WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 21, 808-828. Research Article ISSN 2277–7105

SCREENING OF LUPEOL ON PARTIAL SCIATIC NERVE LIGATION INDUCED NEUROPATHIC PAIN IN WISTAR RAT

***[1]Gaikwad Ashlesha S., [2]Kamble H. V., [3]Ghodake S. R. and [4]Bhintade Charushila**

[1]Student, Department of Pharmacology, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgn Pharata, Pune, Savitribai Phule Pune University.
[2]Principal, Department of Pharmacology, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgn Pharata, Pune, Savitribai Phule Pune University.
[3]Assistant Professor, Department of Pharmaceutics, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgn Pharata, Pune, Savitribai Phule Pune University.
[4]Student, Department of Pharmacology, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgn Pharata, Pune, Savitribai Phule Pune University.

Article Received on
07 September 2024,
Revised on 28 Sept. 2024,
Accepted on 18 October 2024
DOI: 10.20959/wjpr202421-34358

***Corresponding Author**
Gaikwad Ashlesha S.
Student, Department of Pharmacology, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgn Pharata, Pune, Savitribai Phule Pune University.

ABSTRACT

The current study focuses on evaluating the effects of Lupeol on neuropathic pain induced by partial sciatic nerve ligation (PSNL) in Wistar rats. Neuropathic pain, characterized by sensory dysfunction and chronic pain, significantly impairs quality of life. The research aims to induce neuropathic pain using PSNL in rats and assess pain using various behavioural and biochemical parameters, including the Von Frey filament test (mechanical allodynia), Inclined Plane test (muscle strength), Hot Plate test (thermal hyperalgesia), and Randall-Sellito test (pain threshold). Additionally, biochemical markers and histological analyses will provide insight into nerve injury and neuroinflammation. The study builds on Lupeol's known anti-inflammatory, anti-arthritic, and neuroprotective properties, exploring its potential role in modulating neuropathic pain through peripheral and central sensitization mechanisms. Lupeol is hypothesized to reduce pain by inhibiting neuroinflammation, peripheral sensitization, and promoting neuroplasticity, offering a promising therapeutic approach to neuropathic pain management.

KEYWORDS: Lupeol, Neuropathic pain, hyperalgesia, sciatic nerve, allodynia.

1. INTRODUCTION

The international association for the study of pain defines neuropathic pain as pain initiated or caused by primary lesion or dysfunction to the nervous system.[1] The neuropathic pain has been affecting the quality of life of people since early centuries and the research on neuropathic pain is carried out by the National Institute of Neurological Disorders and Stroke (NINDS). Neuropathic pain is mainly caused due to the malfunction in the nervous system which causes transmission of very unpleasant stimuli which causes a persisting pain. Early symptoms of neuropathic pain subject patient to normal pain but as the pain persist certain diagnostic test can be performed which can conform the neuropathic pain.[2]

Neuropathic pain is difficult to confirm as its symptoms can change as the time passes. Neuropathic pain is a symptom of an underlying disease, but as the severity of pain increase, it becomes more important to manage the symptoms with the underline disease. Neuropathic pain is caused due to damage to the peripheral nerve. When there is damage to the peripheral nerve the peripheral nerve fibre stimulates a pain response to the CNS and also can lead to changes in the activity of the dorsal horn neuron.[2]

The causes of neuropathic pain include diabetes, HIV infection shingles multiple sclerosis, cancer, spinal cord injury, stroke, and also condition like lumbar or cervical radiculopathies and traumatic or postsurgical nerve injuries. The patient suffering from neuropathic pain can experience a variety of pain stimuli like some patients may experience a loss of sensation or numbness which is mainly caused by the small unmyelinated C fibre. Some patients may experience over-sensitivity to light touch or pressure (Allodynia) some other sensations include burning pain and shooting and lancing pain which is mainly caused due to damage to the myelinated beta two fibre. And this is what brings challenges to the treatment of neuropathic pain.

Chronic pain due to neuropathic has a reported prevalence ranging from 0.9 % to 17.9% the prevalence of neuropathic pain in patients with cancer varies from 19.% to 39% The incidence of neuropathic pain associated with post-therapeutic neuralgia ranges from 3.9 to 42 per 1000,000 people year, the incidence of sciatica over 1 year is reported to be 9.3% the 1-month prevalence of sciatica range from 0.4% to 16.4%, Neuropathic pain as a feature of chronic pain ranged from 1.1 to 17.9%, in children's Fabry disease and erythromelalgia can be the cause for the neuropathic pain, also CRPS (complex regional pain syndrome) all over the world almost 6.5 % of 8.8 % of the females are suffering from neuropathic pain,

economically disadvantaged males had the highest burden of neuropathic pain. Neuropathic pain was associated with low-income unemployment, neuropathic pain was associated with hypertension, diabetes, bowel disorder and mood disorder.[3]

Neuropathic pain not only affects but deteriorates the quality of life of the person. The management of neuropathic pain is quite complex and response to the medication is inadequate, and the side effect of the present medication is common which includes sedation, dry mouth, blurred vision, weight gain, urinary retention, dizziness and peripheral edema. Thus, it is important to find a new drug which is more effective with low toxicity profile.

Lupeol is a triterpene which is a member of the phytosterol family. Triterpenes are mainly found in fruits, cereals example-mango, strawberry, Tamarindus indica, Allanblackia monoticola, Zanthoxylum riedelianum. Triterpenes are largely derived from vegetable oils, cereals, and fruits. Daily intake of triterpenes is estimated to be 250 mg per day, but in the Mediterranean countries, the diet mainly includes olive oil which is a rich source of triterpenes, and the intake there may reach 400 mg/kg/day. The chemical formula of lupeol is $C_{30}H_{50}O$. Lupeol has a proven anti-inflammatory action more than a potentials anti-inflammatory drug indomethacin. Lupeol has also shown to reduce inflammation in a preclinical study of arthritis and bronchial asthma. Lupeol also has proven effect of modulating several inflammatory molecules 15-lipoxygenase (15-SLO), Tumour necrosis factor α (TNFα), Interleukin β (ILβ), prostaglandin E2 (PGE2), cytokines (IL-2, IL-4, IL-5, IL-6, IL-13, IFN-γ-Th1) myeloperoxidase, macrophages, and T-lymphocytes. So, this study is proposed in the hypothesis that lupeol would serve as a test compound to treat neuropathic pain.

