International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1277-1285 www.ijprajournal.com ISSN: 2249-7781

Investigation of Phytochemical activity in Calotropis Procera Plant

Anjali[1*], Harish Sharma[1], Gyanesh Kumar Sahu[1]
[1*] *Rungta Institute of Pharmaceutical Sciences and Research, Kurud road Kohka, Bhilai (C.G.)*
[1] *MJ College of Pharmacy, Ayyapa Nagar, (C.G)*
[1] *Shri Shankracharya Technical Campus, Shri Shankaracharya Group of Institution, Faculty of Pharmaceutical Sciences, Junwani, Bhilai (C.G.)*

Date Of Submission: 01-03-2021 Date Of Acceptance: 14-03-2021

ABSTRACT: - The latex too constrains inflammatory reply provoked by several inflammatory intermediaries. Also, it has also been established to take antioxidant and anti-hyperglycaemic activity. Since ancient time, natural products have been played a cardinal person in sighting of present drugs, comprising centrally interim treatments. Numerous scientists have established aids of natural medicines in obstinate patients of anxiety, depression and epilepsy. Analgesic agents beleaguered at the spinal cord equal comprise local anaesthetics (produce non-specific conduction blockade) and opioids (act on opioid receptors). The acute toxicity study revealed safely of the extract up to a dose of 2000 mg/kg in mice. The ethanolic, petroleum ether and chloroform extract of leaves of C. procera at a dose of 100, 200 and 500 mg/kg was selected for analgesic activity.

Keywords: - Inflammatory, Anti-hyperglycaemic activity, Anaesthetics, Analgesic activity.

I. INTRODUCTION

Pain is a normal term that explains troublesome sensations in the anatomy. It branch from bracing of the nervous arrangement. Strain can area from aggravating to attenuate, and it can perceive like a acute stabbing or a dull ache. Strain can also be characterize as flutter, stinging, sore, and pinching[1]. The stem barks of Calotropis proceraharvests resin and wax. The leaves part of C. Procerahas reported to contains taraxasterly acetate, uzarigenin, calotropin, pinoresinol, medioresinol, calactin, calacitnic acid, calacitnic acid methyl ester, 19-carboxyl-calacitnic methyl ester, drummondol, 15b-hydroxycalotrin, the C_{11}bicylic lactone norisopenoid, the rare diphenyl furfuran lignan, salicifoliol and 19-nor- and 18,20-epoxy-cardenolides[2]. More ever, the root bark and plants of Calotropis procera are used for several societies of central India as a medicinalcause for jaundice. The chloroform extracts of the root has been exposed to exhibition defensive action alongside carbon tetrachloride persuaded liver damage[3]. Ethanolic extract of C. Procera has been exposed anti-inflammatory, analgesic, antipyretic, and neuromuscular spoiling action[4].

C. procera or sweet akand belongs to family: Asclepiadaceae is innate to India and cultivate well in minor hills at 900m altitude[1]. Thus in review of potential use of plant in folklore for the treatment of CNS diseases and isolation of centrally active substances it was that important to systematically evaluate the analgesic activity of Caltrops procera[5].

1.1 Pain: - Pain is a normal term that explains troublesome sensations in the anatomy. It branch from bracing of the nervous arrangement. Strain can area from aggravating to attenuate, and it can perceive like a acute stabbing or a dull ache. Strain can also be characterize as flutter, stinging, sore, and pinching.

1.2 Types of pain

1 Acute pain
2 Chronic pain
3 Somatic pain
4 Visceral pain
5 Cutaneous pain
6 Neuropathic pain
7 Phantom limb pain

1.2.1 Acute pain is definite as short-term but dangerous ching that derives on speedily but last solitary for a passingera of spell. Acute pain is the form's notice of current injury to tissue or illness. It is frequently fast and piercing shadowed by painful agony[6]. Severe ache is central in one part earlier charming rather feast out. These types of pain replies well to medicines.

