

Experimental animal studies on analgesic and anti-nociceptive activity of *Allium sativum* (Garlic) powder

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ABSTRACT

Background: *Allium sativum* has the analgesic and antinociceptive potential to emerge as a safer alternative drug having no troublesome adverse effects.

Aims: To estimate the analgesic and anti-nociceptive effects of *Allium sativum* powder (ASP) in animal models; and to compare the effects between central and peripheral nociceptive models with that of other established analgesic drugs.

Materials and Methods: Albino rats and mice were used for studying analgesic and anti-nociceptive activity of *Allium sativum* powder at doses 75, 150 and 300 mg/kg orally. Various models viz. acetic acid induced writhing model, Eddy's hot plate for analgesic study and formalin-induced paw licking model were used for anti-nociceptive study.

Results: In acetic acid induced writhing model, effect of ASP was better than the control. In the hot plate model, maximum effect was observed at 60 min at a dose of 300 mg/kg, which was higher than the control. In formalin-induced paw licking model, ASP completely abolished the early phase at 150 and 300 mg/kg and in the late phase, the effect of ASP (300 mg/kg) was higher than control.

Conclusion: ASP is effective in both non-narcotic and narcotic models of nociception, suggesting its possible action via peripheral and central mechanism. It also abolishes the early phase in formalin-induced paw licking model. Hence, ASP can be developed as a potent analgesic and anti-nociceptive agent.

Keywords: analgesic, *Allium sativum*, acetic acid, Eddy's hot plate, formalin

INTRODUCTION

Pain is an unpleasant sensation that can be either acute or chronic and that is a consequence of complex neurochemical processes in the peripheral and central nervous system.¹ Drugs used to relieve pain are opioid (morphine like) and nonopioid (aspirin like) analgesic group of drugs. The introduction of these drugs has revolutionized the treatment of pain. The amazing efficacy of opioid and NSAIDS in painful inflammatory conditions has paved the way for the introduction and use of newer analgesic agents. However, the safety factor in respect of both the analgesic drugs has been rather intriguing and hence a definite need is visualized for the introduction of safer analgesic drugs having no troublesome adverse effects.

Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs.²

Furthermore, it is also interesting to note that several natural products especially indigenous drugs have also been investigated for antinociceptive potential. In fact, opium has originally been described as one of the ingredients of poppy (*Papaver somniferum*) capsule.³

Allium sativum (garlic) is one of the plant substances, used as an indigenous remedy for certain ailments. Its effect as an immunomodulatory and anti-inflammatory, antithrombotic, lipid-lowering, antitumoral and antioxidant properties have been demonstrated.⁴ There are several studies on ethanolic, methanolic extracts of *Allium sativum* but studies on the *Allium sativum* powder (ASP) are sparse.

Hence, in the light of the aforementioned development, ASP has been taken up for the investigative study of its analgesic potential. Specific objectives of the study were to estimate the analgesic and anti-nociceptive effects of ASP in

animal models; and to compare the effects between central and peripheral nociceptive models with that of established analgesic drugs like pentazocine and indomethacin. This study also emphasizes the importance and feasibility of the usage of indigenous preparations in clinical medicine.

MATERIAL AND METHODS

ASP obtained from garlic bulbs has been taken up for the investigation of analgesic and anti-nociceptive activity.

Processing of ASP: Garlic bulbs were obtained from Spicex Chemicals Private Ltd, Mysore. Bulbs are separated, deskinning, and dried with the help of an instrument having heating system on one side and a fan on the other side, maintaining hot air current at 50°C for 6 hours, then powdered in a mixer-grinder at 1,850 RPM for 3 min. The fine powder thus obtained is kept in air tight containers. The yield of garlic powder from the garlic bulbs after the processing was found to be 250 gm/kg of garlic bulbs.

Test dose: A pilot study was conducted with different doses (50 mg/kg, 75 mg/kg, 150 mg/kg, 200 and 300 mg/kg) to assess the appropriate dose for the study. The analgesic activity was observed at few doses and the same was used in the study.