The animal model used in this study is partial sciatic nerve ligation (PSNL) induced neuropathic pain which is a proven effective model in the induction of neuropathic pain. PSNL leads to damage of myelinated fibre and deterioration of unmyelinated axon which produces never injury which in turn activates the immune response and leads to activation of microglia and astrocytes. The glia play a vital role in the release of cytokines and substance P. The increased levels of substance P lead to induction of neuropathic pain induction. The pain stimulated after PSNL depends upon the amount of nerve trapped in the ligation.

Based on the above perspectives, the present study is designed to screen lupeol in PSNL induced neuropathy in Wistar rats. In order to evaluate its molecular mechanism of action, docking studies and determination of molecular markers in sciatic nerves were planned.

2. LITERATURE REVIEW

Neuropathic pain significantly affects the quality of life of patients and their families. It can lead to other serious conditions such as depression, mood disorders, and sleep disturbances. This type of chronic pain alters a person's view of their overall health, negatively influencing relationships and social interactions. The World Health Organization (WHO) supports the Global Burden of Disease study, which highlights two key metrics: disability-adjusted life years (DALYs) and years lived with disability (YLD). These metrics assess the impact of long-term health problems, with lower back pain and neck pain being among the top contributors to YLD globally.[12] For instance, lower back pain accounted for 83 million DALYs and 10.7% of YLD.

Pathophysiology of Neuropathic Pain

Central Sensitization Central sensitization occurs when nociceptive neurons become hyperactive. It is largely dependent on the activation of N-methyl-D-aspartate (NMDA) glutamatergic receptors in the dorsal horn of the spinal cord. This process results in neural hyperactivity and increased sensitivity of A-beta and A-delta fibers, making normally non-painful stimuli painful, such as burning or pricking sensations.

Central Disinhibition In this mechanism, the downregulation of inhibitory neurotransmitters like GABA and glycine occurs in the spinal cord's dorsal horn. This downregulation often follows nerve damage, leading to mechanical and thermal hyperalgesia.

Nerve Fiber Damage A nerve injury activates macrophages that release inflammatory mediators such as cytokines and TNF-alpha, and microglia in the CNS, which also release immune modulators. This process lowers the activation threshold and increases the excitability of peripheral nerve endings, contributing to various types of pain, including spontaneous and evoked pain.

Ectopic Nerve Activity Spontaneous pain can arise from ectopic impulses generated in the nociceptive pathways, often seen in conditions like stump pain or diabetic neuropathy. In

neuropathic pain, increased expression of voltage-gated sodium channels, particularly after nerve injury, contributes to this ectopic activity, leading to heightened sensitivity.

Microglia and Neuropathic Pain Microglia, the immune cells of the CNS, play a key role in neuropathic pain by responding to nerve injury and releasing immune modulators such as IL-1β and TNF-α. These cells contribute to the development and maintenance of neuropathic pain by altering neural activity in the dorsal horn.

Diagnosis of Neuropathic Pain

1. **History**: A detailed patient history is crucial in identifying symptoms of neuropathic pain, such as shooting, burning, or pricking sensations, as well as numbness or tingling. This step helps approach a diagnosis with a reasonable degree of certainty.
2. **Clinical Examination**: Sensory changes are assessed using tools like cotton wool, brushes, or toothpicks. Differences in pain sensation during this examination help identify the likelihood of neuropathic pain.
3. **Confirmatory Tests**: Imaging tests such as MRI can help confirm the presence of stroke, multiple sclerosis, or spinal cord injuries. Skin biopsies may also be used to identify lesions or disorders of the somatosensory system.[4]

Present Treatments for Neuropathic Pain

1. **Antidepressants**

Antidepressants have been used since the 1960s to treat neuropathic pain. Tricyclic antidepressants (TCAs), such as nortriptyline and imipramine, are often effective, but their anticholinergic side effects require careful monitoring, including ECGs. Other antidepressants, such as duloxetine and venlafaxine, have shown efficacy in treating polyneuropathy. While TCAs primarily affect serotonin and norepinephrine reuptake, their analgesic effects are independent of their antidepressant properties.

2. **Calcium Channel Alpha-2-Delta Ligands**

Gabapentin and pregabalin bind to calcium channels, reducing neurotransmitter release. These drugs are commonly used for peripheral and central neuropathic pain, with pregabalin showing efficacy in central neuropathic conditions.

3. Antiepileptic Drugs

Antiepileptic drugs, such as gabapentin, lamotrigine, and phenytoin, have been used to treat neuropathic pain since 1968. Gabapentin acts on GABA and calcium subunits, while lamotrigine inhibits sodium channels and reduces glutamate release. Phenytoin has shown effectiveness in Fabry disease. Common side effects include somnolence, dizziness, and drowsiness.[4]

Source of Lupeol

Soybean, tomato, henna, olive, carrot, cucumber, African pepper, black tea, bitter root, aloe, Olive fruit, Mango fruit, Aloe leaves, Elm plant, Japanese pear (Shinko) etc. are some main sources of Lupeol and are used by people all over the world.

Botanical Name

Aloe vera, Hemidesmus indicus, Apocynum cannabinum, Juniperus communis Cajanus cajan, Lawsonia stib, Calendula officinalis, Lycopersicon esculentum, Camellia sinensis, Morus alba, Capsicum annuum, Olea Europe, Cassia fistula.

Structure

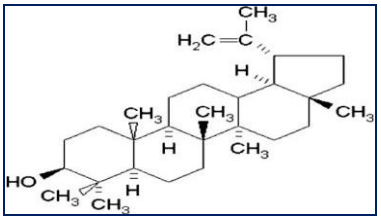

Figure 1: Structure of Lupeol.

(https://www.researchgate.net/figure/Chemical-structure-of-lupeol_fig1_233993789)

The chemical formula of Lupeol is C30H50O and the melting point is 215-216^{0}C. The molecular weight of lupeol is 426.7174g/mol. In an IR study on Lupeol revealed the presence of an olefinic moiety at 1640 cm -1 and a presence of a hydroxyl moiety at 3235 cm-1. In the study of H NMR, it was found that Lupeol contain 7 methyl singlet and it is a pentacyclic triterpenoid.

Activity of Lupeol

Lupeol exhibits various properties like anti-inflammatory, anti-cancer, anti-cancer, anti-arthritis, anti-diabetic, against heart diseases, against renal toxicity and hepatic toxicity.[5]

Figure 2: Different activities of Lupeol.

