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An experimental evaluation of Anti-Inflammatory and Analgesic Activity of *Allium Cepa* Linn. and *Allium Ascalonicum* Linn. - A comparative study

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ABSTRACT

Introduction: The drugs possessing *Vataghna* property are much indicated in classics for managing *Shotha* and *Vedana.* Cost-effective, widely available, and potent drugs should be encouraged over costly ones. The present study was undertaken based on this, focusing on the bulb of *Allium cepa* Linn. & *Allium ascalonicum* Linn. to evaluate the anti-inflammatory and analgesic activities employing carrageenan induced paw oedema in wistar albino rats and hotplate tests in mice, respectively. **Materials and Methods:** Fresh juice of *Allium cepa* Linn. and *Allium ascaloncum* Linn. was given to rats and mice orally to observe anti-inflammatory and analgesic activity, and observed for a day. Anti-inflammatory activity was evaluated using carrageenan induced paw edema model in wistar albino rats. Analgesic activity was evaluated using Hot plate method in mice. Experimental animals were divided into 4 groups, Group A as control was given distilled water and; Group B with standard drug, Paracetamol suspension, Group T 1 as test drug 1 with *Swarasa* of *Allium cepa* Linn; and Group T 2 as test drug II with *Swarasa* of *Allium ascalonicum* Linn. as per the calculated doses respectively. **Results:** Animals treated with the test drugs showed a significant reduction in paw oedema and had analgesic effects compared to the control group. The result obtained was also assessed by a one-way-ANOVA test. **Discussion:** The experiment concludes that both the test drugs have anti-inflammatory and analgesic capabilities. However, anti-inflammatory and analgesic effect was seen better in *Allium ascalonicum* Linn. than *Allium cepa* Linn. No adverse effects were noted in the study.

Key words: *Allium cepa Linn, Allium ascalonicum Linn, Anti-inflammatory, Analgesic*

INTRODUCTION

The *Allium cepa* Linn and *Allium ascalonicum* Linn are perennial herbs that are widely grown all over India and used simultaneously as vegetables and for various medicinal purposes. Traditionally these two plants are used for *Vataroga*, *Rasayana*, *Krimiroga* (worm infestation) *Sopha* (oedema*), Vedanasthapana* (analgesic)[1] etc.

When tissues are damaged (e.g., due to trauma or infection), the body activates an acute inflammatory response. Blood vessels dilate, increasing blood flow to the damaged site. This causes redness and warmth. Blood vessel walls become more permeable, allowing fluid, proteins, and white blood cells to migrate from the circulation to the injured tissue. The influx of fluids and cells results in swelling**.** Injuries activate glial cells and immune cells in the PNS, leading to the release of pro-inflammatory mediators. These mediators sensitise nociceptors, contributing to pain.[2] *Shotha* (oedema), believed to be caused by *Srotodushti*, an irregularity of the body's channels, a pathological feature of various degenerative disorders, including diabetes and arthritis associated with *Vedana* (pain).

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The present study focuses on the pharmacological effectiveness of *Allium cepa* Linn. (*Palandu*) and *Allium ascalonicum* Linn. (*Grinjanka*), in experimental conditions in Wistar rats and swiss albino mice.

Aim and Objectives

To experimentally evaluate Anti-inflammatory and Analgesic activity of *Allium cepa* Linn. and *Allium ascalonicum* Linn.

Materials and Methods

Healthy, active, disease free, adult Wistar albino rats (weighing between 150-200 g) and Swiss mice (weighing between 25–30 g) of either gender satisfying the inclusion criteria were randomly selected. Animals were housed in cages with free access to water and standard laboratory meal in a temperature-controlled room with air conditioning and a 12-hour light/dark cycle. They were allowed to acclimatize to the laboratory conditions for a period of one week, and kept fasting overnight prior to the experiment. For evaluation of both analgesic and anti-inflammatory activities, animals were divided into the following four groups (*n* = six in each group): Group A- control group: Treated with normal saline; Group B- standard group: Treated with Paracetamol suspension IP; Group Trial 1: Treated with Fresh juice of *Allium cepa* Linn; Group Trial 2: Treated with Fresh juice of *Allium ascalonicum* Linn. The trial drugs were cleaned and pounded well in *Khalvayantra* (mortar and pestle). The *Kalka* (paste) obtained is filtered through a clean cotton cloth to collect the *Swarasa (fresh juice)* and used for administration. The duration of the study was 1 day.

