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Reduced nociceptive responses in mice with alloxan induced hyperglycemia after garlic (*Allium sativum* Linn.) treatment

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Administration of ethanol (95%) extract (45 mg/kg body wt/day for 28 days) of garlic (*A. sativum*) to alloxan induced diabetic (ALX-D) mice significantly lowered the serum glucose levels, nociceptive response in tail-flick, hotplate, allodynia, formalin test and relative thickness, weight of hind paw in formalin induced Paw oedema test, over 28 days, thus, showing the reversal trend in hyperglycemia and hyperalgesia compared to ALX-D mice. The reversal of hyperglycemia and hyperalgesia was progressive and more effective as duration of extract administration increased. The results suggest therapeutic potential of ethanol extract of garlic for anti-hyperglycemic and anti-nociceptive effects in diabetes.

Allium species have been studied for their therapeutic uses as antibiotic, antioxidant, antiatherogenic, anticancer, antidiabetic[1], fibrinolytic[2] and anti-inflammatory effects[3]. Garlic has been used as a folk medicine against diabetes in Norway and middle Europe[4]. In recent years it has received particular attention because of its use in treatment of diabetes[5]. The hypoglycemic effect of garlic was demonstrated in normal rats[6], alloxan diabetic (ALX-D) rats[7] and ALX-D rabbits[8].

Neuropathy is one of the common complications in diabetes. The peripheral neuropathy may be either painful or painless[9]. An increase in pain threshold level in tail-flick and hot plate latencies was observed in diabetic rats and mice[10-12], while reduction in pain threshold level in diabetic animals[13], and no alterations in pain perception in streptozotocin treated diabetic (STZ-D) animals were reported[14]. Thus, the experimental evidence is contradictory in pain perception.

The present study has been conducted to assess the effect of garlic extract on nociceptive responses in ALX-D mice.

Materials and Methods

Male Swiss albino mice (30±2 g), procured from National Institution of Nutrition, Hyderabad were maintained at room temperature with pellets (Lipton India Ltd. Bangalore.) and water *ad libitum*. The animals were divided into following 3 groups of 6 each. Group-I = normal mice (controls); Group-II = ALX-D mice and Group-III = ALX-D mice treated with garlic extract.

Diabetes was induced by sc injection of alloxan monohydrate (Loba Chemie, Indo Austranal Co., Mumbai, India), 45 mg/kg body wt; 2.8 *M* dissolved in 0.3 ml of distilled water. Mice became diabetic after 24 hr and were maintained by supplementation of alloxan on 1st, 7th, 14th, 21st and 28th days.

Garlic extract—Fresh bulbs of garlic obtained locally were peeled off, sliced into pieces and dried under shade at room temperature for 2-3 days. The dried pieces were reduced to a moderately fine powder using disintegrator. The powder was extracted in Soxhlet apparatus with 95% ethanol solvent for 24 hr. The extract was concentrated under vacuum (Rota vapour). The residue obtained was dissolved in distilled water and administered orally at 45 mg/kg body wt/day dose in 0.3 ml distilled water for 28 days to test the analgesic and hypoglycemic activities.

The blood was drawn after 18 hr from fasting animals from orbital sinus on 1st, 7th, 14th, 21st, 28th, days and the serum glucose levels were estimated in all the three groups of mice by O-Toluidene method[16]. In the third group garlic extract was administered 4 hr prior to the serum glucose estimation.

Nociceptive tests—Tail-flick[17], hotplate[18], allodynia[19], formalin[20] and formalin induced paw oedema tests were conducted in all the groups. The results were analysed statistically by applying the Two way ANOVA (factorial method), followed by multiple

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comparison test. The level of significance were observed between all the three groups, between the days and with in the groups at $P<0.05$ and <0.001.

Results and Discussion

The ALX-D (group II) mice had showed a significant ($P<0.001$) decrease in the body weight throughout the experiment. The garlic extract treatment gradually recovered the weight loss in ALX-D group of mice. The serum glucose levels were significantly elevated in ALX-D group on 1st, 7th, 14th, 21st and 28th day when compared with control. Garlic treatment resulted in arrest of progressive increase of the serum glucose levels in ALX-D group.