1.2.2 Chronic pain was initially distinct as hurt that has taken 6 months or lengthier. It is now

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1277-1285 www.ijprajournal.com ISSN: 2249-7781

definite as hurt that perseveres leng their than the usual path of period related through a certain form of wound[7]. This continuous or sporadic ting has often outlasted its drive, as it fixes not assistance the form to stop wound. It is frequently additional problematic to luxury than severe discomfort[8]. The knowledge of physical agony can be gather edrendering to the basis and connect ednociceptors (pain noticingneurons)[9]. The practice of physical pain can be grouped according to the source and related nociceptors (pain detecting neurons).

1.2.3 Somatic pain createsaftersinews, muscles, frames, plasma vessels and even nerves themselves. It is noticed with somatic nociceptors[10]. The shortage of pain receptors in these partscropsa leaden, poorly-localized ache of lengthierperiod than cutaneous pain; examples comprisesprains and broken bones[11].

1.2.4 Visceral pain The solidity of intuitive nociceptors is <1% in contrast with somatic afferents and the cortical plotting of instinctual afferents is too less focused. So, visceral pain is illrestricted, wordy and frequently in the midline, with the exclusion of joins and the mesentery[12].

The qualitative natural pain is also dissimilarsince the innards are complex to swelling. It also seems that afferent gritsreply in a classi fiedstyle to strength of inspiration rather than to individual stimulating methods[13]. Visceral pain also display saltitudinal summary, so that if a great area is encouraged, the pain verge is dropped, this does not happen in cutaneous nociception. Visceral pain can also be mentioned to situate far absent from the spring of stimulus. It is regularly segmental and insincere and often shows hyperalgesia[14].

1.2.5 Cutaneous pain is begun by wound to the skin or apparent tissues. Cutaneous nociceptors axe just lower the crust and due to the greatawareness of couragefinishes, food a well-defined, limited pain of short period. Examples of wounds that yield cutaneous throbbingcomprise paper cuts, minor cuts, minor (first degree) burns and cuts[15].

1.2.6 Neuropathic pain or "neuralgia" canhappen as anoutcome of wrong or bug to the nerve tissue himself. This can disturb the skill of the sensual nerves to conveyprecisegen to the thalamus and henceforth the brain understands soreincentives smooth however here is no clear or recognized physiologic reason for the soreness[16].

1.2.7 Phantom limb pain is the feeling of pain from a member that has been misplaced or since which anindividualnoleng their obtains physical indications. It is aknowled generally generally stated by amputees and quadriplegics[17].

1.3 Concentric contraction

1.3.1 A concentric contraction or shortening contraction

It is a kind of muscle reduction in which the muscles cut though making power. This happens when the strength produced by the muscle surpasses the load contrasting its shrinkage[18].

Throughout a concentric narrowing, a muscle is moved to pactcon ferring to the down hill wire mechanism. This occurs during the size of the might, making a power at the musculo-tendinous intersection, producing the power to cut and altering the viewpoint of the combined[19]. In relative to the prod, a concentric reduction of the biceps would cause the arm to bend at the elbow as the hand moved from the leg to the shoulder (a biceps curl). A concentric shrinkage of the triceps would alteration the angle of the multiparty in the conflicting way, uncurling the arm and poignant the hands near the leg[20].

1.3.2 Eccentric contraction

Through outa precorrection (lengthening contraction), the muscleleng thens while below strain due to disparate force bigger than the muscle causes. Rather than occupied to pull a joint in the path of the muscle reduction, the muscle performances to brake the joint at the end of a program or thenregulator the repositioning of a load[21]. This can occur unwillingly (e.g., when attempting to move a heaviness too heavy for the muscle to boost) or willingly (e.g., when the muscle is 'smoothing out' a crusade). Ended the short-term, forexercise connecting both strange and concentric contractions seem to upsurge muscular forte more than exercise with concentric reductions alone. Though, exercise-induced muscle injury is also better during expansion reductions[22].

Throughout an odd reduction of the biceps muscle, the elbow twitches the program while bent and then uncurls as the hand changesabsent from the assume[23]. During an eccentric reduction of the triceps muscle, the elbow shocks the driveconventional and then curves as the hand changes to the shoulder[24]. Desmin titin, and other z-line proteins are complicated in eccentric reductions, but their device is poorly assumed in judgment to cross-bridge cycling in concentric shrinkages.