Anti-inflammatory screening

The inflammatory reaction is readily produced in rats as oedema with the help of the irritant substance carrageenan. When injected in the dorsum of the foot of the rats, they produce acute paw oedema within a few minutes of injection. Carrageenan is a sulphated polysaccharide obtained from seaweed, and by the release of histamine, 5 HT, Bradykinin, and prostaglandins, it produces inflammation and oedema. The rats were weighed, and a mark was made at the tibio-tarsal joint of the left hind paw. Then, they were dosed orally with a control drug (2ml/kg), standard drug (11.5mg/kg).4ml and the test drug 1 (3.6mg/kg) and test drug 2 (3.6mg/kg) respectively before 1hr of injection.[3] Basal readings were recorded and then 0.05 ml of 1% of carrageenan was injected into the sub plantar region of the left hind paw. After 1 hr of injection, the volume of the injected paw was measured by digital plethysmograph and recording repeated at an interval of 15, 30, 60, 120 minutes. Each time, the paw is dipped to the fixed mark to restore the constant p a w volume.

Analgesic screening

In this method, heat is used as a source of pain. Animals are individually placed on a hot plate maintained at a constant temperature (55°C), and the reaction of animals, such as paw licking or jump response, is taken as the endpoint. Analgesics increases the reaction time. The basal reaction time was noted by hind paw licking or jump response (whichever appears first) in animals placed on a hot plate maintained at a constant temperature. Typically, animals show such a response in 6-8sec. A cut period of 15 sec is observed to avoid damage to the paws. Then, they were dosed orally with a control drug (2ml/kg), standard drug (2.5mg/kg) .1ml and the test drug 1 (0.16ml/25g) and test drug 2 (0.16ml/25g) respectively before 1hr,[4] the reaction time of animals on the hot plate were observed on 15, 30, 60, and 120 minutes after drug administration. As the reaction time increased with drugs, 15 sec was taken as the maximum analgesia, and animals were removed from the plate to avoid injury to the paws.

Observations and Results

Statistical Analysis

The statistical significance ($p < 0.05$ and $p < 0.01$) was compared among the control and experimental groups by using IBM SPSS 24 software. Analysis of variance (ANOVA) followed by the Dunnett's t-test was used to determine the anti-inflammatory of the trial drugs compared to the control group. A repeated-measures ANOVA with a Greenhouse-Geisser correction followed by post hoc tests using the Bonferroni correction was applied to analyse the analgesic effect of the trial drugs at various time periods.

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Anti-inflammatory Effect

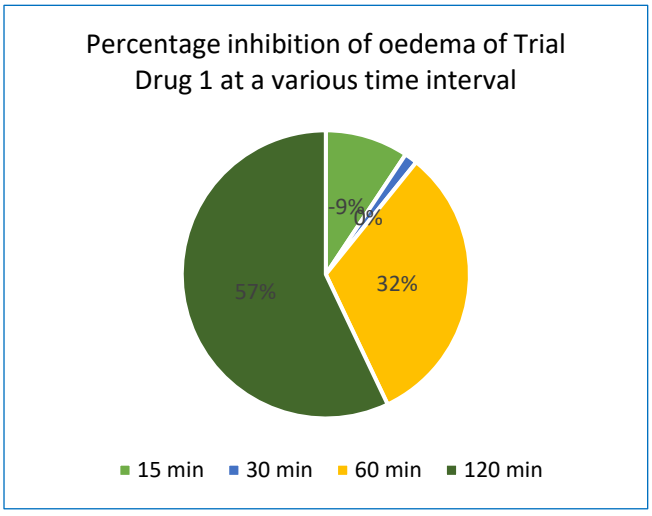
The anti-inflammatory effect of the trial drugs T1 and T2 at different periods are shown in Table 1.

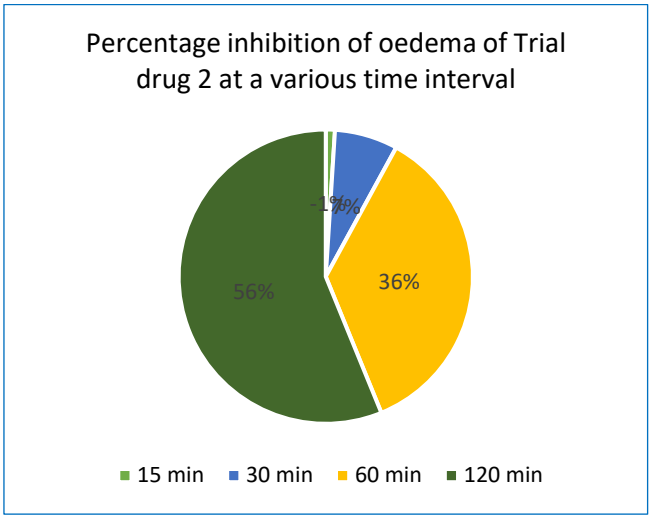
Time (Minutes)	Standard	Drug T1		Drug T2	
	Paw oedema volume (ml)	Paw oedema volume (ml)	Percent inhibition	Paw oedema volume (ml)	Percent inhibition
15	0.55±0.01**	0.57±0.01**	-4.24	0.55±0.01**	-0.60
30	0.76±0.01**	0.77±0.01**	-0.66	0.73±0.01**	4.17
60	0.96±0.01**	0.82±0.01**	14.58	0.75±0.01**	21.53
120	1.07±0.02**	0.79±0.01**	25.89	0.71±0.01**	33.70

