

## INVESTIGATION OF PHYTOCHEMICAL ACTIVITY BY CALOTROPIS PROCERA

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### 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.

## **1. 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<sup>1</sup>. 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<sub>11</sub>bicyclic lactone norisopenoid, the rare diphenyl furfuran lignan, salicifoliol and 19-nor- and 18,20-epoxy-cardenolides<sup>2</sup>. 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<sup>3</sup>. Ethanolic extract of *C. Procera* has been exposed anti-inflammatory, analgesic, antipyretic, and neuromuscular spoiling action<sup>4</sup>.

*C. procera* or sweet akand belongs to family: *Asclepiadaceae* is innate to India and cultivate well in minor hills at 900m altitude<sup>1</sup>. 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*<sup>5</sup>.

**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 thing 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<sup>6</sup>. 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 definite as hurt that perseveres leng their than the usual path of period related through a certain form of wound<sup>7</sup>. 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<sup>8</sup>. The knowledge of physical agony can be gather edrendering to the basis and connect ednociceptors (pain noticingneurons)<sup>9</sup>. 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<sup>10</sup>. The shortage of pain receptors in these partscropsa leaden, poorly-localized ache of lengthierperiod than cutaneous pain; examples comprisesprains and broken bones<sup>11</sup>.

**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 joints and the mesentery<sup>12</sup>.

The qualitative natural pain is also dissimilar since the innards are complex to swelling. It also seems that afferent gritsreply in a classifedstyle to strength of inspiration rather than to individual stimulating methods<sup>13</sup>. 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<sup>14</sup>.

**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 throbbing comprise paper cuts, minor cuts, minor (first degree) burns and cuts<sup>15</sup>.

**1.2.6 Neuropathic pain** or "neuralgia" can happen as an outcome of wrong or bug to the nerve tissue himself. This can disturb the skill of the sensual nerves to convey precise gen to the thalamus and henceforth the brain understands sore incentives smooth however here is no clear or recognized physiologic reason for the soreness<sup>16</sup>.

**1.2.7 Phantom limb pain** is the feeling of pain from a member that has been misplaced or since which an individual no longer obtains physical indications. It is acknowledged generally generally stated by amputees and quadriplegics<sup>17</sup>.

### **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<sup>18</sup>.

Throughout a concentric narrowing, a muscle is moved to pact con 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<sup>19</sup>. 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<sup>20</sup>.

#### **1.3.2 Eccentric contraction**

Through outa precorrection (lengthening contraction), the muscle lengthens 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 then regulator the repositioning of a load<sup>21</sup>. 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, fore exercise connecting both strange and concentric contractions seem to

upsurge muscular force more than exercise with concentric reductions alone. Though, exercise-induced muscle injury is also better during expansion reductions<sup>22</sup>.

Throughout an odd reduction of the biceps muscle, the elbow twitches the program while bent and then uncurls as the hand changes absent from the assume<sup>23</sup>. During an eccentric reduction of the triceps muscle, the elbow shocks the drive conventional and then curves as the hand changes to the shoulder<sup>24</sup>. 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 deemed an undesirable amount of mechanical labour, (work is being done on the muscle), biological vigour (in fat, glucose or ATP) is still disbursed, though 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<sup>25</sup>.

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<sup>26</sup>. 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<sup>27</sup>. 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 delayed 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<sup>28</sup>. While unaccustomed heavy eccentric contractions can easily lead to overtraining, moderate training may confer protection against injury<sup>29</sup>.

## **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<sup>30</sup>.

### **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<sup>31</sup>. 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, and rodents<sup>32</sup>.

### **1.4.2 Brain**

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

### **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<sup>35</sup>.

### **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<sup>36</sup>. 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<sup>37</sup>. 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<sup>38</sup>.

## **2. Methods**

### **2.1 Acetic acid- induced writhing (Chemical method)**

This was carried out in groups of mice (n=5) by noting 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.