Though the influence is deed an undesirable amount of mechanical labour, (work is being done on the muscle), biological vigour (in fat, glucose or ATP) is still disbursed, though

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1277-1285 www.ijprajournal.com ISSN: 2249-7781

less than would be paid out during a concentric shrivelling of the same force. For example, one expends more energy going up a flight of stairs than going down the same flight[25].

Strength experience in hefty eccentric loading suffer greater damage when laden (such as during muscle building or strength training exercise) as compared to concentric loading. When eccentric contractions are used in weight training, they are normally called negatives[26]. During a concentric contraction, muscle fibres slide across each other, pulling the Z-lines together. During an eccentric contraction, the filaments slide past each other the opposite way, though the actual movement of the myosin heads during an eccentric contraction is not known[27]. Exercise featuring a heavy eccentric load can actually support a greater weight (muscles are approximately 40% stronger during eccentric contractions than during concentric contractions) and also results in greater muscular damage and elayed onset muscle soreness one to two days after training. Exercise that incorporates both eccentric and concentric muscular contractions (i.e., involving a strong contraction and a controlled lowering of the weight) can produce greater gains in strength than concentric contractions alone[28]. While unaccustomed heavy eccentric contractions can easily lead to overtraining, moderate training may confer protection against injury[29].

1.4 Exploratory Behaviour

It is a quality related to inquisitive thinking such as investigation, and learning, evident by observation in human and many animal species. The term can also be used to denote the behaviour itself being caused by the emotion of curiosity[30].

1.4.1 Causes

Although many living beings have an innate capability of curiosity, it should not be categorized as an instinct because it is not a fixed action pattern; rather it is an innate basic emotion because, while curiosity can be expressed in many ways, the expression of an instinct is typically more fixed and less flexible[31]. Curiosity is common to human beings at all ages from infancy through adulthood, and is easy to observe in many other animal species. These include apes, cats, androdents[32].

1.4.2 Brain

Although the phenomenon of curiosity is widely regarded, its neural correlates still remain relatively unknown[33]. However, recent studies have provided insight into the neurological mechanisms that may be associated with curiosity, such aslearning, memory, and motivation. Such research aims to transition the study of curiosity from a speculative realm to one of more scientific credibility[34].

1.4.3 Neurological aspects

Due to the complexity of the subject, focusing on specific neural processes within curiosity can help in better understanding the phenomenon of curiosity as a whole[35].

1.4.4 Attention

Attention is the cognitive process by which one can selectively focus and concentrate on particular stimuli in the surrounding environment. There may be many stimuli in the surrounding area, but as there are limited cognitive and sensory resources, attention allows the brain to better focus on what it perceives to be the most important or relevant of these stimuli[36].

Figure1: - Calotropis Procera Plant

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1277-1285 www.ijprajournal.com ISSN: 2249-7781

Scientists can measure the amount of attention an individual devotes to a stimulus by tracking eye movements. Organisms focus their eyes on stimuli that are particularly stimulating or engaging; the more attention a stimulus garners, the more frequent the eye will be directed towards that stimulus[37]. Normal individuals will look at new stimuli at least two to three times more often than familiar or repetitive stimuli. Exciting or novel stimuli demand more attention than stimuli perceived as boring[38].

II. METHODS

2.1 Acetic acid- induced writhing (Chemical method)

This was carried out in groups of mice (n=5) by nothing the writhing responses produced by intra peritoneal administration of 1% acetic acid (0.1 ml/ 10g) 15min after intra peritoneal injection of either control vehicle or ethanolic extract of C. procera (in different doses) were compare against the standard analgesic aspirin (200mg/kg). The number of writhes produced in these animals was counted for 30 min.

2.2 Tail flick method (Thermal method)

Analgesic activity was recorded by using analgesia meter. The rats were placed in rat holder, with its tail coming out through a slot in the lid. The tail was kept on bride of analgesia meter called jacket with an electrically heated nichrome wire, underneath. The tail received radiant heat from wire, heated by passing current of 6 mA. The time taken for withdrawal of tail after switching on the current was taken as latent period, in scene of tail flicking response and was consider as index nociception. The cut off time for determination of latent period was taken as 30s to avoid injury to skin. Three tail flick latencies were measured (Basal reaction time) per rat at each time interval and he means of tail-flick latencies were used for statistical analysis. After recording the basal reaction time in group of rats (n=5) at least 3 consecutive trial were selected for further experimentation and were administrated i.p. either control vehicle C. procera extract (in different doses) or pentazocin was used as reference standard and were tested 30 min later.

