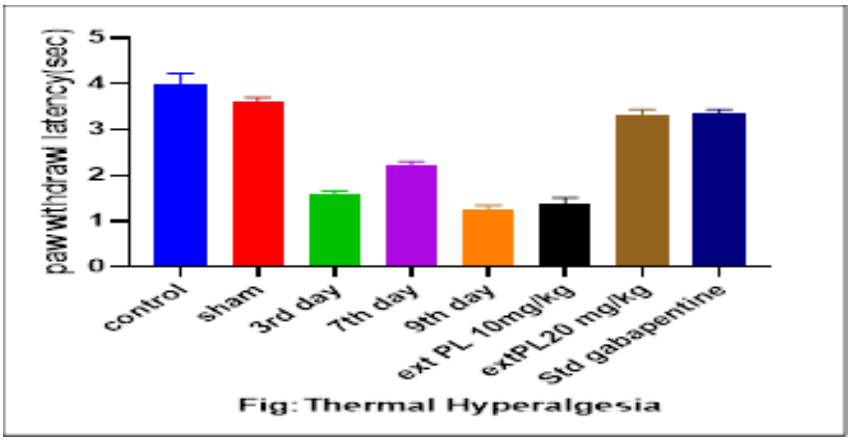

Fig: Thermal Hyperalgesia

Effect on Mechanical Hyperalgesia assessed by pin prick test on 3rd day and 9th day after surgery; All the data is expressed in Mean ± SD. The statistical significance were tested by one way ANOVA followed by Bonferroni's post hoc test using graph pad prism version 8.1. The level of significance used are; * p>0.05 # Mean ±SD expressed as P value P more than 0.05 but P less than ****p< 0.0001.

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Effect on Mechanical Hyperalgesia evaluated by pin prick test

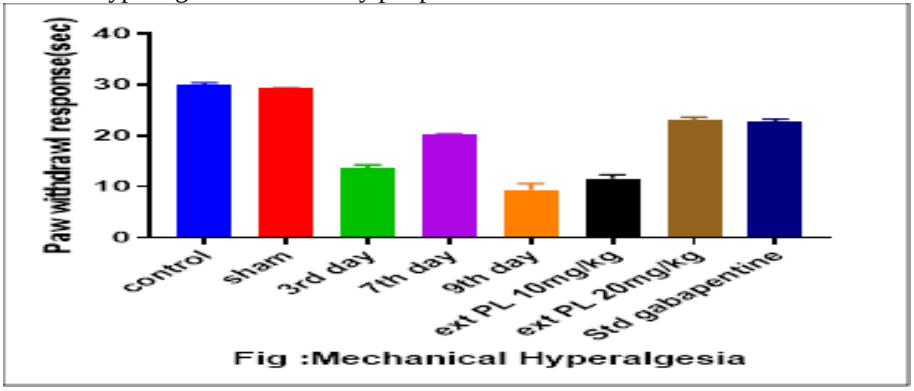


Fig :Mechanical Hyperalgesia

Effect on Mechanical Hyperalgesia assessed by pin prick test on 3rd day and 9th day after surgery; all the data is expressed in Mean ± SD. The statistical significance were tested by one way ANOVA followed by Bonferroni's post hoc test using graph pad prism version 8.1. The level of significance used are; * p>0.05 # Mean ±SD expressed as P value P more than 0.05 but P less than ****p< 0.0001.

- **Effect on Cold allodynia evaluated by acetone drop test**

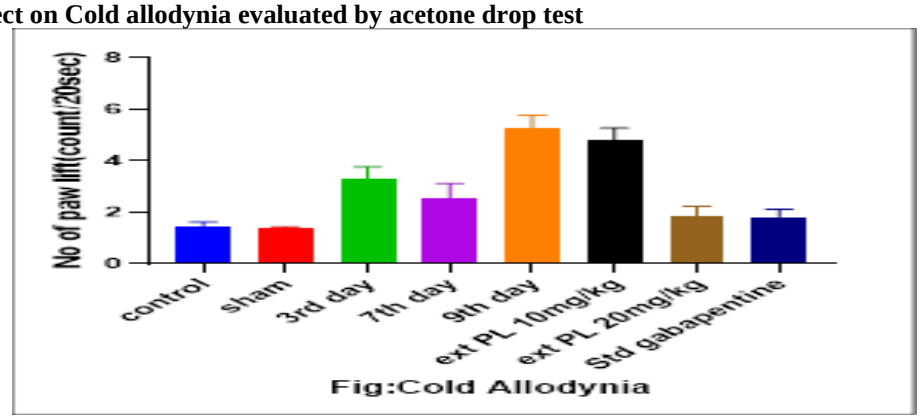
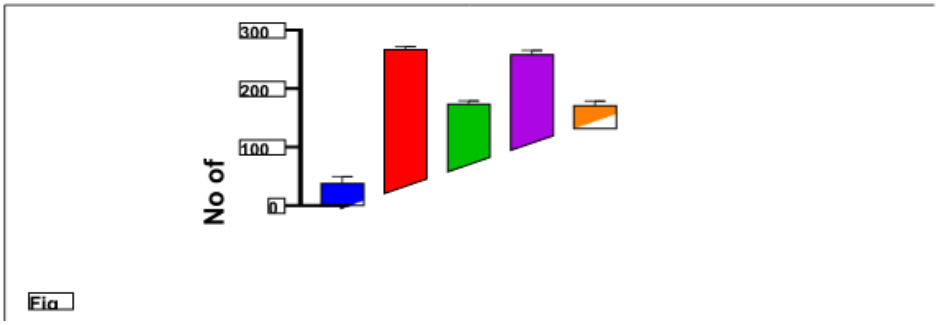