Phytochemical test

ASP was subjected to standard phytochemical screening tests for various constituents.

Chemicals: Indomethacin 10mg/kg and pentazocine 10mg/kg, ASP, normal saline and other chemicals were of analytical grade.

Animals: Adult Albino mice of either sex weighing between (20-30 g) or Adult Albino rats of either sex weighing between (120-130 g) were used for the study. The animals were procured from animal research laboratory, National Institute of Mental Health and Neuro Sciences, Bangalore and housed in the animal house of the institute in 5 groups, at an ambient temperature of 25±1°C with ad libitum

access to food and water. The study protocol was approved by Institutional Animal Ethics Committee.

Treatment schedule: The analgesic and anti-nociceptive activities were examined by using the *Acetic acid induced writhing test, Eddy's hot plate model and Formalin-induced paw licking model*. Animals were divided into five groups, with each group consisting of six animals. Group 1 received vehicle (normal saline); group 2 received indomethacin / pentazocine (10 mg/kg orally), groups 3, 4 and 5 received ASP -75,150 and 300 mg/kg orally respectively.

Acetic acid induced writhing in rats

Adult albino rats were randomized into five groups of 6 each. The analgesic activity of ASP was assessed using writhing test.⁵ Control, standard and test groups were treated with vehicle, indomethacin (10 mg/kg) and ASP (75,150 and 300mg/kg) orally respectively, 60 minutes prior to the test. Acetic acid solutions (10 ml/kg, 0.6% in normal saline) was injected intraperitoneally and were observed for the (writhes) contraction of abdominal muscles for 10 min. Number of writhes were counted to assess analgesic activity of various groups, and was expressed as the percentage inhibition of abdominal constrictions between control group and ASP treated group animals.

Thermally-induced pain in mice

This is one of the most commonly used methods for evaluating central analgesic activity of a drug.⁶ In this method heat was used as a source of pain. Mice were being divided into 5 groups of six each. First group served as a control, second group served as the standard (Pentazocine 10 mg/kg, intraperitoneally), while the third, fourth and fifth groups received 75,150 and 300 mg/kg of ASP respectively. After 1 hour, animals were individually placed on a hotplate maintained at a temperature of 55± 0.5°C, and were placed not more than 15 seconds (cut off time) on the hotplate, in order to avoid damage to the paws.

The time taken to flick the hind paw or lick or jump from the hot plate was considered as the reaction time of the particular animal. The reaction time was recorded at 0, 15, 30, 45, 60, 90, and 120 min. An analgesic increases the reaction time. Percent decrease in reaction time was taken as index of pain perception at each interval.

Formalin- induced paw licking model in mice

Adult albino mice randomized into five groups of 6 each were not fed for a day. The control group received 10ml/kg of distilled water orally, standard group received indomethacin (10 mg/kg orally) and the test groups received ASP at doses 75, 150 and 300 mg/kg, orally respectively. Formalin solution (20 µl of 2.5%) was injected subcutaneously under the surface of the right hind paw of each mouse and the responses were observed for half an hour.⁷ The duration of time spent licking the injected paw was recorded and was indicative of pain. The first phase of the analgesic activity normally peaked at 5 minutes representing the neurogenic response and the second phase representing inflammatory pain response after 15-30 minutes of formalin injection. This suggests formalin test has two distinctive phases possibly reflecting different types of pain.

Statistical analysis:

The statistical analysis of data was done using one-way analysis of variance by using the SPSS software (version 11.5). P< 0.01 was considered as highly significant.

RESULTS

Acetic acid induced writhing model in mice

As shown in Table - 1, the ASP produced significant (P<0.01) reduction in the number of writhing in mice in dose dependent manner. At 100 and 200 mg/kg, the percent reduction of writhing was 57.44% and 72.10% respectively, as compared to the control group, whereas the standard drug indomethacin (10 mg/kg) showed a reduction of 91.38%.

