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Research Paper

Evaluation of The Analgesic Activity of Garlic Extract in Some Animal Models in Rats.

Medical Science

KEYWORDS : Endothelial Cell Count (ECC), manual SICS, phacoemulsification, specular microscopy.

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ABSTRACT

Background : Garlic has been used for various diseases from ancient times because of its anti-inflammatory ,anti arthritic, anti-oxidant, dyslipidemic, anti-cancer, anti-infective properties . Because of the above mentioned properties in the present study Garlic extract (GE) preparation was evaluated for its analgesic activity by making use of different central and peripheral pain models. So this study was undertaken to evaluate the analgesic activity of Garlic extract (GE) in established experimental central and peripheral pain models in rats .Methods and Material: The analgesic activity of GE was assessed by employing different pain models such as i)Hot plate and tail clip tests for central analgesia ii) 4% sodium chloride induced writhing as peripheral analgesic model . The percentage inhibition of writhes and prolongation of reaction time were the parameters of evaluation.

The results obtained were analyzed by ANOVA and Student's unpaired "t"- test . Results: GE treatment (200 mg/kg and 300 mg/kg) reduced writhing episodes significantly in 4%NaCl induced writhing in rats as compared to control indicating its analgesic effect. The highest percentage inhibition of pain was seen with 300mg/kg of GE. GE treatment, in Hot plate & Tail - clip methods significantly prolonged the reaction time at 60 min & 90 minutes.Conclusions: Garlic extract (GE) was found to be effective in all three models of experimental pain. However it is less potent than standard analgesic drugs and could be employed safely in higher doses.

Introduction:

Pain is the most common symptom for seeking the physician[1] advice. As long-term use of conventional analgesics is associated with significant toxicity. Hence there is a need for new analgesics. Garlic is a global natural herb with medicinal properties[2] like hypoglycemic[3], memory enhancing[4], anti-oxidant[5], antidepressant[6], anaesthetic[7], wound healing[8] and anti-inflammatory[9] in various animal models. In addition onion extract have been suggested to possess analgesic effects. Hence ethanolic extract of garlic was chosen for present study. Garlic extract was found to exert anti-nociceptive action in models of both superficial and visceral pain.

Material and Methods:

Animals

Sprague Dawley albino rats of either sex (200-250g) were used for this study. Rats were housed in groups of three; in standard big polypropylene cages measuring 40×27.5×13.5 cm.The animals had free access to food and water ad libitum. The experimental protocol was approved by the "Institutional Animal Ethics Committee" [IAEC]. Diclofenac (India chemical Co., New Delhi) Pentazocine (Fortwin, Ranbaxy) were used for this study.

The garlic plants with bulbs were authenticated from Botanical survey of India, Pune Maharashtra. About 500g of garlic bulb were utilized in both first and second rounds of extraction. The bulbs were dried in hot air oven at 60°C till moisture content was removed (1 hour).Soxhlet apparatus assembled and thimble made .Extraction was done with 70 % ethanol (70 ml of 99.9 % ethanol and add distilled water to make it 70%) for 72 hours.[11]The extract was concentrated with the help of a vacuum evaporator under reduced pressure and stored in deep freezer and utilized at the time of study. Stock solution concentration 100mg/ml was utilized.

A pilot study was conducted with garlic extract (75mg/kg, 100mg/kg, 150 mg/kg, 200mg/kg, 250 mg/kg, 300mg/kg, 400mg/kg) was done to assess the appropriate dose of the study. The analgesic activity was observed at few doses and the same was used for the final study.

Experimental evaluation of Analgesic activity :Prior to employing the different methods to study the analgesic activity (physical, thermal and chemical models of pain) a preliminary screening was done. Those rats showing reaction time of less than 8 seconds were included in the study and the rest were excluded.

Group I: Control group treated with distilled water Group II: Test group treated with GE 100mg/kg body weight p.o. Group III: Test group treated with GE 200mg/kg body weight p.o. Group IV: Test group treated with GE 300mg/kg body weight p.o. Group V:Diclofenac 10mg/kg body weight orally for peripheral pain models .Pentazocine 10mg/kg body weight i.p. for both central pain models : 4% NaCl induced Writhing Method :Analgesic activity of GE was assessed by counting the number of writhes induced by 1ml/kg of 4% Na Cl.[12] Animals that do not exhibit writhing within 30 seconds were discarded. GE in 100mg/kg, 200mg/kg, 300mg/kg doses & Diclofenac sodium 10mg/kg was administered p.o. to the different treatment groups 60 min before the 4 % NaCl administration. The number of writhes was observed for about 10 minutes and the animals showing no response were defined analgesic positive. Percentage inhibition of the number of writhes was taken as index of analgesia.% inhibition = Nc -Nt/Nc × 100. Nc = mean no: of writhes in the control group. Nt = mean no: of writhes in the test group. No response= analgesic positive Writhing response = analgesic negative .