Values are shown as mean±SEM. **p<0.01 compared with control

RESULTS

The results indicated that the degree of inflammation significantly reduced with increase in time after the administration of the anti-inflammatory trial drugs T1 and T2. It can also be noted that the anti-inflammatory effect of trial drug T2 was significantly greater at each time period compared to the trial drug 1. The test drug T2 showed a 57% inhibition of oedema in comparison to test drug T1 which showed 56% of inhibition. The maximum amount of inhibition was noted at 120 minutes.





Anti-inflammatory effect of drugs T1 and T2 at different periods is shown in Figure 1.

Effect of Analgesic Drugs

The Analgesic effect of the trial drugs T1 and T2 at different periods are shown in Table .2

Treatment	Threshold time				
	At 0 minute	After 15 minutes	After 30 minutes	After 60 minutes	After 120 minutes
Control	3.61 ± 0.01*	3.62 ± 0.02	3.61 ± 0.02	3.62 ± 0.01	3.62 ± 0.01
Standard	3.61 ± 0.11*	4.30 ± 0.02*	5.37 ± 0.02*	6.28 ± 0.04*	8.77 ± 0.07*
T1	3.61 ± 0.11*	3.72 ± 0.02*	4.37 ± 0.04*	4.77 ± 0.03*	5.10 ± 0.05*
T2	3.61 ± 0.13*	3.74 ± 0.02*	4.44 ± 0.02*	4.87 ± 0.02*	5.43 ± 0.03*

The results are represented by mean ± SEM (n = 6). * p < 0.01 compared to control group.

RESULT

The Table 2 revealed that the pain thresholds in the group treated with Drug T1 significantly increased over time from 0.110 seconds at 15 minutes of drug administration with the highest tolerance level of 1.49 seconds at 120 minutes, suggesting that the drug T1 was most effective at 120 minutes. The pain thresholds

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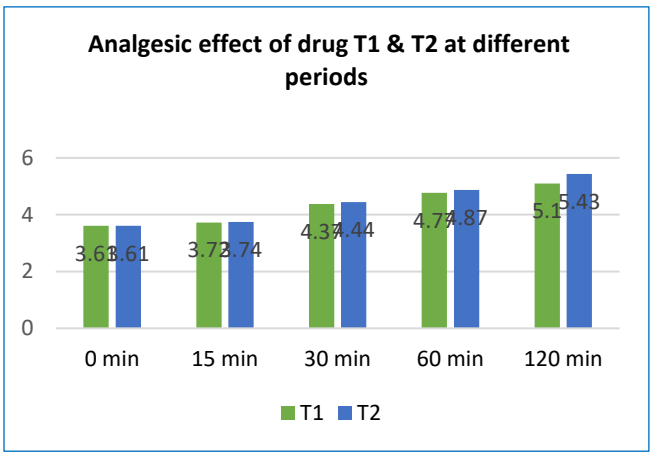
in the group treated with Drug T2 significantly increased over time from 0.130 seconds at 15 minutes of drug administration to 1.82 seconds at 120 minutes, suggesting that the drug T2 was most effective at 120 minutes.

The analgesic effect of drug T1 & T2 at different periods is depicted in Figure 2



Analgesic effect of drugs T1 and T2 at different periods is shown in Figure 2.

