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# Antinociceptive activity of *Karpasa Beeja*

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## ABSTRACT

**Background:** *Karpasa Beeja* (cotton seeds) is a drug which is been used since *Samhita* period, it has *Vatahara* and *Vedanasthapana* properties. *Acharya Charaka* includes *Karpasa* in *Brimhaniya Gana* while *Acharya Susruta* includes it in *Vatasamsamana Varga.* Cotton seeds are a rich source of cattle feed. It is also widely used in preparation of Ayurvedic medicines. The present study is highly relevant in searching experimentally for its Anti-nociceptive activity in cotton seeds and its pharmacognostical, phytochemical, HPLC+TLC profile. **Aim and objective:** An experimental evaluation of *Karpasa beeja* for its Antinociception activity. **Results:** Acetic acid induced writhing test, mice was treated with paracetamol (72.5mg/Kg) and test *Karpasa Beeja* drug *Kashaya* (12.48ml/kg,) and distilled water as control group in a dose of 1ml. The efficacy of test substances for analgesic activity was evaluated in the swiss albino mice. The analgesic activity of *Karpasa Beeja Kashaya* was studied in swiss albino mice by administering the test substance orally. The writhing effect was induced by 1% V/V glacial acetic acid solution in mice pre-treated with test substance. The number of abdominal writhing were measure over 20 min after injection of acetic acid. Finally, percentage inhibition was calculated. **Conclusion:** In this test substance it is found that the percentage inhibition of pain is 38.89 % hence it can be concluded that the test substance is known to possess analgesic activity.

**Key words:** *Karpasa beeja, Gossypium hirsutum, Analgesic, Anti-nociceptive, HPLC+TLC*

## INTRODUCTION

*Vedana* is often considered as a synonym of *Vyadhi* or disease. This may be because of the fact that almost all the diseases are associated with one or another type of pain. The concept of *Vedana* in *Ayurveda*, has a theoretical and philosophical background apart from its clinical importance. Pain is commonly referred to as *Vedana*. But *Vedana* is a much broader concept than merely pain. *Vedana* (*Samvedanam- Amarakosam)* literally means perception. This may be a pleasant one (*Sukham)* or an unpleasant one (*Dukha). Sukha* and *Dukha* are *Gunas* attributed to *Atma.* This perception may come from *Sarira, Manas* or *Atma*. The unpleasant feeling, whatever may be its seat is called as *Roga* or *Vikara*. The objective of *Chikitsa* or treatment is to overcome this *Ruja* the *Roga*. No other symptom may have got as much importance as the symptom pain as it is the most distressing and disturbing of all symptoms. *Vedana*, when considered in a broader sense, each *Dosha* produces its own type of *Vedana*. For e.g., *Daha* attributed to *Pitta*; *Toda*, *Ruja* are attributed to *Vata* and *Kandu*, *Supti* are attributed to *Kapha*. But in a clinical point of view, patient refers to pain as *Vedana*. This *Vedana* is especially attributed to *Vata*. Even this *Vedana* is of different permutations and combinations. *Vedana* is a purely subjective experience and only the person who experiences it can explain it. Our *Acharyas* have tried their best to put the different types of pain into objective terms by the use of certain similies like *Ankusavat*, *Vriscikadagdamiva*, *Soola* etc. but the relevance of such similies in the modern era are now questionable because in olden days people were familiar with the pain caused by the bite of *Vriscika* or that caused by an *Ankusa* but now people are less accustomed with such injuries. The subjectivity of pain not only lies in its type but also in its severity. *Caraka* in his *Vimanastana* explains about

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*Guru Rogi* and *Laghu Rogi.* The *Gurutva* or *Laghutva* of *Vyadhi* depends on the *Satva* and *Bala* of the *Rogi*. *Vedana* is primarily considered as an attribute of *Vata* though *Pitta* and *Kapha* also manifests their own types of *Vedana.*

Pain is a submodality of somatic sensation. The word "pain" is used to describe a wide range of unpleasant sensory and emotional experiences associated with actual or potential tissue damage. Nature has made sure that pain is a signal, we cannot ignore. In pain the information is transmitted to the CNS via three major pathways. Most ailments of the body cause pain. The ability to diagnose different diseases depends to a great extent on the knowledge of the different qualities and causes of pain. Sensitivity and reactivity to noxious stimuli are essential to the wellbeing and survival of an organism. Pain travels through redundant pathways, ensuring to inform the subject: "Get out of this situation immediately." Without these attributes, the organism has no means to prevent or minimize tissue injury. Individuals congenitally insensitive to pain are easily injured and most of them die at an early age. For thousands of years, physicians have tried to treat pain without knowing the details of the ways in which pain is signalled from the injured part of the body to the brain, or the ways in which any of their remedies worked. Recent discoveries about how the body detects, transmits, and reacts to painful stimuli, have allowed physicians to relieve both acute and chronic pain.