III. EVALUATION PARAMETERS

In this work, methodology for the validation of medicinal plants with action on the analgesic activity has been applied, especially methodology which is used to investigate chronic pain activity since the search for new compounds of natural origin with analgesic activity is important in western world.

3.1 Extraction of Plant Materials

The percentage yield of the successive extraction of Calotropis proceraleavesare presented in Table 3.1.

Table 3.1 Description of different extracts of Calotropisprocera leaves.

Extracts	Colour	Odour	Consistency	% Yield(w/w)
Petroleum Ether Extract	Faint Yellow	Characteristic	Dry	3.5
Chloroform Extract	Dark Yellow	Characteristic	Dry	3.8
Ethanol Extract	Faint Yellow	Characteristic	Sticky	9.27

3.2 Phyto chemical Screening:

Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds and tannins in the C. procera extract.

Table- 3.2: Estimation of Phyto chemical analysis of different extract of C. procera.

Sr. No	Chemical test		PEE	CE	EE
1	Test for Alkaloids	Hager's Test	-ve	-ve	-ve
		Mayer's Test	-ve	-ve	-ve
		Dragendroff's Test	-ve	-ve	+ve
		Wagner's Test	-ve	-ve	-ve

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1277-1285 www.ijprajournal.com ISSN: 2249-7781

Sr. No	Chemical test		PEE	CE	EE
2	Test for carbohydrates	Molisch's Test	+ve	+ve	-ve
		Fehling's Test	-ve	-ve	+ve
		Barford's Test	-ve	-ve	-ve
		Benedict's Test	-ve	-ve	+ve
3	Test for cardiac glycosides	Baljet test	-ve	-ve	+ve
		Legal test	-ve	-ve	+ve
4	Test for Anthraquinone glycosides	Modified Borntrager's test	-ve	-ve	-ve
		Borntrager's test	-ve	-ve	-ve
5	Test for saponins glycosides	Foam test	-ve	-ve	+ve
		Hemolytic test	-ve	-ve	-ve
6	Test for fixed oil	Stain Test	+ve	+ve	-ve
7	Test for Proteins and Amino acids	Millons's Test	-ve	-ve	-ve
		Biuret Test	-ve	-ve	-ve
		Ninhydrin Test	-ve	-ve	-ve
8	Test for Phytosterols and triterpenoids	Liebermann-Burchard Test	-ve	-ve	+ve
		Salkowski Test	-ve	-ve	-ve
9	Test for flavonoids	Shinoda test	-ve	-ve	+ve
10	Test for tannin	Lead acetate solution	-ve	-ve	-ve
		5% $Fecl_3$ solution	-ve	-ve	-ve

PEE= Petroleum Ether Extract, **CE =** Chloroform Extract, **EE =** Ethanol Extract

3.3 Acute Toxicity Study:

The acute toxicity study revealed safely of the extract up to a dose of 2000 mg/kg dose level in mice. So that $1/20^{th}$, $1/10^{th}$ and $1/4^{th}$ (i.e. 100mg/kg, 200 mg/kg and 500 mg/kg orally) was selected for analgesic activity. For analgesic activity of petroleum ether extract, chloroform extract and ethanolic extract of leaves of C. procera was prepared in distilled water for oral route of administration.

Table 3.3 Preliminary acute toxicity levels of crude extracts.

Group	Extracts Dose Levels (mg/kg)			N	N^0 dead PEE	N^0 dead CE	N^0 dead EE
	PEE	CE	EE				
Group 1	100	100	100	6	0	0	0
Group 2	200	200	200	6	0	0	0
Group 3	500	500	500	6	0	0	0
Group 4	1000	1000	1000	6	0	0	0
Group 5	2000	2000	2000	6	1	2	1

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1277-1285 www.ijprajournal.com ISSN: 2249-7781

Control	1 ml dH_2O	1 ml dH_2O	1 ml dH_2O	6	0	0	0

PEE = Petroleum Ether Extract, **CE** =Chloroform Extract, **EE** =Ethanolic Extract

3.4 Analgesic Activity:

One way ANOVA revealed a significant effect of all treatment groups in acetic acid induced writhing test as well as tail flick method. The post-hoc analysis by Tukey's test revealed significant effect of all dose of C. procera (P<0.001) on analgesic activity in mice.