Toxicity of Lupeol

Toxicity study conducted on Lupeol suggested that Lupeol is a nontoxic drug and does not cause any systemic toxicity at a dose range of 30 to 2000 mg\kg in animals.

Bioavailability of Lupeol

Lupeol is a compound with high bioavailability. But synthetic derivative was observed to have increased pharmacological efficacy then Lupeol.

3. OBJECTIVES

To screen the effect of lupeol on mediators of neuropathic pain using in vivo models.

To induce neuropathic pain in Wistar rats using partial sciatic nerve ligation.

To screen the induction of neuropathic pain in Wistar rats using pain parameters.

4. RESEARCH METHOD / METHODOLOGY

Materials

Chemicals

- Xylazine(75mg/kg)
- Antibiotics- Povidone Iodine ointment.

All chemicals are analytical grade. Xylazine were dissolved in cold normal physiological saline and test and standard drugs were dissolved in distilled water. Antibiotic were given under supervision of veterinarian.

Surgical:-7-0 nylon suture, Scissors, cotton.

Test compounds:- Gabapentin (Standard drug), Lupeol (Test compound).

Animals

56 Male Wistar rats (weighing 150-180 g) were obtained from the National Institute of Biosciences, Pune (India). The animals were housed in polypropylene cages & maintained at 24±1°C, with relative humidity 45-55% and 12:12 h dark/light cycle. The animals had free access to standard pellet (Nav Maharashtra Chakan Oil Mills Ltd., Pune) and water ad libitum.

Instruments:- Von frey filament, Inclined Plane, Hot Plate, Randall Sellito, Weighing Balance.

Software utilized

Several online servers, viz. admet SAR, Molinspiration and Medchem designer were accessed to predict the molecular properties, toxicity and bioactivity of the Lupeol.

Bioactivity score Prediction

Lupeol bioactivity was tested by measuring GPCR ligand activity percentage, ion channel modulator, ligand nuclear receptor, kinase inhibitor, protease inhibitor, enzyme inhibitor.All these parameters were contrasted with the regular medication gabapentin by utilizing an electronic web database, molinspiration medication likeliness (www.molinspiration.com), and measured drug likeliness scores of lupeol.

Bioactivity score prediction Steps

a) Go to :- http://www.molinspiration.com
b) In search box → add smiley/ structure of TMCA → and search.
c) The result part of Bioactivity score consist of score which is generated based on the interaction of Lupeol with different receptors. Highest value indicates good interaction of TMCA with that receptor.

Molecular properties prediction

Lipinski 's rule of five (RO5) has been used to assess drug similarity and/or to determine whether a chemical compound with a certain pharmacological or biological activity has properties that would make it an orally active drug such as human moiety. Using Medchem Model (www. simulations-plus.com), important molecular properties such as molecular weight, hydrogen bond donor, hydrogen bond acceptor, logP of lupeol.

In vivo studies

Rats were anesthetized with and Xylazine 75mg/kg and the left sciatic nerve was exposed at the high thigh level. Briefly, one-third to one-half of the left sciatic nerve was tightly ligated using 7-0 nylon suture. Sham surgery in age matched animals was performed exposing the left sciatic nerve but no ligation was made. The muscle layers were closed with 4-0 nylon suture and the skin was sutured.

Table 1: Grouping of animals.

S.no	Groups	No. of animals	Treatment
1.	Vehicle control group	08	Vehicle
2.	Sham control group	08	Vehicle
3.	PSNL group	08	Vehicle
4.	Standard group	08	Gabapentin (60mg/kg)
5.	Treatment group 1	08	Lupeol 20mg/kg
6.	Treatment group 2	08	Lupeol 100mg/kg
7.	Treatment group 3	08	Lupeol 200 mg/kg

Experimental Design

After completion of quarantine period of 1 week, animals were randomly divided into 7 groups of 8 animals each as follows.

Group 1: Vehicle Control group: Animals did not undergo PSNL and were administered only with vehicle for 28 days.

Group 2: Sham Control group: the sciatic nerve of sham animals was exposed but not ligated and the animals were administered with vehicle for 28 days.

Group 3: PSNL control- PSNL was performed on this group and were administered with vehicle for 28 days.

Group 4: Standard Treated group: 60 mg/kg gabapentin was administered to the PSNL animals from day 8 to day 28 once daily p.o.

Group 5: Lupeol (20 mg/kg) Treated: lupeol 20 mg/kg was administered to the PSNL animals from day 8 to day 28 once daily p.o.

Group 6: Lupeol (100 mg/kg) Treated: lupeol 100 mg/kg was administered to the PSNL animals from day 8 to day 28 once daily p.o

Group 7: Lupeol (200 mg/kg) Treated: lupeol 200 mg/kg mg/kg was administered to the PSNL animals from day 8 to day 28 once daily p.o.

Procedure

All groups except vehicle group and sham group were induced with neuropathic pain by PSNL. On day 0, Rats were anesthetized with and xylazine 75mg/kg and the left sciatic nerve was exposed at high thigh level. Briefly one-third to one-half of left sciatic nerve was tightly ligated using 7-0 nylon suture. The parameters were taken before surgery on day 0 and after surgery on 4, 8, 12, 16, 20,24 and 28th day. The respective treatment was started from day 8th to 28th day and the effect of standard and test compound were analysed using different behaviour parameters.

Parameters Recorded

Von frey filament

Von Frey filament gives the idea about mechanical threshold in the paw of the animal. The animals were placed on the perforated bed of Von Frey apparatus. The other set of Von Frey monofilament was applied to the rat left hind paw with the force of 1.04 to 63.2 g, in a perpendicular manner .Response to the touch, such as licking the paw, transient vocalization, jumping, shaking the paw, biting at the probe or the stimulated paw response, was considered positive.[6]

Inclined Plane Test

The inclined plane test was performed to assess muscular strength using a sliding apparatus. Each rat was placed on a plastic plate, inclined at 30°, and the angle of the plate will be increased at a rate of 2° per sec. The maximum inclination will be confirmed when a rat could maintain itself on the plane for at least 5 sec.