Fig:Cold Allodynia

Effect on cold allodynia assessed by acetone drop test on 3rd day and 9th day after surgery; All the data is expressed in Mean ± SD. The statistical significance were tested by one way ANOVA followed by Bonferroni's post hoc test using graph pad prism version 8.1. The level of significance used are; * p>0.05 # Mean ±SD expressed as P value P more than 0.05 but P less than ****p< 0.0001.

- **Effect of Inflammatory Pain**

Effect of Plumbago extract activity against carrageenan induced licking



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Effect of Plumbago roots extract activity against carrageenan induced licking. All the data is expressed in Mean ± SD. The statistical significance were tested by one way ANOVA followed by Bonferroni's post hoc test using graph pad prism version 8.1. The level of significance used are; * p>0.05 # Mean ±SD expressed as P value P more than 0.05 but P less than ****p< 0.0001.

- **Effect of Nociceptive pain Parameters**

Effect of Plumbago roots extract on Hot Plate

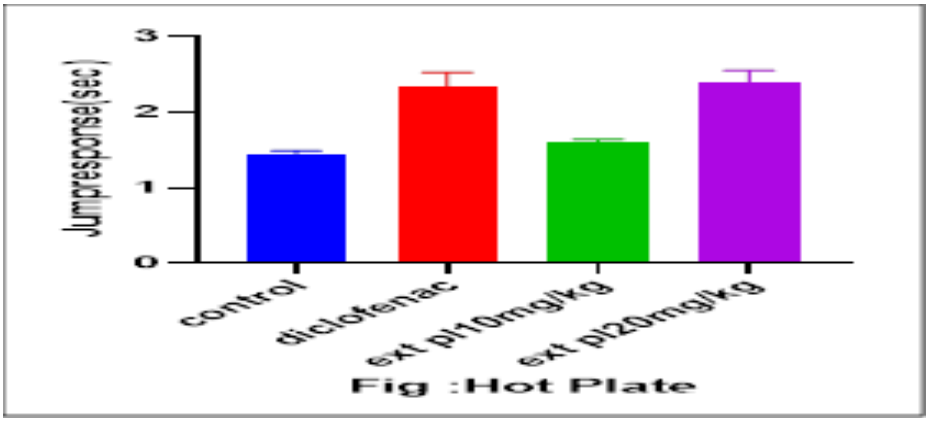

Fig :Hot Plate

Effect of Plumbago roots extract on Hot Plate. All the data is expressed in Mean ± SD. The statistical significance were tested by one way ANOVA followed by Bonferroni's post hoc test using graph pad prism version 8.1. The level of significance used are; * p>0.05 # Mean ±SD expressed as P value P more than 0.05 but P less than ****p< 0.0001.

- **Effect of Plumbago roots extract on Tail Flick**

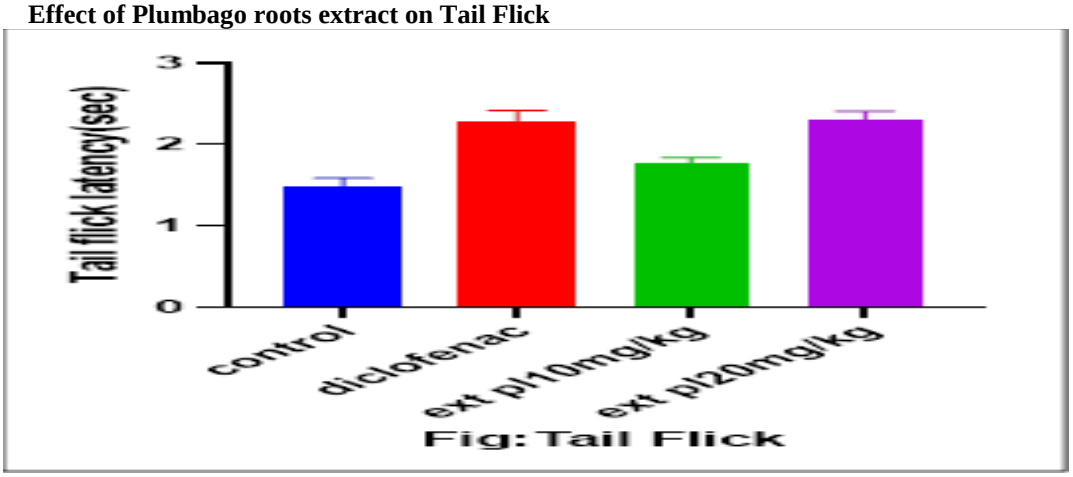

Fig: Tail Flick

Effect of Plumbago roots extract on Tail Flick. All the data is expressed in Mean ± SD. The statistical significance were tested by one way ANOVA followed by Bonferroni's post hoc test using graph pad prism version 8.1. The level of significance used are; * p>0.05 # Mean ±SD expressed as P value P more than 0.05 but P less than ****p< 0.0001.