Table 1: Analgesic activity of ASP in albino mice in acetic acid induced writhing method

Groups	Drugs	Dose mg/kg (p.o)	No. of Writhing	Percent reduction (%)
I	Control	-	47±1.57	0.00
II	Indomethacin	10	4.73±1.45*	91.38
III	ASP	75	31.18±0.79	33.65
IV		150	20.00±0.53*	57.44
V		300	13.11±0.82*	72.10

Values are expressed in terms of mean ± SEM, n = 6 in each group, *p< 0.01 statistically highly significant as compared with control group. ASP =Allium Sativum Powder.

Thermally-induced pain in mice

In this model, the reaction time in ASP treated group increased significantly (P<0.01) in comparison to the control group. The maximum effect was observed at the highest dose viz. 200 mg/kg at 60 min which showed a reaction time of 16.5 sec, whereas the standard drug pentazocin (10mg/kg) showed a reaction time of 17.2 sec. The extract also showed dose and time dependent activity -Table 2.

Table 2: Analgesic activity of ASP in Eddy's hot plate model in mice

Groups	Drugs (p.o)	Dose mg/kg	Reaction time in seconds				
			0 min	30 min	60 min	90 min	120min
I	Control	-	3.89±0.15	4.10±0.07	4.60±0.17	4.30±0.25	3.59±0.12
II	Indomethacin	10	12.6±0.42*	14.6±0.81*	17.2±0.64*	14.2±0.47*	13.6±0.53*
III	ASP	75	6.55±0.25*	9.12±0.65*	11.7±0.45*	12.2±0.85*	12.5±0.35*
IV		150	8.80±0.12*	11.7±0.65*	13.7±0.55*	14.0±0.47*	13.2±0.34*
V		300	10.6±0.52*	13.6±0.67*	16.5±0.33*	13.5±0.38*	12.4±0.54*

Values are expressed in terms of mean ± SEM, n = 6 in each group, *p< 0.01 statistically highly significant as compared with control group. ASP =Allium Sativum Powder.

Formalin- induced paw licking model in mice

Administration of ASP at 50 and 100 mg/kg caused reduction in duration of paw-licking (43.8 and 35.11 sec), as compared to the control group (57.2 sec). Higher doses of ASP at 200 mg/kg showed complete abolishment of the early phase

indicated by absence of paw licking after the formalin injection. However, the standard drug-indomethacin (10 mg/kg) exhibited a reduction of paw licking time of 25.4 sec only in the early phase. In late phase, the administration of ASP decreased the duration of paw licking dose dependently from 70.37 sec at 50 mg/kg to 48.84 sec at 200 mg/kg in the late phase. On the other hand, indomethacin (10 mg/kg) exhibited a reduction of paw licking time of 33.84 sec in the late phase. The effect of ASP at 200 mg/kg was comparable to standard drug-Table 3.

Groups	Drugs	Dose mg/kg (p.o)	Early phase (duration of paw licking in seconds)	Late phase (duration of paw licking in seconds)
I	Control	-	57.2±1.57	135.20± 1.10
II	Indomethacin	10	25.4±1.45*	41.85±0.34*
III	ASP	75	43.8±0.79*	70.37±1.12*
IV		150	39.00±0.53*	61.31±1.11*
V		300	0	49.84±0.45*

Values are expressed in terms of mean ± SEM, n = 6 in each group, *p< 0.01 statistically highly significant as compared with control group. ASP =Allium Sativum Powder.

DISCUSSION

Analgesics are medications used to relieve pain without reducing the consciousness of the patient. They work by reducing the amount of pain felt and this is generally achieved by interfering with the way the pain message is transmitted by the nerves. We carried out the study using different experimental models to evaluate peripheral and central analgesic and anti-nociceptive activity of ASP.