Levine *et al.*[13] demonstrated prolonged tail-flick latencies to radiant heat in diabetic mice. While, Itmar

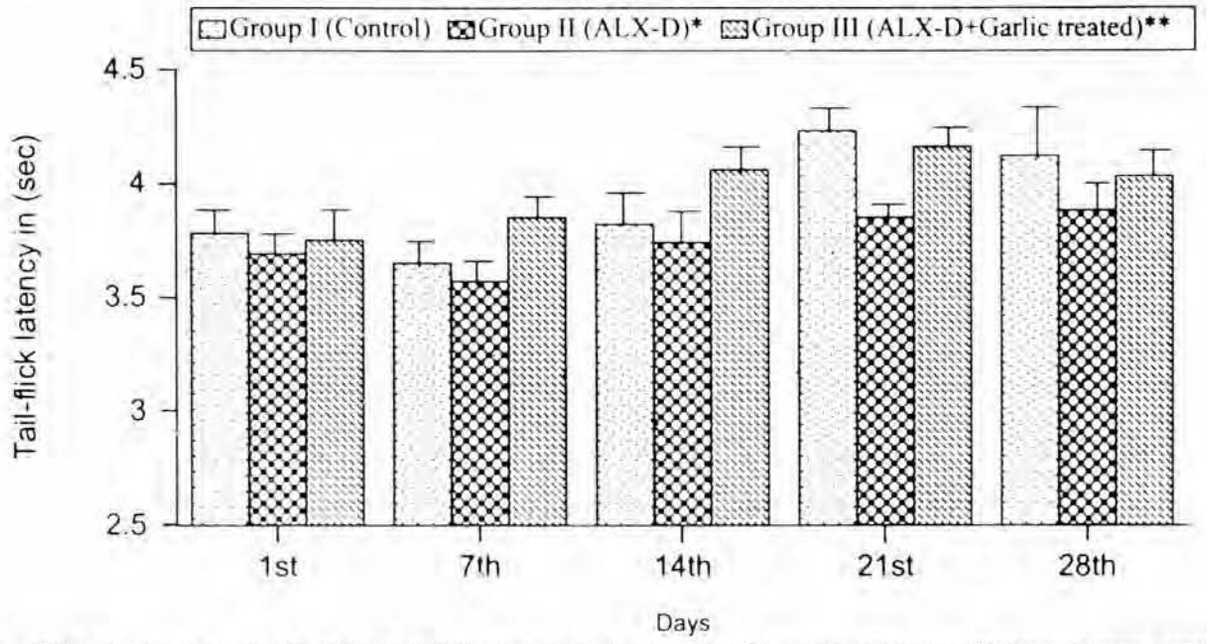

Fig. 1—Effect of ethanol extract of garlic on tail-flick response in alloxan induced hyperglycemic mice [$P<0.001$, between control and ALX-D(*) and ALX-D and ALX-D+garlic (**)]