Hot Plate Test

Hot plates test is to detect the thermal allodynia after induction of neuropathic pain, the temperature of the hot plate was maintained at 52 ^{0} C. the rats were placed on the hot plate and heat tolerance of the animals were observed, any sign of licking or jumping was considered as response . The time interval between each experiment maintained was 5 min. A 15-sec cut off time was used to minimize tissue damage. Animals presenting baseline latencies higher than 20-sec will be excluded.[7]

Randall Sellito

The mechanical paw withdrawal threshold of the animal was checked by using Randall Sellito. The paw of animals was placed on to the region, the weight applied, and passed

through scale. The reading at which the animal pulls his leg towards the body was noted. The scale reading starts from 1, while (1 = 32 g). Therefore, readings were analysed in the form of g into the results section.[8]

Standard drug (Gabapentin)

Gabapentin, (1-(amino methyl) cyclohexane acetic acid), is an anticonvulsant structurally related to the neurotransmitter gamma-aminobutyric acid (GABA). Like GABA, gabapentin is lipophilic and thus able to quickly move across the blood-brain barrier (Bennett and Simpson, 2004) and is accepted as first-line therapy for peripheral neuropathic pain, such as diabetic neuropathy and postherpetic neuralgia[1] Gabapentin's mechanism of action is primarily attributed to its binding to the α2-ÿ1 subunit on presynaptic voltage-gated calcium channels throughout the peripheral and central nervous systems, modulating neurotransmitter release.[9]

5. RESULT / FINDINGS

ADMET Prediction Profile

A good drug candidate is absorbed in required time and well distributed throughout the system for its effective metabolism and action. Toxicity is another very important factor which often overshadows the ADME behaviour. Failure of drugs at clinical trial stage due to adverse effects generated because of their toxicity proves very expensive and detrimental in the drug development process. In silico drug-likeness prediction along with further ADME/Tox tools presents an array of opportunities which help in accelerating the discovery of new targets and ultimately lead to compounds with predicted biological activity.

Table 2: ADMET Prediction Profile of Lupeol.

Absorption		
Blood-Brain Barrier	BBB+	0.9592
Human Intestinal Absorption	HIA+	0.9974
P-glycoprotein Substrate	Substrate	0.6969
Renal Organic Cation Transporter	Non-inhibitor	0.7710
Distribution		
Subcellular localization	lysosome	0.5245
Metabolism		
CYP450 2C9 substrate	Non- substrate	0.8184
CYP450 2D6 substrate	Non-substrate	0.9047
CYP450 1A2 inhibitor	Non- inhibitor	0.8619
CYP450 2C9 inhibitor	Non- inhibitor	0.8200
CYP450 2D6 Inhibitor	Non- inhibitor	0.9506
CYP450 2C19 Inhibitor	Non- inhibitor	0.7320

Toxicity		
AMES Toxicity	Non-AMES toxic	0.9420
Biodegradation	Not Ready biodegradable	0.9793
Acute Oral Toxicity	III	0.8578
Carcinogenicity (Three-class)	Non-required	0.5755
Aqueous solubility	-4.4139	LogS
Rat Acute Toxicity	3.3838	LD50, mol/kg

Effect of Partial Sciatic nerve ligation on the mechanical threshold of Wistar rats

Von Frey Filament apparatus was used to assay the effect of PSNL & lupeol on the mechanical threshold of animals. On day 0 i.e. before surgery the vehicle, sham, and the PSNL group animals showed no difference in the mechanical threshold and were tolerant to the sensation. On day 4 the vehicle group showed some tolerance, and the sham group animals showed a little sensitivity to the stimuli .The treatment group 3 i.e. lupeol 200mg group animals showed a highly significant decrease in the mechanical threshold on day 4 i.e. (57.22%) ($p<0.001$). On day 8,12,16,20,24,28 the lupeol 200mg group animals showed a significant lowering in the mechanical threshold by 61.96 %, on day 8, 75.64% on day 12, 79.30 % on day 16,80.54 % on day 20, 79.05 % on day 24 and 80.38 % on day 28 i.e. ($p<0.001$). The increase in the sensitivity might be due to due to the neuropathic pain induced due to the PSNL which leads to a highly significant decrease in the mechanical threshold.

Table 3: Effect of Partial Sciatic nerve ligation on the Mechanical Threshold of Wistar rats.

Table 3.1

Days	Mechanical threshold (g)						
	Vc	**Sham**	**Psnl**	**Standard (gabapentin)**	**Lupeol 20 gm**	**Lupeol 100 gm**	**Lupeol 200 gm**
0	4.96 ±0.22	4.48 ±0.21	4.19 ±0.24	5.01 ±0.23	5.2 ±0.42	5.31±0.23	5.44 ±0.28
4	4.89 ±0.23	4.27 ±0.32	0.78 ±0.36	4.3 ±0.23	4.41 ±0.12	4.45±0.42	4.53 ±0.41
8	4.53 ±0.24	4.25 ±0.42	0.68 ±0.12	4.35 ±0.45	4.4 ±0.23	4.56±0.38	4.64 ±0.35
12	4.45 ±0.41	4.58 ±0.35	0.69 ±0.24	4.6 ±0.35	4.71 ±0.25	4.79±0.34	4.82 ±0.25
16	4.25 ±0.32	4.41 ±0.35	0.8 ±0.29	4.5 ±0.41	4.66 ±0.39	4.78±0.43	4.86 ±0.47
20	4.64 ±0.41	4.8 ±0.48	0.6 ±0.36	5.2 ±0.35	5.23 ±0.37	5.33±0.35	5.39 ±0.38
24	4.47 ±0.12	4.53 ±0.39	0.61 ±0.45	4.59 ±0.34	4.66 ±0.41	4.81±0.39	4.87 ±0.39
28	4.58 ±0.35	4.55 ±0.37	0.49 ±0.38	4.68 ±0.39	4.72 ±0.39	4.88±0.45	4.94 ±0.47

VC- Vehicle Control, SC – Sham Control, PSNL-Partial Sciatic Nerve Ligated group.