Antinociception is the action or process of blocking the detection of painful or injurious stimulus sensory neurons. Pain is termed nociceptive (nocer - to injure or to hurt in Latin), and nociceptive means sensitive to noxious stimuli. Noxious stimuli are stimuli that elicit tissue damage and activate nociceptors. Nociceptors are sensory receptors that detect signals from damaged tissue or the threat of damage and indirectly also respond to chemicals released from the damaged tissue. Nociceptors are free (bare) nerve endings found in the skin, muscle, joints, bone and viscera. Recently, it was found that nerve endings contain transient receptor potential (TRP) channels that sense and detect damage. The TRP channels are similar to voltage gated potassium channels or nucleotide gated channels, having 6 transmembrane domains with a pore between domains 5 and 6. They transduce a variety of noxious stimuli into receptor potentials, which in turn initiate action potential in the pain nerve fibers. This action potential is transmitted to the spinal cord and makes a synaptic connection in lamina I and II. The cell bodies of nociceptors are mainly in the dorsal root and trigeminal ganglia. No nociceptors are found inside the CNS. Nociceptors are not uniformly sensitive. They fall into several categories, depending on their responses to mechanical, thermal, and chemical stimulation liberated by the damage, tumor, and inflammation. So anti-nociception can be called as peripheral analgesics activity. Many of the peripheral analgesics possess anti-inflammatory property and in some cases antipyretic activity. For many of them the mode of action has been elucidated as an inhibition of cyclooxygenase in the prostaglandin pathway. It can be tested by using Writhing test.

The word *Karpasa* in *Sanskrit* denotes the cotton plant or the cotton tree. The use of *Karpasa* as medicine was not seen in the *Vedic* period. Only a very few descriptions about it was seen in the *Samhita* period. The identity of the plant mentioned in *Samhita* is also controversial because synonyms are used and also while checking the context of mentioning it creates a doubt whether the mentioning is about cotton plant. Elaborate descriptions of *Karpasa* are seen from the *Nighantu* period onwards. *Karpasa Beeja* (cotton seeds) is *Vatahara* and has got *Vedana Sthapana* property according to *Ayurvedic* classics, which gave the base for choosing the study drug for these activities. *Acharya Caraka* includes *Karpasa* in *Brimhaṇiya Gana*[2] while *Acharya Susruta* includes it *Vata Samsamana Varga.*[3] *Karpasa Beeja* has *Snigdha Guṇa, Madhura Rasa*, *Madhura Vipaka*, *Kincit Ushna Virya* and *Vataharakarma*.

## Materials and Methods

Cotton seeds were authenticated and purchased from Calicut. The *Kaṣhaya* of Cotton seeds were prepared at Invivo Biosciences Bengaluru 154 Kodigehalli Village, Magadi road Bengaluru-560091 as per the classical

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reference of *Sarangadhara Samhita*, *Madhyamakhaṇda*, *Kashaya Kalpana*. During experimentation, on everyday basis, Cotton seeds (*Gossypium hirsutum* Linn.) were weighed separately over accurate digital weighing machine in the quantity of 20 grams each. Then the seeds were pounded using a metal mortar and pestle and made into moderately crushed seeds. Distilled water was taken 16 times that of weighed drug which is 320ml Then, the pounded drugs were transferred separately into two stainless steel cooking bowls, 320ml of water was added to each of the bowls and kept for boiling over low flame reducing to 1/8th to obtain the quantity of 40 ml of *Kashaya* each.

Total 18 healthy Swiss albino mice weighing between 120-250gms will be taken and divided randomly into three groups, each containing six rats, maintained under a constant 12hr light and dark cycle at 22-24 and at 45%-55% relative humidity. Animals were feed with Pelleted rodent from VRK nutrition solutions. Deep bore well water passed through charcoal filters and exposed to UV rays and water in polypropylene water bottles were provided to the animals. The Animal Ethical committee has approved for experiment on animals. The experimental protocol was approved on 25/02/2022 by the Institutional Animal Ethical Committee (IAEC).

**Table 1: Group Allocation**

| Group No | Treatment group | Dose | No. of animals | Animal numbers | |
|---|---|---|---|---|---|
| 1 | Control | - | 6 | 1 | 6 |
| 2 | Positive control Paracetamol suspension) | 100mg/kg | 6 | 7 | 12 |
| 3 | *Kashaya* of cotton seeds -Therapeutic Dose | 12.48 ml/kg | 6 | 13 | 18 |

To the group 1, distilled water administered to serve as control. Group 2 was taken as the standard and administered with aqueous suspension standard drug Paracetamol, (100mg/kg body weight).