3.4.1 Acetic acid-induced writhing response

The first study showed that the application of different doses of extracts had significant analgesic effects in the animals under investigation. The results of doses 200 and 500 mg/Kg were significant and comparable with the effect of aspirinin analgesic activity (Table 3.4.1).

Table 3.4.1- Effect of C. procera leaves extract on acetic acid induced writhing in mice

Group	Treatment	Dose	Mean No. of writhing
I	Control	0.1 ml/10g	76.40±7.86
II	Aspirin	200mg/kg	10.40±3.21***
III	Calotropis procera **(PEE)**	100 mg/kg	65.20±4.60*
IV	Calotropis procera (**PEE**)	200mg/kg	46.20±3.42***
V	Calotropis procera (**PEE**)	500mg/kg	30.60±2.88***
VI	Calotropis procera (**CE**)	100 mg/kg	54.35±2.65*
VII	Calotropis procera (**CE**)	200mg/kg	39.22±2.22***
VIII	Calotropis procera (**CE**)	500mg/kg	27.55±1.75***
IX	Calotropis procera (**EE**)	100mg/kg	61.33±3.83*
X	Calotropis procera (**EE**)	200mg/kg	45.15±2.45***
XI	Calotropis procera (**EE**)	500mg/kg	23.45±2.65***

PEE = Petroleum Ether Extract
CE =Chloroform Extract
EE =Ethanolic Extract
Data represents mean ± SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (n=5), values are compared with control animals, p<0.05. *P<0.01, **P<0.001, ***P<0.0001.

3.4.2 Tail Immersion Method

The result of tail immersion test in mice is presented in Table 6.6. The result shows that the extract at the dose of 500mg/kg and the reference drug Pentazocin significantly (P = 0.0001) increased the PRT when compared to the negative group (Group 1). At the doses of 100 and 200 mg/kg, the extract did not show any significant increase in PRT, although there was a marginal increase in the mean PRT from7.80±1.92to 13.40±1.14*** for petroleum ether extract of Calotropis procera.

Table 3.4.2: Effect of C. procera leaves extract on tail flick latent period in rats.

Group	Treatment	Dose (mg/kg)	Mean latent period in rats		
			Initial	After 30 min	After 60 min
I	Control	0.1 ml/10g	8.00±0.71	8.2±0.84	8.40±0.55
II	Pentazocin	10mg/kg	8.20±0.84	16.60±1.34***	18.60±1.14***
III	Calotropis procera**(PEE)**	100mg/kg	8.00±0.71	9.80±0.84	11.60±1.14***
IV	Calotropis procera**(PEE)**	200mg/kg	7.80±1.92	11.60±1.14**	13.40±1.14***
V	Calotropis procera**(PEE)**	500mg/kg	7.60±1.14	15.40±1.14***	16.40±1.14***

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1277-1285 www.ijprajournal.com ISSN: 2249-7781

Group	Treatment	Dose (mg/kg)	Mean latent period in rats		
			Initial	After 30 min	After 60 min
VI	Calotropis procera**(CE)**	100mg/kg	7.95±0.72	8.90±0.80	10.55±1.24***
VII	Calotropis procera**(CE)**	200mg/kg	7.55±1.05	10.45±1.25**	15.25±1.25***
VIII	Calotropis procera**(CE)**	500mg/kg	7.40±1.11	14.74±1.03***	15.23±1.79***
IX	Calotropis procera**(EE)**	100mg/kg	8.25±0.92	10.05±0.55	12.00±1.25***
X	Calotropis procera**(EE)**	200mg/kg	7.25±1.20	10.25±1.20**	12.04±1.36***
XI	Calotropis procera**(EE)**	500mg/kg	8.74±1.12	16.35±1.20***	15.35±1.25***

Data represents mean±SD; one-way of analysis of variance, ANOVA followed by Tukey's multiple Comparison Test (n=5), values are compared with control animals, p<0.05. *P<0.01, **P<0.001, ***P<0.0001.
PEE = Petroleum Ether Extract
CE = Chloroform Extract
EE = Ethanolic Extract

IV. DISCUSSIONS

Preliminary phytochemical study of the petroleum ether, ethanolic and ethanolic extract of Calotropis procera,showed the presence of saponins, terpens, phenols, alkaloids, tannins and flavonoids.