Data expressed as the mean ±S.E.M. n= 8 rats

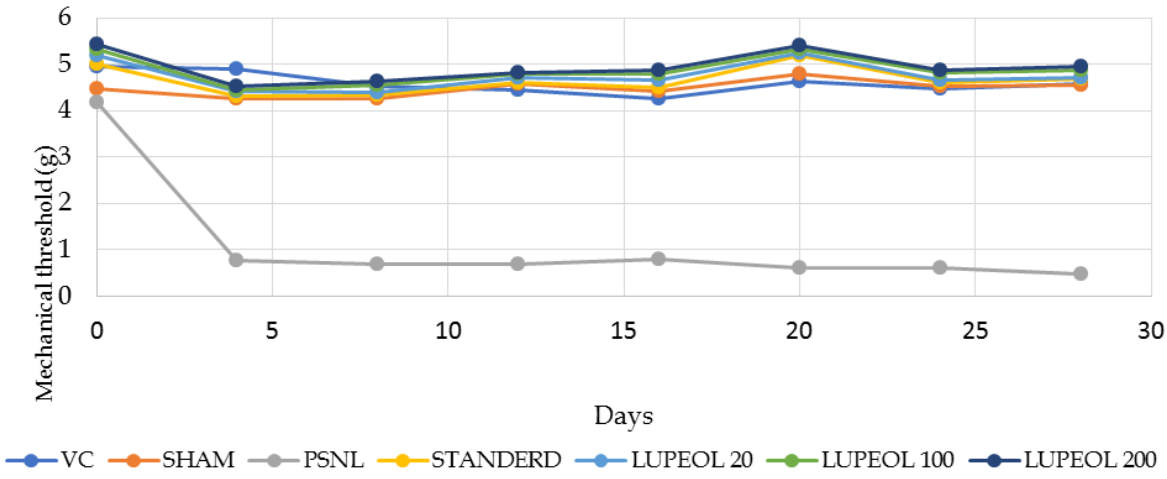

Figure 3: Effect of Partial Sciatic nerve ligation on the mechanical threshold of Wistar rats.

- **Effect of Partial Sciatic nerve ligation on paw withdrawal threshold of Wistar rats**

Randal sellito apparatus was used to assay the effect of partial sciatic nerve ligation on the paw withdrawal threshold of the animals. On day 0 the vehicle, sham, and the PSNL group showed no significant difference and were tolerant to pressure up to 200 g i.e. 6.81%. on day 4 after the surgery moderate significant decrease in the paw withdrawal threshold was seen i.e. 81.42 % ($p<0.01$) in PSNL group when compared with Sham group . On day 12 also moderate significant decrease in the paw withdrawal threshold was seen i.e. 83.80 %, whereas on day 16 (86.66%), 20 (88.11%),24 (87.5%), 28 (91.11%) highly significant decrease in the paw withdrawal threshold was observed i.e. ($p<0.001$).

Table 4: Effect of Partial Sciatic nerve ligation on paw withdrawal threshold of Wistar rats.

Table 4.1

Paw withdrawal threshold (g)							
Days	**Vc**	**Sham**	**Psnl**	**Standard (gabapentin)**	**Lupeol 20 mg**	**Lupeol 100 mg**	**Lupeol 200 mg**
0	190 ±5.56	190.25±5.56	190.87±6.23	191.87 ±5.23	193.87±5.65	196.87±7.23	199.87±8.32
4	189.3 ±6.54	192.25±6.45	82.375±6.23	84.375±10.23	87.375±9.65	89.375±8.24	91.375±6.25
8	191 ±9.56	191.25± 8.23	72.45 ±9.25	75.45 ±6.35	78.45 ±7.23	81.45 ±5.21	83.45 ±8.54
12	185.3 ±6.23	185.8 ±9.24	45.25 ±7.23	46.25 ±8.21	49.25 ±8.25	52.25 ±6.36	55.25±10.25
16	191.7 ±8.56	189.62±5.32	39.25 ±8.54	41.25 ±11.23	42.25 ±9.54	45.25± 8.54	49.25 ±9.65
20	190.3 ±7.45	191.5 ±8.65	37.25±12.54	39.25 ±13.25	41.25 ±7.23	42.25± 9.35	47.25 ±7.25
24	190.6 ±9.56	188.62±10.56	39.5 ±15.21	42.5 ± 9.56	45.5 ±8.54	46.5± 11.25	50.5 ±6.28
28	191.2±10.28	189.25±12.56	37.12 ±8.14	40.12 ±5.87	41.12 ±6.32	43.12±10.56	47.12±11.12

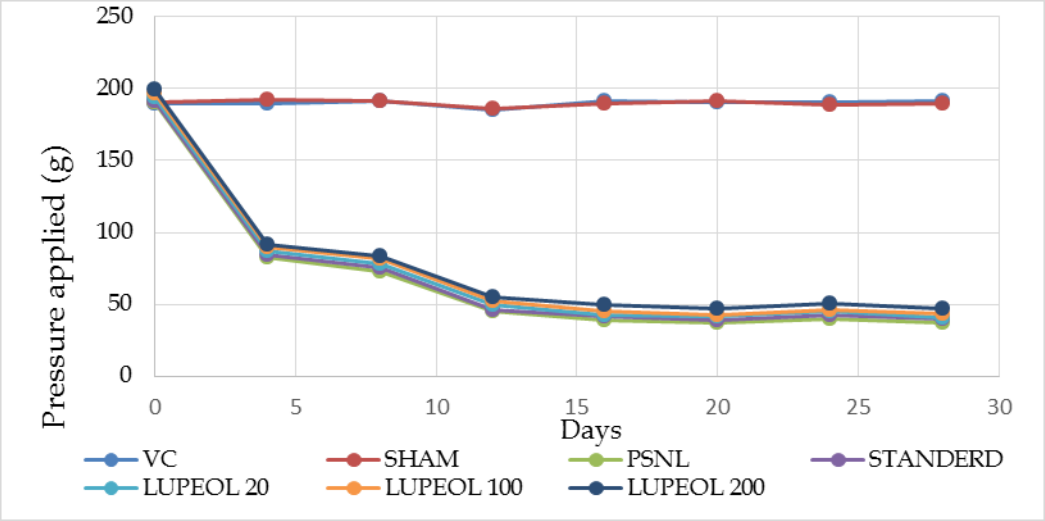

Figure 4-Effect of Partial Sciatic nerve ligation on paw withdrawal threshold of Wistar rats.

- **Effect of Partial Sciatic nerve ligation on heat sensitivity of Wistar rats**

Hot plate apparatus was used to assay the effect of PSNL on the heat sensitivity of the animals. the hot plate was adjusted to 52 °C and the cut off time 15 sec was set to avoid burning to the animal .on day 0 no significant difference was found between the vehicle, Sham and the PSNL (8.4%) group animals, on day 4 after surgery highly significant difference was seen between the sham control and the PSNL control group ($p<0.001$) i.e. 54.16 % decrease in the response time, after surgery it was observed that the heat sensitivity of the PSNL group was found to be increased. on day 8,12,16,20,24,28 a highly significant difference was seen in sham and the PSNL group i.e. ($p<0.001$) the response time on day 8 was decreased by 57.89 %, on day 12 by 58.2 %, on day16 by 68.3%, on day 20 by 64.67 %, on day 24 by 70.37% and on day 28 by 69.25.