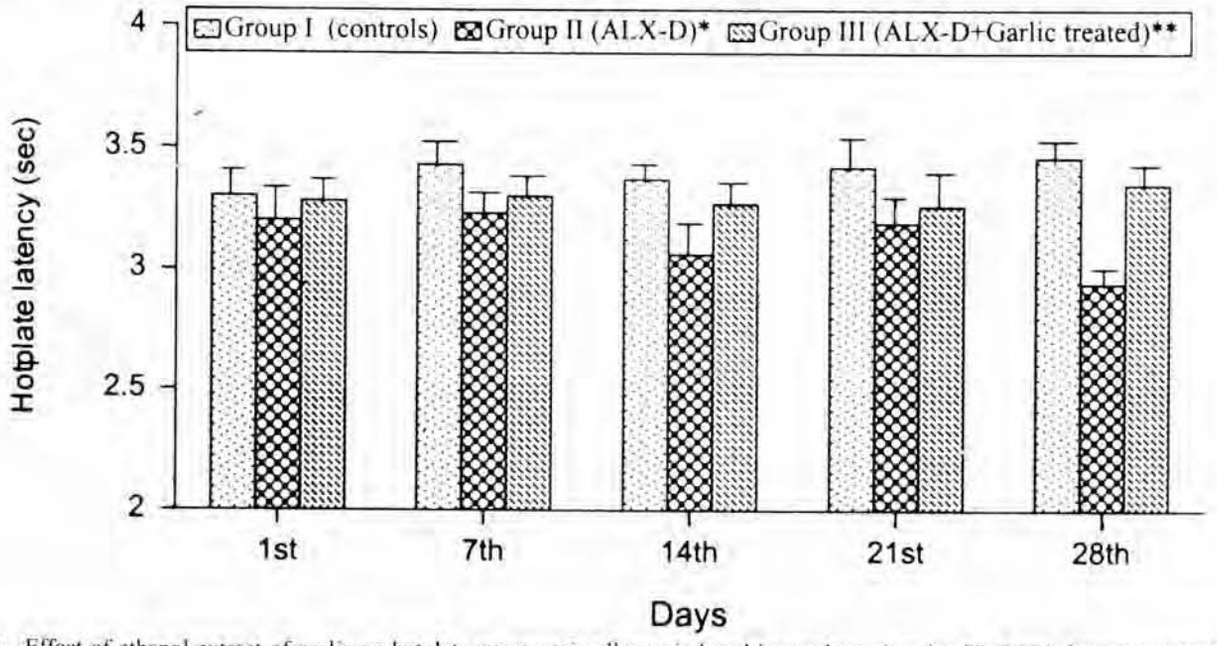

Fig. 2—Effect of ethanol extract of garlic on hotplate response in alloxan induced hyperglycemic mice [$P<0.001$, between control and ALX-D (*) and ALX-D and ALX-D+garlic (**)]

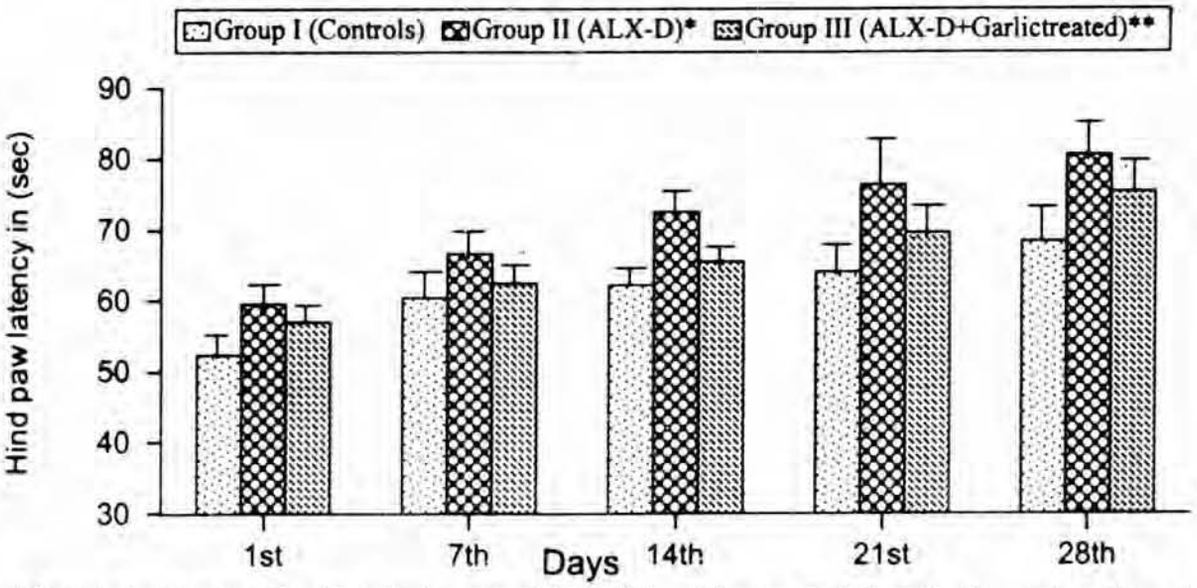

Fig. 3—Effect of ethanol extract of garlic on hind paw cumulative withdrawal duration of allodynia in alloxan induced hyperglycemic mice [$P<0.001$, between control and ALX-D (*) and ALX-D and ALX-D+garlic (**)]