Group 3 were administered with test drug cotton seed *Kashaya,* for all test groups *Kaṣhaya* was administered once daily for 7 consecutive days. On the 7$^{th}$ day 1 hour after the administration of drug acetic acid in a dose of 1 ml/100g body weight (1%v/v solution) was injected intra peritoneal to each mouse. Each mouse was placed in separate per specs boxes under observation immediately after the acetic acid injection and the number of abdominal constrictions (writhes) were counted over a period of 15 mins for each animal. (Analgesic effect was recorded by counting the number of writhing syndrome after the injection of acetic acid for a period of 15 minutes). For scoring purposes, a writhe was indicated by stretching of the abdomen by simultaneous stretching of at least one hind limb. The latency of onset and number of writhing was noted. The mean number of writhes and latency of onset of writhing by the trial groups, standard group & control group were calculated and compared statistically.

Percentage of inhibition was evaluated using the formula: average writhes in the test group minus writhes in the control group divided by writhes in the control group times 100. The time period with the greatest percent of inhibition was considered as peak time.

The experimental data were expressed as mean ± SEM. The data obtained was analyzed by using one way analysis of variance (ANOVA) followed by POST HOC TUKEY KRAMER TEST for determining the level of significance of the observed effects. A “P value” of less than 0.05 was considered statistically significant. Graph Pad In Stat-3 and SPSS graph pad was used for statistical analysis of the generated data.

## Observation and Results

**Table 2: No. of writhing in Acetic acid induced writhing test**

| Group | Animal No. | Writhing response | % Inhibition |
|---|---|---|---|
| **Control** | 1 | 32 | |
| | 2 | 35 | |
| | 3 | 30 | |

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| | | | |
|---|---|---|---|
| | 4 | 28 | |
| | 5 | 38 | |
| | 6 | 35 | |
| **Mean** | | **33.00** | |
| **SEM** | | **1.51** | |
| **Standard** | 7 | 15 | **56.06** |
| | 12 | 18 | |
| **Mean** | | **14.50** | |
| **SEM** | | **1.31** | |
| **Test** | 13 | 20 | **38.89** |
| | 14 | 23 | |
| | 15 | 18 | |
| | 16 | 20 | |
| | 17 | 17 | |
| | 18 | 23 | |
| **Mean** | | **20.17** | |
| **SEM** | | **1.01** | |

**Graph 1: No. of writhing in acetic acid induced writhing test**

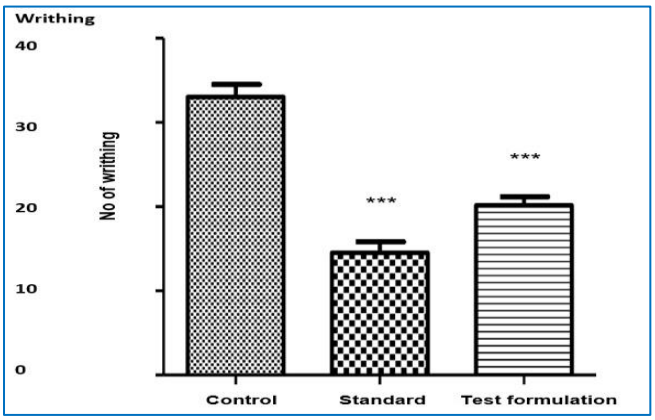


**Table 3: Descriptives of No. of writhing in each group.**

| Groups | Mean | Std. error |
|---|---|---|
| Control | 33 | 18.26 |
| Standard | 14.50 | 1.31 |
| *Karpasabeeja* | 20.17 | 1.01 |

The efficacy of test substances for analgesic activity was evaluated in the Swiss albino mice. The analgesic activity of *Karpasa Beeja Kashayam* was studied in Swiss albino mice by administering the test substance orally. The writhing effect was induced by 1% V/V glacial acetic acid solution in mice pre-treated with test substance. The number of abdominal writhing were measure over 20 min after injection of acetic acid. Finally, percentage inhibition was calculated.

## Discussion

Acetic acid induced writing test was done to find out the central and peripheral analgesic activity of cotton seeds.

Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which enhances inflammatory pain by increasing capillary permeability. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response.

The observation was done in two parts that is latency of onset of writhing as well as number of writhing. The latency of onset was considerably increased by the test drug than the standard drug but it was not statistically significant. While the test drugs showed statistically significant decrease in the number of abdominal constrictions and stretching.