Table 5- Effect of Partial Sciatic nerve ligation on heat sensitivity of Wistar rats

Table 5.1

Days	Response time (s)						
	Vc	Sham	Psnl	Standard (gabapentin)	Lupeol 20 mg	Lupeol 100 mg	Lupeol 200 mg
0	10.62 ±0.56	10.73 ±0.23	9.74±0.77	9.68 ±0.23	9.64 ±0.56	9.54 ±0.25	9.44±0.23
4	10.61 ±0.55	9.68 ±0.51	4.47±0.78	4.45 ±0.25	4.57 ±0.54	4.47 ±0.53	4.37±0.52
8	±9.35 ±0.48	9.5 ±0.71	4.01±0.25	3.89 ±0.45	3.21 ±0.87	3.01 ±0.54	2.8 ±0.57
12	10.46 ±0.62	9.25 ±0.23	3.8 ±0.45	3.5 ±0.56	3.5 ±0.45	3.3 ±0.56	3.1 ±0.87
16	10.66 ±0.84	9.84 ±0.52	3.41±0.52	3.2 ±0.54	3.2 ±0.56	3.11 ±0.84	2.9 ±0.56
20	10.36 ±0.72	9.24 ±0.48	3.57±0.87	3.37 ±0.84	3.37 ±0.87	3.27 ±0.77	3.1 ±0.74
24	10.15 ±0.52	10.15 ±0.89	2.8 ±0.45	2.8 ±0.25	2.7 ±0.74	2.6 ±0.54	2.4 ±0.65
28	10.01 ±0.23	10.01 ±0.84	2.91±0.56	2.8 ±0.86	2.61 ±0.66	2.51 ±0.68	2.35±0.84

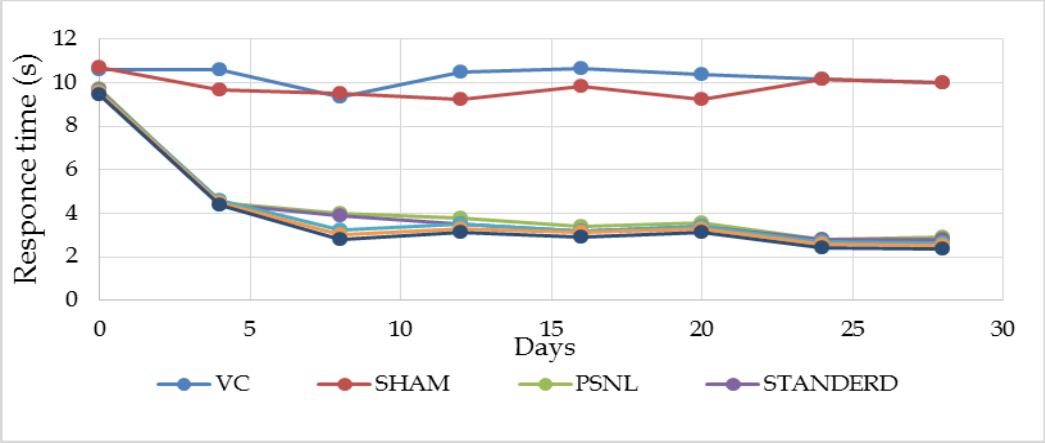

Figure 5-Effect of Partial Sciatic nerve ligation on heat sensitivity of Wistar rats.

- **Effect of Partial Sciatic nerve ligation on grip strength of Wistar rats**

Inclined plane apparatus was used to assay the grip strength of the animals. The inclined plane was set at 30^0 and each sec 2^0 rise in the angle was made. On day 0 i.e. before surgery no significant difference was seen between the Sham and the PSNL groups. On day 4 a highly significant decrease ($p<0.001$) in the grip strength was in observed in treatment group 3 i.e. Lupeol 200 mg group, the grip strength was decreased by 13.79 %, which confirmed the nerve pain. On day 8,12,16,20 further gradual decrease in the grip strength was observed i.e. on day 8 the grip strength was decreased by 14.50 % on day 12 by 22.94 %, on day 20 by 31.95%, whereas on day 24 (30.29 %) and 28 (32.45%) a no further decrease in the grip straight was observed comparing the past days, but had a highly significant decrease in grip strength when compared with standard control group.

Table- 6Effect of Partial Sciatic nerve ligation on grip strength of Wistar rats

Table 6.1.

Days	Grip strength(∘)						
	Vc	Sham	Psnl	Standard (gabapentin)	Lupeol 20 mg	Lupeol 100 mg	Lupeol 200 mg
0	80.61 ±3.33	72 ±3.21	77.75±3.28	78.75 ±3.56	79.75±2.56	80.75 ±2.62	82.75 ±2.56
4	80.62 ±2.30	73.37±2.56	63.25±2.25	64.25 ±2.54	65.25±3.85	66.25 ±3.21	68.25 ±3.84
8	79.37 ±2.56	70.37 ±2.54	60.12±2.54	63.12 ±2.34	65.12±2.78	67.125±2.13	69.12±2.25
12	86.25 ±2.48	72.62 ±2.89	55.75 ±2.36	58.75 ±3.25	60.75±3.68	62.75 ±2.58	64.75 ±3.21
16	81.25 ±3.25	67.37 ±2.65	52.1 ±2.58	53.1 ±3.65	55.1 ±2.41	57.1 ±3.54	59.1 ±2.54
20	80.62 ±2.84	63.37 ±3.54	43.12 ±2.84	45.12 ±2.84	46.12±3.89	48.12 ±2.69	49.12 ±2.87
24	81.25 ±2.45	65.87 ±3.45	45.37 ±3.56	46.37 ±2.39	48.37±2.87	49.37 ±2.54	51.37 ±2.54
28	82.25 ±2.66	65.5 ±2.33	40.75 ±2.55	42.75 ±3.78	43.75±3.26	46.75 ±3.55	49.75 ±3.54

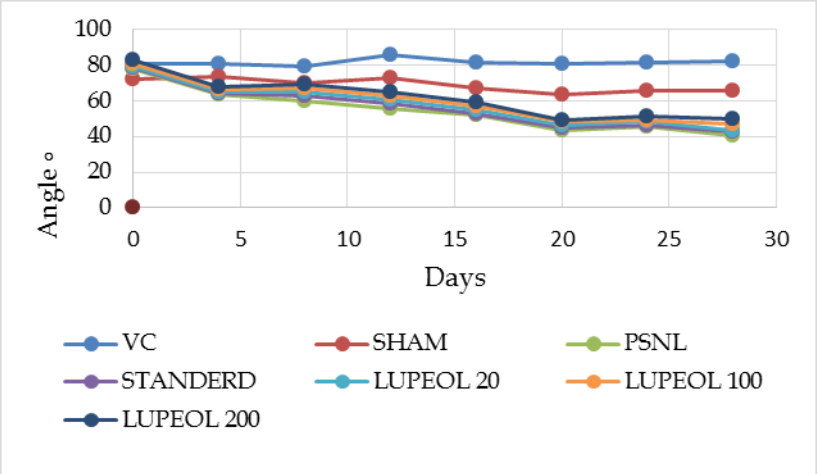

Figure 6: Effect of Partial Sciatic nerve ligation on grip strength of Wistar rats.