The acute toxicity study revealed safely of the extract up to a dose of 2000 mg/kg in mice. The ethanolic, petroleum ether and chloroform extract of leaves of C. procera at a dose of 100, 200 and 500 mg/kg was selected for analgesic activity.

Different extracts (Petroleum ether, chloroform and ethanolic) of leaves of C. procera were investigated for analgesic activity by using different models. The analgesic properties were studied onacetic acid induced writhing and tail flick latent period in rats. The result shows that the ethanolic extract of the leaves of C. procera was found to be effective.

REFERENCES

[1]. Bhutada P, Mundhada Y, Bansod K, Dixit P, Umathe S, Mundhada D. Anticonvulsant activity of berberine, an isoquinoline alkaloid in mice. Epilepsy Behav. 2010; 3: 207-210.
[2]. Bum EN, Taiwe GS, Nkainsa LA, Moto FC, SekeEtet PF, Hiana IR, Bailabar T, RouyatouSeyni P, Rakotonirina A, Rakotonirina SV. Validation of anticonvulsant and sedative activity of six medicinal plants. Epilepsy Behav. 2009; 14:454–458.
[3]. Carlini EA, Plants and the central nervous system. Pharmacol. Biochem. Behav. 2003; 3:501–512.
[4]. Chen Y, Wang HD, Xia X, Kung HF, Pan Y, Kong LD. Behavioral and biochemical studies of total furocoumarins from seeds of Psoraleacorylifolia in the chronic mild stress model of depression in mice. Phytomedicine 2007; 14: 523–529.
[5]. Anonymous, The Wealth of India, New Delhi, India: National Institute of Scientific andIndustrial Research 3; 1998.
[6]. Kirtikar K, Basu BD. Indian Medicinal Plants. 7th ed. Dehradun: International book distributors; 2001.
[7]. Pathak K and Argal A. CNS activity of Calotropis gigantea roots. J. Ethnopharmacol. 2006; 106:142–145.
[8]. Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrhoeal activity of Calotropis gigantean r.br. in experimental animals. J. Pharm. Pharmaceut. Sci. 2004; 7:70–75.
[9]. Pathak AK, Argal A. Analgesic activity of Calotropis gigantea flower. Fitoterapia. 2007; 78:40-42.
[10]. Srivastava SR, Keshri G, Bhargavan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of Calotropis gigantea Linn. in rats. Contraception. 2007; 75: 318–322.
[11]. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, Publication and Information Directorate, Council of Scientific and Industrial Research Publication (CSIR), New Delhi 1992.