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- **Effect of Plumbago extract on Acetic acid Induced writhing**

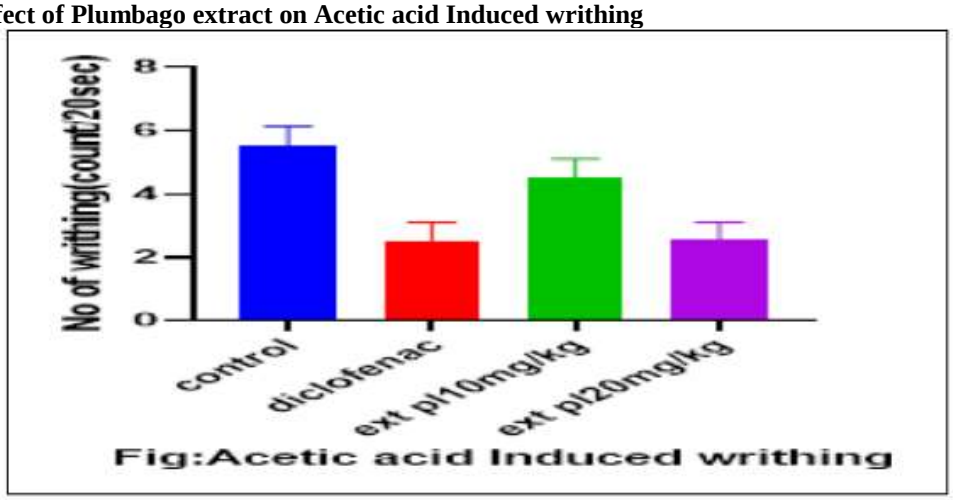

Fig:Acetic acid Induced writhing

Effect of Plumbago roots extract on Acetic acid Induced writhing. All the data is expressed in Mean ± SD. The statistical significance were tested by one way ANOVA followed by Bonferroni's post hoc test using graph pad prism version 8.1. The level of significance used are; * p>0.05 # Mean ±SD expressed as P value P more than 0.05 but P less than ****p< 0.0001.

III. DISCUSSION:

The present study describes the role of plumbago plant roots extract in neuropathic pain (NP), pathophysiology of NP, phytoconstituents and mechanism of action plumbago as well as treatment strategy for NP.

Mechanistic studies revealed that these activities of PL are related to its ability to modulate the NF-κb activation pathway which in turn induces S-G2/M cell cycle arrest through the induction of p21 (an inhibitor of cyclin-dependent kinase) changes redox status of cell and inhibits the enzyme NADPH oxidase. The anti-inflammatory and analgesic activities of roots of Plumbago zeylanica and in bioassay-guided isolation of anti-inflammatory and antinociceptive compound from this plant. However, the mechanism underlying the anti- inflammatory action of plumbagin remains unknown. Because NF-κb plays a pivotal role in inflammation and PL has the ability to modulate NF-κb in cancer cells, we therefore hypothesize that PL could suppress experimental inflammation through the inhibition of NF- κB activation. The anti-inflammatory activity of PL was examined in the rat paw edema NF- κb models induced by commonly used carrageenan and other phlogistic agents, and the role of NF-κb pathway and the proinflammatory mediators COX, iNOS, TNF- α and IL-6 were examined.

The transcription of proinflammatory mediators, such as inducible nitric-oxide synthase (iNOS), cyclooxygenase (COX) 2, tumor necrosis factor (TNF) , and IL-6. These mediators play important roles in the mediation, propagation, and extension of a local or systemic inflammatory process and can cause further activation of NF-κB, subsequently increasing further production of these proinflammatory mediators via positive feedback mechanisms .

Plumbagin can be use to inhibit production of these proinflammatory mediators and inhibition of these mediators is beneficial for the treatment of inflammatory diseases such as neuropathic pain and has become an important strategy for suppressing inflammation as is the case in nonsteroidal anti-inflammatory drugs.

IV. CONCLUSION:

Effect of Plumbago extract CCI Induced Neuropathic pain parameters reduce mechanical stimulation, thermal hyperalgesia and cold allodynia associated with neuropathic pain it shows analgesic effect.

Effect of Plumbago extract it shows activity against carrageenan inject rat paw oedema and decrease the no of licking of rat paw it shows anti-inflammatory activity.

Plumbago extract it shows activity against nociceptive pain in rats it show various types of pain models like hot plate, tail flick, acetic acid induced writhing it reduces pain and it shows analgesic effect.

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