6. DISCUSSION

Neuropathic pain can be defined as the pain initiated or caused by a primary lesion or dysfunction in the nervous system. Neuropathic pain has been affecting lives of people and the prevalence of neuropathic pain range from 0.9% to 17.9%. The cancer patient suffering for neuropathic pain ranges from 19.0% to 39.1%. The patient suffering from trigeminal neuralgia are found to be 12.6 to 28.9 per 100000 per year. Also, the impact neuropathic pain puts on the patient's life are more devastating. Neuropathic pain imposes a burden on the patient suffering from pain and also on the family Neuropathic pain also lead to sever disorder like depression, sleep disturbance and mood disorder. Pain changes the perception of the patient about his general health and also affects relationship and interaction with people. WHO sponsored Global Burden of Disease study has given two terms i.e. disability adjusted life year and (YLD) years lived with disability. WHO defines disability as any short term or long-term health loss. Lower back Pain, Neck Pain contributes for 5 of top 10 condition which are responsible for most YLD globally. Lower Back Pain contributed to 83 million DALYS and 10.7% of YLD. The mechanism how neuropathic pain takes place can be divided into four broad classes;

1) Central Sensitization 2) Central disinhibition 3) Damage to the nerve fibre 4) Ectopic nerve activity.

There various other factors that add up for the pathophysiology of neuropathic pain which include microglia and astrocytes, P2X4RS, IRF8, P38, T-cell, BDNF. In the case of neuropathic pain, the treatment option is limited and the present treatment drug have an ample amount of side-effect and have shown effect in very few patients suffering from

neuropathic pain. 50% of recovery from the pain is considered to be a successful therapy. taking all this point under consideration and the changeless faced by the patient suffering from neuropathic pain, we have worked in the path of screening a test compound which would possibly decrease the pain in the neuropathic pain condition and would have a very low side-effect profile. The test compound used in the study is a triterpene named as Lupeol which have proven to be an effective as anti-arthritic agent, an anti-microbial agent, Anti-Protozoal agent, antimutagenic agent, anti-diabetic agent, cardioprotective agent, skin protective agent, and a hepatoprotective agent .Lupeol has also shown anti-inflammatory action more than that the NSAID drug indomethacin. Lupeol was selected as a test compound as it has the ability to inhibit proinflammatory cytokine which play a major role in pathogenesis of neuropathic pain. Lupeol has the ability to inhibit TNF alpha, IL1 beta, IRF8 and interferon gamma. Inhibiting this co-factor would eventually lead in stoppage of the inflammatory process which lead to nerve degeneration and nerve damage, inhibition of this mediators would also decrease the intensity of pain and improve the quality of life of people. Even if regular NSIAD do not work in neuropathic pain due to their pathway of action, Lupeol does not act by the same pathway, it acts by a different pathway which is not still clear and shows its action. Other drug which like TNF alpha inhibitors, IL1 beta inhibitors have shown to have promising effect in neuropathic pain, but study was conducted on such drug that would act on number of pathogenic factors at once. To check the likeliness of lupeol to be a lead compound, Insilco testing of lupeol was carried out, structure of lupeol was subjected to ADMET SAR and Molinspiration software.

Bioactivity Parameters

The best conformation of the ligand in binding pocked indicated that the postulated binding site located between loops extended from β-sheet and α-helix. This pose did not show any hydrogen bond interactions with amino acids constituted the active site; however close lipophilic interactions have been found as depicted in the figure. Which signifies that Lupeol Inhibit proinflammatory mediators like TNF-α and IL-1β by acting on caspase-1, the hypotheses mechanism through which Lupeol could be acting might be by two ways 1) by inhibiting the caspase -1 which serves as interleukin -1-β-converting enzyme which eventually lead to blockage of activation of IL-1 β and IL18.

Inhibition of IL-1 β lead to decrease in mechanical allodynia, short term memory disorder, and depression as stated by Gui et al.,2016. It also inhibit IL-18 which are expressed in

macrophages and also serve as a activating source for IFN-ɤ, IFN-ϒ and T-helper cell and T-cells are also involved in causing neuropathic mechanical hypersensitivity in adult rat. To justify this sentence I like to focus on a study which was conducted in neonate rats and adult rats, neuropathic pain was induced in the animals and observation where taken and they conclude that the neonate rats do not suffer from neuropathic pain but the adult rats do this takes place due to the reduced microglia signalling in the neonatal dorsal horn and this lead to a reduction in the P38 signalling, ERK cascade and CD68 expression, deficiency of hypersensitivity in the neonate rat due to inactivation and infiltration of T-cell in neonate rat. Once the ADMET and the drug likeliness was confirmed 54 male Wistar rat where subjected to partial sciatic nerve ligation to induce neuropathic pain. To assay if neuropathic pain was induced in the animals different behavioural parameter where taken, the parameter mainly include Vonfrey apparatus to measure the mechanical threshold, Hot Plate apparatus to check the heat sensitivity, Inclined plane to check the grip strength of animals, and the Randal Sillito apparatus to check the pressure sensitivity of the left leg of the animlas. in the behavioural test of mechanical threshold it was observed that on day 0 the animals of all three group i.e. the vehicle group, the sham control group and the PSNL group showed the same mechanical threshold but the reading takes on day 4 show a highly significant decrease in the mechanical threshold and the same consecutive effect was seen on day 8,12,16,20,24, and 28 which might be due to the central sensitization that takes place in neuropathic pain. due to the injury to the sciatic nerve the hyper excitability of nociceptive neuron takes place. This mainly occur due to the release of the excitatory amino acid which travel through the primary nociceptive afferent fibre and are carried to the spinal dorsal horn and lead to changes in the voltage gated channels which in turn lead to neuronal hyperexcitability and increase in the mechanical sensitivity of A delta and A beta fibre and activation of second order nociceptive neuron so due this reason the mechanical threshold decreases in neuropathic pain and the stimuli like pricking becomes pain full. The hot plate analysis was mainly done to check the heat sensitivity of the animals. On day 0 the three groups showed no significant difference in the readings but on day 4 i.e. after surgery of the PSNL Group, showed significant decrease in the time to which they were tolerant to heat and the same decrease in the tolerance heat was seen on the following days which indicate that the animals had generated heat sensitivity, it mainly take place due to increase various receptor protein like transient receptor potential (TRPV1). TRPV1 is located on the peripheral nociceptive ending in the subtype region and is activated by heat stimulation at 41 C^0. In the nerve injury the TRPV1 is downregulated but up regulated in the C-fibre which is not injured. So due to the expression of TRPV1 and