This model as mentioned earlier is for predicting analgesic effect with peripheral mode of action, The writhing observed in this model is mediated by formation and release of phlogistic mediators prostaglandin and bradykinin. The observed effect may be due to decrease in the formation of these mediators. It can also be due to up regulation of the receptor activity of these mediators. Pain, being the most unpleasant sensory and emotional experience, needs utmost attention and treatment. In this study *Karpasa Beeja Kashaya*[5] was used to treat the pain induced by chemical agent named acetic acid. Acetic acid induced writhing in mice attributed visceral pain elicited by triggering localized inflammatory response resulting from release of free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and

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prostaglandin biosynthesis. In this study it was found that *Karpasa Beeja Kashaya* at the dose of 12.48mL/kg body weight showed analgesic activity. The % inhibition of pain is 38.8% in *Karpasa Beeja Kashayam* when compared against control. The analgesic activity of the test substance may be due to the presence of analgesic principles acting through prostaglandin pathways or otherwise. Further fractionation of the test substance in different solvent may help in understanding the mechanism of analgesic activity.

Order of anti-nociceptive activity of test sample Paracetamol > *Karpasa Beeja Kashaya*

Writhing behaviour highly significant results were obtained in central analgesic models where the test drug was comparable or showed less effect than the standard drug paracetamol.

## CONCLUSION

In the experimental study cotton seed *Kaṣhayas* showed significant anti-nociceptive effects. Highly significant results were obtained in anti-nociceptive models where the test drug was comparable or showed less effect than the standard drug paracetamol.

**Fig 1: *Karpasa* Plant**

**Fig 2: *Karpasa* Flower**

**Fig 3: *Gossypium hirsutum*** L. Seed **A.** Entire View, **B.** Longitudinal Section, **C.** Cross Section

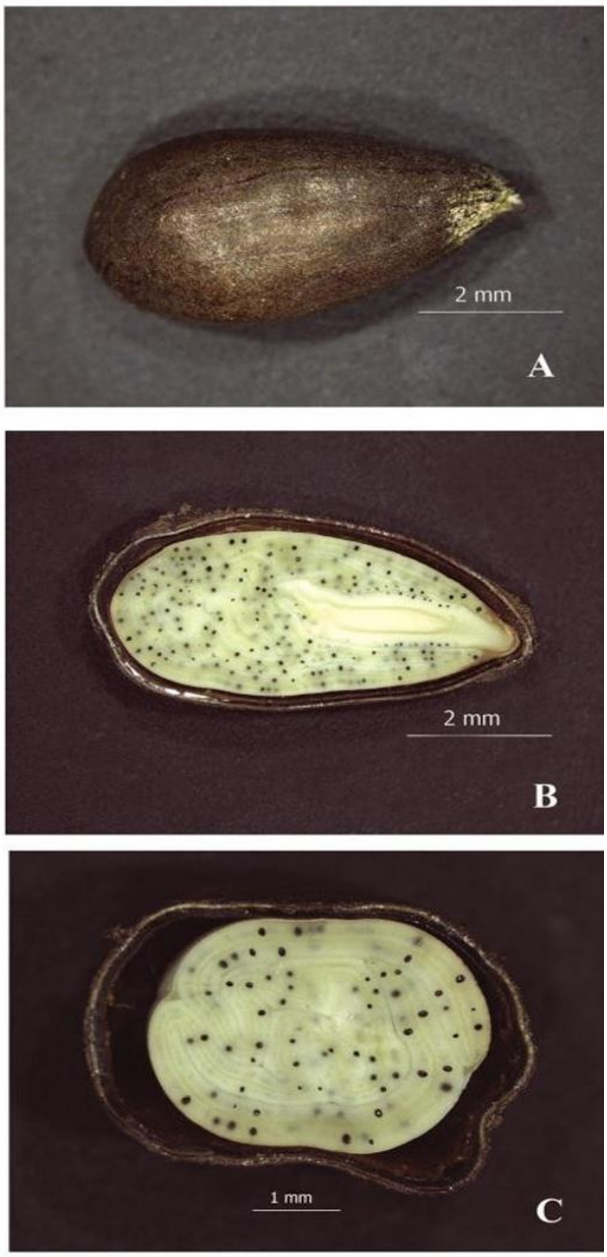
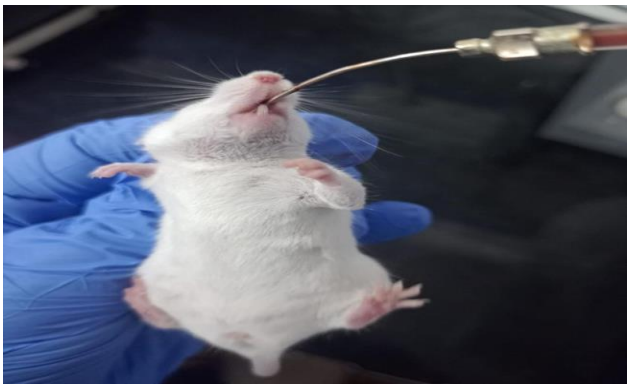



**Fig 4: Experimental study**



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