ASP showed analgesic activity both centrally or peripherally. The plant is supposed have the phytoconstituents which inhibit cyclooxygenase enzyme for producing analgesia peripherally or act on central opioid receptors for producing analgesia centrally. Standard drug indomethacin act on cyclooxygenase pathway of prostaglandins synthesis.⁸

Acetic acid induced writhing response in mice is reliable and affords rapid evaluation of peripheral

type of analgesic action. Pain sensation in this writhing method is elicited by triggering localized inflammatory response resulting in release of free arachidonic acid from tissue phospholipids via cyclooxygenase (COX), and prostaglandin biosynthesis.^{9,10} Acetic acid is believed to act indirectly by inducing the release of prostaglandins and other mediators into the peritoneum which in turn stimulate nociceptive neurons sensitive to analgesic anti-inflammatory drugs. The mechanism of analgesic activity of ASP could be probably due to the blockade of pain mediators, which excite pain nerve endings similar to that of indomethacin and NSAIDs. Thus, the reduction in the number of writhing suggests that ASP might exert anti-nociceptive activity by inhibition of cyclooxygenase in peripheral tissue.

The hot-plate method is one of the most common tests considered to be selective for the centrally acting drugs. Thermally-induced pain in mice measures the complex response to a non-inflammatory, acute nociceptive input used for studying central nociceptive activity.¹¹ Nociceptive reaction toward thermal stimuli in mice is a well-validated model for detection of opiate analgesics as well as several types of analgesic drugs from spinal origin.¹² An agent that causes a prolongation of the hot plate latency using thermally-induced pain in mice must be acting centrally is an established fact.¹³ NSAIDs inhibit only peripheral pain whereas narcotic analgesics block both.^{14,15} ASP has shown inhibition of both types of pain. The analgesic effect of the plants in both models reveals that they have dual action through central and peripheral mechanism.¹¹

Normally, the fine afferent C- and A- fibers are activated by brief, high intensity stimuli, which do not induce any tissue damage. However, during inflammation, the afferent fibers can be activated by lower intensity stimuli and the pain produced differs in quality and persistence. Formalin induced pain is caused by peripheral tissue inflammation. It involves a phase of inflammation wherein a variety of chemical mediators alter the functions of peripheral afferent fibers.¹⁶

In the present study, the formalin-induced paw

licking model comprises of early phase (immediately after injection) seems to be caused by C-fiber activation due to the peripheral stimulus and late phase (starting approximately 20 min after formalin injection) appears to depend on the combination of an inflammatory reaction. The formalin resulted in a progressive biphasic behavioral response such as licking and biting of the injected paw in all the experimental groups. The beginning time point of the phase 2 response in these experimental groups was 15 min after the formalin injection.

ASP completely abolishes the early phase at the dose 200 mg/kg, suggesting complete inactivation of C- fiber in the early phase. ASP decreased the reaction time in dose dependent manner in the late phase also, which might suggest that ASP causes partial inactivation of NMDA and non-NMDA receptors.

Phytochemical screening of ASP: It revealed the presence of tannins, flavonoids, aromatic acids, reducing sugars and saponins.¹⁷ One of the most biologically active compounds, allicin (diallyl thiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes alliin to allicin which is further metabolized to vinyldithiines. Ajoene is another chemical constituent thought to be most important to health.

Previous studies have shown the anti-inflammatory, analgesic and anti-convulsant

activity of methanolic extracts of Allium sativum.¹⁸

In contrast to various extracts, the natural powder form can be easily prepared and can serve as an efficient household remedy for common ailments.

CONCLUSION

A number of studies on Allium sativum, or its major active principles, have shown an antihyperlipidaemic, antibacterial and anti rheumatic properties. In the present study, ASP has shown promising results in experimental algesia. It can be interpreted that ASP possesses promising analgesic and anti-nociceptive properties, possibly exerts its effect through diverse mechanisms that may involve both central pain inhibitory mechanism as well as peripheral pathways through inhibition of prostaglandin synthesis. ASP may serve as a potential adjuvant for management of various painful conditions. The results obtained by this study cannot be directly extrapolated to humans; further studies are required to establish the effect on pain perception in humans.

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