## **3 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 procera leaves are presented in Table 3.1.

**Table 3.1 Description of different extracts of Calotropis procera 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 |
| 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 |

| Sr. No | Chemical test                           |                               | PEE | CE  | EE  |
|--------|---|-------------------------------|-----|-----|-----|
| 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 <sub>3</sub> 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<sup>th</sup>, 1/10<sup>th</sup> and 1/4<sup>th</sup> (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<br>Dose Levels (mg/kg) |                           |                           | N | N <sup>o</sup> dead<br>PEE | N <sup>o</sup> dead<br>CE | N <sup>o</sup> dead<br>EE |
|----------------|---------------------------------|---------------------------|---------------------------|---|----------------------------|---------------------------|---------------------------|
|                | PEE                             | CE                        | EE                        |   |                            |                           |                           |
| <b>Group 1</b> | 100                             | 100                       | 100                       | 6 | 0                          | 0                         | 0                         |
| <b>Group 2</b> | 200                             | 200                       | 200                       | 6 | 0                          | 0                         | 0                         |
| <b>Group 3</b> | 500                             | 500                       | 500                       | 6 | 0                          | 0                         | 0                         |
| <b>Group 4</b> | 1000                            | 1000                      | 1000                      | 6 | 0                          | 0                         | 0                         |
| <b>Group 5</b> | 2000                            | 2000                      | 2000                      | 6 | 1                          | 2                         | 1                         |
| <b>Control</b> | 1 ml dH <sub>2</sub> O          | 1 ml<br>dH <sub>2</sub> O | 1 ml<br>dH <sub>2</sub> O | 6 | 0                          | 0                         | 0                         |

PEE = Petroleum Ether Extract, CE =Chloroform Extract, EE =Ethanollic 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 from  $7.80 \pm 1.92$  to  $13.40 \pm 1.14^{***}$  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   | $8.00 \pm 0.71$            | $8.2 \pm 0.84$         | $8.40 \pm 0.55$        |
| II    | Pentazocin                      | 10mg/kg      | $8.20 \pm 0.84$            | $16.60 \pm 1.34^{***}$ | $18.60 \pm 1.14^{***}$ |
| III   | <i>Calotropis procera</i> (PEE) | 100mg/kg     | $8.00 \pm 0.71$            | $9.80 \pm 0.84$        | $11.60 \pm 1.14^{***}$ |
| IV    | <i>Calotropis procera</i> (PEE) | 200mg/kg     | $7.80 \pm 1.92$            | $11.60 \pm 1.14^{**}$  | $13.40 \pm 1.14^{***}$ |
| V     | <i>Calotropis procera</i> (PEE) | 500mg/kg     | $7.60 \pm 1.14$            | $15.40 \pm 1.14^{***}$ | $16.40 \pm 1.14^{***}$ |
| VI    | <i>Calotropis procera</i> (CE)  | 100mg/kg     | $7.95 \pm 0.72$            | $8.90 \pm 0.80$        | $10.55 \pm 1.24^{***}$ |
| VI I  | <i>Calotropis procera</i> (CE)  | 200mg/kg     | $7.55 \pm 1.05$            | $10.45 \pm 1.25^{**}$  | $15.25 \pm 1.25^{***}$ |
| VI II | <i>Calotropis procera</i> (CE)  | 500mg/kg     | $7.40 \pm 1.11$            | $14.74 \pm 1.03^{***}$ | $15.23 \pm 1.79^{***}$ |
| IX    | <i>Calotropis procera</i> (EE)  | 100mg/kg     | $8.25 \pm 0.92$            | $10.05 \pm 0.55$       | $12.00 \pm 1.25^{***}$ |
| X     | <i>Calotropis procera</i> (EE)  | 200mg/kg     | $7.25 \pm 1.20$            | $10.25 \pm 1.20^{**}$  | $12.04 \pm 1.36^{***}$ |
| XI    | <i>Calotropis procera</i> (EE)  | 500mg/kg     | $8.74 \pm 1.12$            | $16.35 \pm 1.20^{***}$ | $15.35 \pm 1.25^{***}$ |

Data represents mean $\pm$ 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

#### **4 Discussions**

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

The acute toxicity study revealed safety 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 on acetic 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.

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