Hotplate Method: Rats were placed on a hot-plate at 55°±5 ° C.[13] The latency to lick the paw was the reaction time. A cut off time of 30 seconds was followed to avoid any thermal injury to the paws. GE was given in the doses of 100mg/kg, 200mg/kg, 300mg/kg per orally at 0 min. The reaction time of the rats was recorded at 30, 60, 90 min after drug administration. The mean of the observed values was considered for statistical analysis.

Haffner's Tail clip Method: An artery clip with thin rubber sleeves was applied to the base of the rats's tail for 30 seconds. The latency period to dislodge the clip by biting was recorded with the help of stopwatch.[13] The garlic extract was given in the doses of 100mg/kg, 200mg/kg, 300mg/kg per orally at 0 min .The reaction time was observed at 30.60,90 min after drug administration. The mean of the observed values was considered for sta-

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tistical analysis.

All data were expressed as mean ±SEM. Statistical analysis was carried out by ANOVA and Student's unpaired "t"- test for peripheral as well as central models. Multiple comparisons were done with Bonferroni's adjusted t-test. The values considered significant at P<0.05 when compared with control.

Results: The Garlic extract (GE) at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg p.o. and diclofenac 10 mg/kg po which was used as standard exhibited a significant reduction (P<0.05) in the number of writhes as compared to control [Table 1] .The percentage inhibition of the number of writhes were 76 % with Garlic extract 200mg/kg and 93 % with Garlic 300mg/kg which was comparable to diclofenac sodium 10 mg/kg.

Table 1: Effect of GE on 4% NaCl induced writhing in rats

Treatment (mg/kg,p.o)	Number of writhes	% inhibition
Control	25.9 ±4.4	0 %
Diclofenac sodium (10)	4.5±2.59*	82%
GE (100)	13±2*	49%
GE(200)	6.1±1.17*	76%
GE (300)	1.8±1.29*	93%

*** P < 0.05 as compared to control Figures are mean ± SD.**

In the hotplate method, GE in the doses of 200 & 300mg/kg, significantly prolonged the reaction time in rats at 60 and 90 minutes as compared to control (p<0.05) [Table 2] whereas Garlic extract in the dose of 100mg/kg significantly prolonged the reaction time as compared to control only at 90 min (p<0.001).Garlic extract at the dose of 300mg/kg showed a significant increase in the reaction time at

60,90 min as compared to pentazocine (p<0.05).Pentazocine showed significant findings from 30 minutes onwards (p<0.01) when compared to control.

Table 2: Effect of GE on latency of paw licking in hot plate method

Latency of paw licking (sec)				
Treatment (mg/kg p.o)	0 min	30 min	60 min	90 min
Control	5.01±1.10	5.13±1.09	5.74±1.07	4.70±1.13
Pentazocine (10mg/kg i.p.)	6.74±1.78	12.24±4.14*	8.56±2.15	7.32±1.74
GE (100)	4.81±1.41	8.58±1.99	8.30±1.50	10.09±2.05 *
GE (200)	6.30±1.36	10.54±2.08 *	11.39±2.23*	11.01±3.53 *
GE (300)	6.39±1.65	12.9±1.92 *	16.23±1.24▲	17.05±1.59▲

***p<0.05, when compared with control group. Fig are Mean±SD**

▲p< 0.05 when compared with pentazocine group.

In Haffner's tail clip method the garlic extract in the doses of 200mg/kg & 300mg/kg, significantly prolonged the reaction time in rats at 60 and 90 minutes as compared to control (p<0.05) ,while Pentazocine showed significant findings from 30 minutes onwards (p<0.05) as compared to control[Table :3] GE 200mg/kg showed results comparable to pentazocine.

Table 3: Effect of GE on latency of tail biting in tail-clip method

Treatment group (mg/kg p.o.)	0min	30min	60min	90min
Control	4.38±2.26	3.63±1.92	3.50±0.93	3.25±1.03
Pentazocine (10 mg/kg i.p.)	4.63±2.72	14.13±5.62*	11.63±7.01*	9.63±5.83
GE-(100)	3.35±2.13	5.41±3.78	5.50±5.46	5.41±3.78
GE-(200)	3.37±1.90	5.33±4.56	9.86±5.54*	9.05±5.02*
GE-(300)	3.96±2.28	13.55±5.98*	14.93±7.15*	8.65±4.74*

*** p<0.05, when compared with Control group. Fig are Mean±SD**

Discussion: Analgesics are medications used to relieve pain of multiple etiology without reducing the consciousness of the patient. This is generally achieved by interfering with the way the pain messageis transmitted by the nerves. The present study was carried out using different experimental pain mode to evaluate the peripheral and central analgesic activity of GE.