DISCUSSION

In *Charaka Sutrasthana, Shothahara Dashaimani,* most drugs in this *Varga* are *Madhura* (sweet), *Katu* (pungent), *Tikta* (bitter) *Rasa* (taste), and *Usna Verya* (hot potency). *In Vedanasthapana Varga*, most drugs possess *Kashaya* (astringent), *Katu* (pungent), *Tiktha* (bitter) *Rasa* (taste), *Tikshna* (sharp), *Snigdha* (unctuous) *Guna* (properties). So, it can be concluded that *Vatakapha Hara Dravyas* are used in the management of *Shodha* (edema) and *Vedhana* (pain). The drug's *Katu Rasa* is *Kaphashamak* (reduce *Kapha Dosha*), *Swayathuhara* (reduce edema), *Vrnanavasadayati* (wound healing), and has *Maraganvivrnoti* (opens up channels) activity which aids in the dilation of *Srothas* (body channels), and so does *Shrothovishodana* (cleanness the body channels), while the *Madhura Rasa* is *Sandhanakara* (promote healing) and *Sthairyakara* (imparts cells integrity), which helps in healing. *Guru* and *Snigdha Guna* does *Vatahara*, and *Tiksna Guna* does *Kaphahara* action. *Ushna Guna* does *Vatahara* and *Kapha Vilayana Karma*. It is noted that *Palandu* is of *Madhura Vipaka* (post digestive effect) and *Grinjanka* is *Katu* in *Vipaka*. *Prabhava* (spefific action) is a unique action exhibited by the *Dravya* (drug) which cannot be explained by its *Rasa, Virya*, and *Vipaka***.** The anti-inflammatory activity of these drugs may be claimed due to their *Vatakaphahara* property. The *Rasayana* (rejuvenation) property of the drug will add to the fast healing of the tissue damage. As *Vata* is the primary *Dosha* in the manifestation of pain, the *Vatahara* property of the drug helps alleviate pain. The *Shothahara* and V*edanasthapana* action observed in this study is due to the test drugs Rasa, *Guna*, *Verya*, *Vipaka*, and *Prabhava*.[5]

Various phytochemicals like quercetin, flavonoids, alkaloids, terpenes, saponins, glycoside are found in both species. Quercetin is a flavanol found in allium species that has potent anti-inflammatory and antioxidant properties. In addition, quercetin can inhibit Tumor necrosis factor (TNF-α) and Interleukin (IL)-1α levels of lipopolysaccharide (LPS)-induced mRNA, which results in reduced apoptotic neuronal cell death caused by microglial activation. Quercetin suppresses the production of inflammatory enzymes (e.g., lipoxygenase (LOX) and cyclooxygenase (COX). It regulates inflammation induced by LPS by inhibiting Src and Syk-mediated phosphatidylinositol-3-Kinase (PI3K)-(p85) tyrosine phosphorylation and subsequent complex formation of Toll-like Receptor 4 (TLR4), which restricts downstream signalling pathway activation in RAW 264.7 cells. The flavonoids found in the sample may inhibit the enzyme prostaglandin synthesis to minimize pain.[6] Several studies showed that the alkaloids suppresses antigen and mitogen-induced lymphocyte proliferation, natural killer cell cytotoxicity, histamine release by mast cells, and interleukin-1 (IL-1).[7] Terpenes reduce proinflammatory levels and can increase the production of some anti-inflammatory cytokines**,** attenuating the inflammatory process, tissue destruction, and disease progression.[8] The significant ameliorative activity of the saponins may be due to inhibition of the mediators of inflammation, such as histamine, serotonin, and prostaglandin, along with its antioxidant property, which inhibits the formation of

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ROS, which also plays a significant role in inflammation.[9] Glycoside were found to induce most of the analgesic effects through cyclooxygenase and lipoxygenase pathway.[10] As a result of the aforesaid, it is possible that the active principles included in the test formulation are functioning through one or more of the processes outlined above.

CONCLUSION

Carrageenan-induced paw oedema is a popular test for anti-inflammatory activity, detecting orally active anti-inflammatory agents through acute inflammation mediators. It is highly sensitive and reproducible, and has significant predictive value for new anti-inflammatory drugs. One hour before the induction of oedema, both the standard and test drugs are given. Oedema is assessed at intervals of 0,15,30,60, and 120 minutes. The drug was administered orally in the form of freshly prepared *Swarasa*. At 0 min and 15min, no significant changes were observed. At 30min, 60min, and 120min, considerable effect was marked with a minimum variation between the two trial groups.

The hot plate test is a behavioural screen to estimate the effects of NCEs (New chemical entities) on the threshold for detecting pain in rodents. It is based on the principle that when rodents are placed on a hot surface, they Will initially demonstrate the aversive effects of the thermal stimulus by licking their paws and, ultimately, by attempts to escape the environment. Substances that alter the nociceptive threshold either increase or decrease the latency to licking/jumping. Foot-licking is more sensitive to analgesic properties, and Allium ascalonicum Linn was moderately better than Allium cepa Linn in producing a better effect. The difference was significant at 15, 30, 60, and 120 minutes after drug administration.

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