[12]. Lhinhatrakool T, Sutthivaiyakit S. 19-nor- and 18, 20-epoxycardenolides from the leaves of Calotropis gigantea. J. Nat. Prod. 2006; 69:1249–1251.
[13]. Kumar VL, Arya S. Medicinal uses and pharmacological properties of Calotropis procera. In: Govil, J.N. (Ed.), Recent Progress in Medicinal Plants, 2006; 11: 373-388.
[14]. Samvatsar S, Diwanji VB. Plant sources for the treatment of jaundice in the tribals of Western Madhya Pradesh of India. Journal of Ethnopharmacology, 2000; 73: 313–316.
[15]. Basu A, Sen T, Ray RN, Nag Chaudhuri AK. Hepatoprotective effects of Calotropis procera root extract on experimental liver damage in animals. Fitoterapia, 1992; 63: 507–514.
[16]. Kumar VL, Basu N. Antiinflammatory activity of the latex of Calotropis procera. Journal of Ethnopharmacology, 1994; 44:123-125.
[17]. Dewan S, Sangraula H, Kumar VL. Preliminary studies on the analgesic activity of latex of Calotropis procera. Journal of Ethnopharmacology, 2000a; 73: 307–311.
[18]. Dewan S, Kumar S, Kumar VL. Antipyretic effect of latex of Calotropis procera. Indian Journal of Pharmacology, 2000b; 32: 252.
[19]. Arya S, Kumar VL. Antiinflammatory efficacy of extracts of latex of Calotropis procera against different mediators of inflammation. Mediators of Inflammation, 2005; 228–232.
[20]. Roy S, Sehgal R, Padhy BM, Kumar VL. Antioxidant and protective effect of latex of Calotropis procera against alloxan-induced diabetes in rats. Journal of Ethnopharmacology, 2005; 102: 470–473.
[21]. Choedon T, Mathan G, Arya S, Kumar VL, Kumar V. Anticancer and cytotoxic properties of the latex of Calotropis procera in a transgenic mouse model of hepatocellular carcinoma. World Journal of Gastroenterology, 2006; 2 12: 2517–2522.
[22]. Mossa JS, Tariq M, Mohsin A, Aqeel A, Al-Yahya MA, Al-Said MS, Rafatullah S. Pharmacological studies on aerial part of Calotropis procera. Am J Chin Med, 1991; 19: 223–231.
[23]. Fields HL, Levine JD: Pain-Mechanisms and management [Medical Progress]. West J Med 1984; 141:347-57.
[24]. Benett P N, Brown M J. Clinical pharmacology. 9th ed. London: Churchill Livingstone; 2003.
[25]. Serpell M**.** Anatomy, physiology and pharmacology of pain. Surgery (Oxford) 2006 October; 24(10): 350-53.
[26]. Irina AS, Gary HD, Michel B, Bushnell MC. Differentiation of Visceral and Cutaneous Pain in the Human Brain. J Neurophysiol 2003;89: 3294–303.
[27]. Bjorn AM, Bengt L. Mode of Action of Spinal Cord Stimulation in Neuropathic Pain. J Pain and Symptom Manage 2006 April; 31: s6- s12.
[28]. http://www.wellcome.ac.uk/en/pain/microsite/medicine2.html.
[29]. Wolfgang K. Opoid induced hyperalgesia-Pathophysiology and clinical relevance. Acute pain 2007; 9: 21-34.
[30]. Faulkner JA. "Terminology for contractions of muscles during shortening, while isometric, and during lengthening". Journal of applied physiology 2003; 95 (2): 455–459.
[31]. Colliander EB, Tesch PA. "Effects of eccentric and concentric muscle actions in resistance training". Acta Physiol. Scand 1990; 140 (1): 31–9.
[32]. Nikolaidis MG, Kyparos A, Spanou C, Paschalis V, Theodorou AA, Vrabas IS. "Redox biology of exercise: an integrative and comparative consideration of some overlooked issues". J. Exp. Biol. 2012; 215 (Pt 10): 1615–25.
[33]. Brooks, G.A; Fahey, T.D. & White, T.P. Exercise Physiology: Human Bioenergetics and Its Applications. (2nd ed.). Mayfield Publishing Co; 1996.
[34]. Alfredson H, Pietilä T, Jonsson P, Lorentzon R. "Heavy-load eccentric calf muscle training for the treatment of chronic Achilles tendinosis". Am J Sports Med 1998; 26 (3): 360–6.
[35]. Satyendra L, Byl N. "Effectiveness of physical therapy for Achilles tendinopathy: An evidence based review of eccentric exercises". Isokinetics and Exercise Science 2006; 14 (1): 71-80.
[36]. Tassinary; Cacioppo. "The Skeletomotor system: surface electromyography". In Cacioppo, John T.; Tassinary, Luois G.; Berntson, Gary G.Handbook of Psychophysiology (Second ed.). Cambridge: Cambridge University Press; 2000.

[37]. Shwedyk E, Balasubramanian R, Scott RN. A nonstationary model for the Electromyogram. IEEE Transactions on Biomedical Engineering 1977; 24 (5): 417–424.
[38]. Saladin Kenneth. Anatomy and Physiology: The Unity of Form and Function. New York: McGraw Hill, 2012.