increased sensation of heat lead to nerve activity at normal body temperature i.e. 38 C^0. Patient suffering from such pain characterized by heat hyperalgesia and an on-going burning pain, similarly the sensitivity to cold stimuli is found to be due to the expression of TRPM8 receptor. Also, the Randal Sillito apparatus was used to test the pressure sensitivity of the rats .before surgery the PSNL group showed same sensitivity to the pressure applied as that of the sham and the vehicle group animals and where able to bearer pressure up to 200 gm. After the surgery of the PSNL group animals the pressure sensitivity of the animals had increased significantly this mainly take place due to nerve lesion is caused it leads to activation of macrophages, which travels into the nerve and the dorsal root ganglion and leads to release of inflammatory mediators like cytokines and TNF alpha . in addition, microglia get activated in the CNS which release various immune modulators which play role in initiation of neuropathic pain such changes lead to reduction in the activation threshold and increase membrane excitability in the peripheral nerve ending. The same mechanism works for the grip strength parameter, due to the pain and the sensitivity generated in the paw the animas is not able to make balance and grip the surface that he was able to grip before, So the animals in the PSNL group showed normal grip strength on day zero and the grip strength decreased significantly on day 4,8,12,16,20,24 and 28 after the surgery due to the generation of neuropathic pain. So, the study carried and the behaviour Parameter taken suggest that the animals have under gone neuropathic pain. The above observation of the behavioural study confirms that the animals have been induced with neuropathic pain and PSNL was found to be a good model for the induction of neuropathic pain, But the further preclinical study would be required for confirming the effect of Lupeol on Neuropathic pain.

8. CONCLUSION

The ADMET and the MOLINSPRIRATON study performed on Lupeol suggest that Lupeol possess good properties of being a Drug and has a low toxicity profile. The docking study carried out further suggest that Lupeol can acts on neuropathic pain through the mechanism of inhibition of apoptosis of neurons or by inhibiting the production of IL-1β both by inhibiting the enzyme Caspase-1. The above observation of the behavioural study confirms that the animals have been induced with neuropathic pain and PSNL was found to be a good model for the induction of neuropathic pain, But the further preclinical study would be required for confirming the effect of Lupeol on Neuropathic pain.

ACKNOWLEDGEMENT

My heartfelt gratitude and reverence to Principal & guide Dr. H.V.Kamble for his guidance, knowledge, insight and support for fulfilment of this project. I would also like to thank S.R.Ghodake, Sir. I do not have enough words to express my gratitude to my family, Shankar, Latika, Swajal for the colossal support and encouragement extended by them for completion of this project. I would also like to thank my friends Shubham, Samta for all the inputs and assistance. Most importantly I would like to express my gratitude to all the animals who sacrificed their lives for fulfilment of this project.

REFERENCES

1. Dworkin, R.H., O'Connor, A.B., Backonja, M., Farrar, J.T., Finnerup, N.B., Jensen, T.S., Kalso, E.A., Loeser, J.D., Miaskowski, C., Nurmikko, T.J., Portenoy, R.K., Rice, A.S.C., Stacey, B.R., Treede, R.D., Turk, D.C., and Wallace, M.S., 2007. Pharmacologic management of neuropathic pain: Evidence-based recommendations. Pain, 132(3): 237–251.
2. Jensen, T.S. and Baron, R., 2003. Translation of symptoms and signs into mechanisms in neuropathic pain. Pain, 102(1): 1-8.
3. Colloca, L., Ludman, T., Bouhassira, D., Baron, R., Dickenson, A.H., Yarnitsky, D., Freeman, R., Truini, A., Attal, N., Finnerup, N.B., Eccleston, C., Kalso, E., Bennett, D.L., Dworkin, R.H., and Raja, S.N., 2017. Neuropathic pain. Nature Reviews Disease Primers, 3: 1–20.
4. Finnerup, N.B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R.H., Gilron, I., Haanpää, M., Hansson, P., Jensen, T.S., Kamerman, P.R., Lund, K., Moore, A., Raja, S.N., Rice, A.S.C., Rowbotham, M., Sena, E., Siddall, P., Smith, B.H., and Wallace, M., 2015. Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. The Lancet Neurology, 14(2): 162–173.
5. Saleem M, Afaq F, Adhami VM, Mukhtar H. Lupeol modulates NF-kappaB and PI3K/ Akt pathways and inhibits skin cancer in CD-1 mice. Oncogene, 2004; 23.
6. Hodges, L.D., Kweifio-Okai, G. and Macrides, T.A., 2003. Antiprotease effect of anti-inflammatory lupeol esters. Molecular and cellular biochemistry, 252(1-2): 97-101.
7. Xiao, W., Boroujerdi, A., Bennett, G.J., and Luo, Z.D., 2007. Chemotherapy-evoked painful peripheral neuropathy: Analgesic effects of gabapentin and effects on expression of the alpha-2-delta type-1 calcium channel subunit. Neuroscience, 144(2): 714–720.

8. Hargreaves, K., Dubner, R., Brown, F., Flores, C., and Joris, J., 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain, 32(1): 77–88.
9. Boyle, Y., Fernando, D., Kurz, H., Miller, S.R., Zucchetto, M., and Storey, J., 2014. The effect of a combination of gabapentin and donepezil in an experimental pain model in healthy volunteers: Results of a randomized controlled trial. Pain, 155(12): 2510–2516.