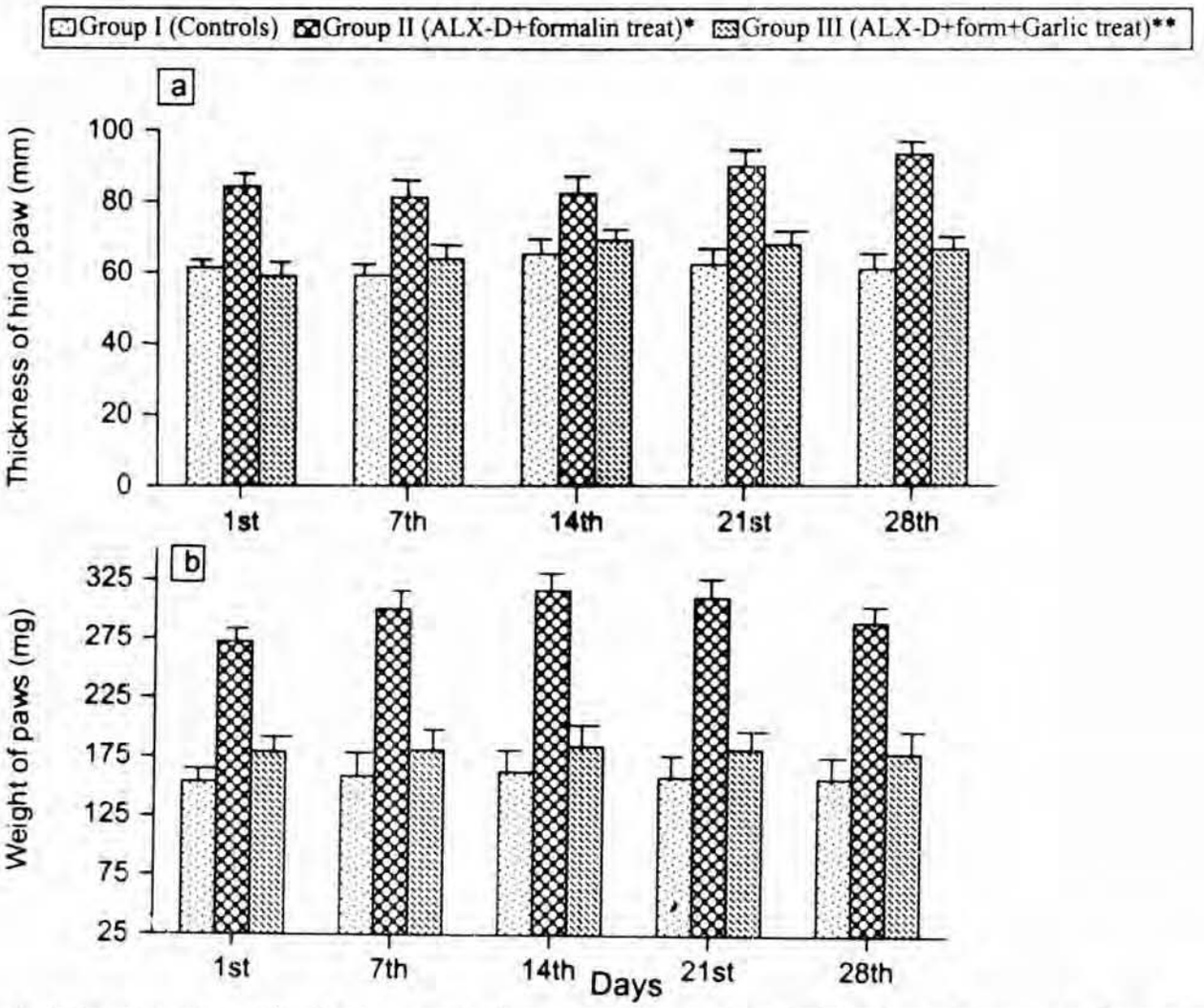

Fig. 4—Effect of (a) garlic and (b) ethanol extract of garlic on paw oedema (a=relative thickness, b=weight average) in alloxan induced hyperglycemic mice [$P<0.001$, between control and ALX-D(*) and ALX-D and ALX-D+garlic (**)]