The garlic extract showed analgesic activity in both central and peripheral models of pain as compared to control and at higher doses comparable to standard. Writhing induced by 4% Na Cl solution in rats is a sensitive and specific test for predicting the peripheral analgesic activity of a compound in man.[12]

Bhavyapraakash Nighantu describes Garlic to be used as 'kookshi shoolahanta' (alleviating pain in abdomen).[14]Intra-peritoneal injection of 4% NaCl, the nociceptive response is due to release of endogenous substances such as bradykinin and prostaglandins and other mediators into the peritoneum, which stimulate the nociceptive endings sensitive to analgesic, anti-inflammatory drugs. In this study we found that administration of GE in the doses of 100, 200 and 300 mg/kg orally 60 min before administration of NaCl resulted in significant decrease in the number of writhes. The percentage inhibition of writhes was found to be 49%, 76% and 93% with the respective doses. Diclofenac in the dose of 10 mg/kg resulted in 82% inhibition in the number of writhes, which was comparable to the dose of 200mg/kg of GE.

A study by Jayanthi et al using Garlic powder in the doses of 75, 150 and 300mg/kg orally 60 min before administration of acetic acid resulted in significant decrease in the number of writhes as compared to control. The percentage inhibition of writhes was 33%, 57% and 72% with the respective doses. Indomethacin in the dose of 10mg/kg resulted in 91% inhibiton in the number of writhes.[15]Farjana et al studied the anti-nociceptive activity of methanolic extract of Allium sativum cloves (MEAS) in acetic acid induced writhing in mice. The extract was given in the doses of 50, 100, 200 and 400 mg/kg and resulted in significant reductions in the number of writhings by 27.6%, 41.4%, 51.7% and 55.2%.[16]In our study, GE given in the dose of 100 and 200 mg/kg resulted in 49 % and 76 % inhibition of writhing. The differences observed might be due to difference in the species (mice

versus rats) or agent used for inducing writhing (NaCl versus acetic acid) or the formulation (powder versus ethanolic extract versus methanolic extract).

The hot-plate method as well as tail-clip method is most commonly used tests, considered to be selective for the centrally acting analgesic drugs. In both these models of experimental pain, GE administered in the doses of 200 & 300 mg/kg per orally, significantly prolonged the reaction time in rats at 60 and 90 min as compared to control. Jayanthi et al studied the analgesic activity of Garlic powder in mice by hot-plate method. They administered Garlic powder in the dose of 75,150 and 300 mg/kg per orally and found a significant increase in reaction time at 30,60, 90 and 120 min. Our findings are consistent with their

observations. We did not find any study exploring the analgesic activity of Garlic in tail-clip method.

The Garlic extract, is less potent as compared to pentazocine or diclofenac, the standard drugs. Acute toxicity LD50 studies of aqueous garlic extract, garlic powder, and alcoholic garlic extract showed no mortality in mice till doses of 5g/kg body weight. Plant extracts with LD50 less than 10mg/kg body weight are considered highly toxic and those with LD50 greater than 50mg/kg body weight are considered non-toxic. [17]Thus garlic can be considered as non toxic and higher doses may be employed. Garlic contains following phytoconstituents like flavanoids, alkaloids and sulfur compounds. The major phytoconstituents are Thioacremonone, Allicin, DADS, DATS, Ajoene & Quercetin. [5]Some of this may inhibit cyclooxygenase [16]peripherally and act on opioid receptors centrally leading to analgesia. The mechanism of analgesic activity of Garlic extract could be probably due to the blockade of inflammatory pain mediators because of its established anti-inflammatory properties in carrageenan induced rat paw edema and cotton pellet induced dry granuloma weight. Moreover the release of PGE2 a byproduct of Arachidonic acid metabolism is directly dependent on generation of free radicals/reactive oxygen species. Allyl cysteine, alliin, allicin, and allyl disulfide are protective sulfur

compounds against free radical damage.[18]Analgesia can result from decline in plasticity at dorsal root via deprivation of substance P or glycine or glutamate from the nerve endings .Garlic extract is reported to decrease Glutamate levels and therefore may result in analgesia in dorsal horn neurons. Ajoene found in garlic has been proposed to inhibit the pain receptors at dorsal root of spinal cord thus resulting in inhibition of pain signal transduction.[10]

Limitations: We have used only 3 models of experimental pain. The analgesic activity of garlic extract should be confirmed in other models of experimental pain. Isolation, purification and characterization of active compounds of the extract were not part of our study. But such studies are necessary and it is possible that the active compound may be equal in potency as compared to standard analgesic drug. We studied only up to 90 min. Prolonged study is needed to find out the exact duration of analgesia. We have not studied the combination of standard drugs with garlic. It might be possible that garlic extract potentiates analgesic activity of standard drugs which may result in lower dosages of standard drugs minimizing the adverse effects of such drugs.

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