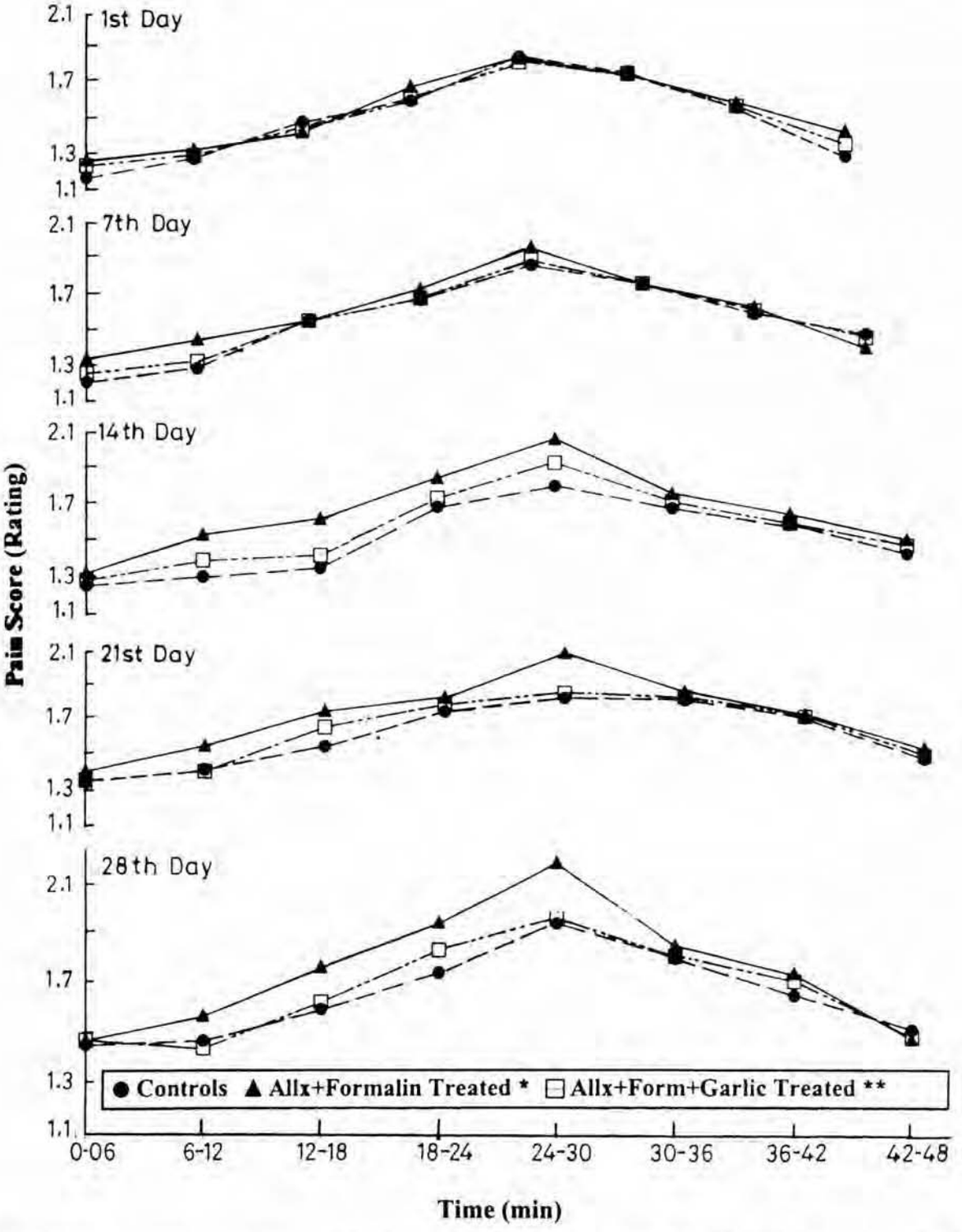

Fig. 5—Effect of ethanol extract of garlic on formalin test in alloxan induced hyperglycemic mice. [$P<0.001$, between control and ALX-D(*) and ALX-D and ALX-D+garlic (**)]

et al.[14] did not find any change in pain perception both in acute and chronic diabetes in rats. In the present study ALX-D group, showed a significant decrease in tail-flick (TFRT) and hotplate latency (HPLs) and allodynia responses. Administration of garlic in ALX-D group resulted in significantly more TFRT, HPLs and allodynia responses when compared to ALX-D group, suggesting reduction of hyperalgesic condition (Figs 1-3). Chu *et al.*[10] reported a higher level of pain threshold in STZ-D male rats in hotplate response. As the alloxan induced hyperalgesia is prevailing in the nociceptive tests, a decrease in pain threshold may be due to a decrease in central and peripheral endogenous opoid levels[13,21].

In ALX-D group the hind paw oedema was significantly increased when compared to controls. Garlic extract significantly ($P<0.001$) decreased thickness and weight of hind paw in ALX-D mice (Figs 4a & b). The results were significant between the groups and between the days ($P<0.001$). The decrease in weight and relative thickness may be due to its anti-inflammatory action through inhibition of inflammatory cell influx of thiosulfinates (allicins) and cepaenes (onion principles) present in it[3]. The above findings corroborate with the anti-inflammatory activity of garlic against carrageenin-induced hind paw oedema in albino rats[22]. In the present study, the formalin pain response increased during first 6 min and then decreased until 18 min; it again showed increased pain intensity between 18-24 min in ALX-D group on 14th, 21st, 28 days. The pain in the formalin model has frequently been reported to be biphasic in nature, indicating the accomplishment of hyperalgesia. The garlic treatment has shown a significant decrease in pain sensitivity in ALX-D group ($P<0.001$) of mice on 7th, 14th, 21st and 28th day of experimentation as shown in Fig. 5. The decrease in pain score in ALX-D mice may be due to its anti-inflammatory activity or also due to the action of modulated centrally acting endogenous opiodergic mechanisms[23].

It is possible that 2 week of hyperglycemia was not sufficient time for the full effects of diabetes on pain perception[11,20,24]. In the present study, long term treatments for one month have shown a significant increase in pain sensitivity to tail-flick, hotplate, allodynia and formalin - test, in ALX-D group indicating the development of hyperalgesia, which is similar to the observation on the diabetic mice as reported[14].

In conclusion, hyperglycemia and hyperalgesia were maintained in the diabetic state by repeated injections of alloxan monohydrate over 4 weeks. The simultaneous garlic treatment of ALX-D group resulted in recovered body weights, and decrease in hyperglycemia and hyperalgesia, which was predominant on the 14th, 21st and 28th days. Further detailed studies using different dosages, fractions of garlic extract and time duration are needed to confirm the potential nociceptive effect of garlic treatment.

References

1 Mathew P T & Augusti K T, *Indian J Biochem Biophys*, 10 (1973) 209.

2 Gupta N N, Mehrotra R M L & Sircar A R, *Indian J Med Res*, 54 (1966) 48.

3 Dorsch W, Schneider E, Bayer T, Brev W & Wagner H, *Int Arch Allergy Appl Immunol*, 92 (1) (1990) 39.

4 Laland P & Havrevold O W, *Z physiol chem*, 221 (1993) 180.

5 Augusti K T, *Indian J Exp Biol*, 34 (1996) 634.

6 Chang M L W & Johnson M A, *J Nutri*, 110 (1980) 931.

7 Sheela C G & Augusti K T, *Indian J Exp Biol*, 30 (1992) 523.

8 Jain R C, Vyas M D & Vyas C R, *Amer J Clin Nut*, 28 (1975) 684.

9 Foster D W, in *Harrison's principles of Internal medicine*, edited by E Braunald, K J Isselbacher, R G Peterdorf, J D Wilson, J B Martin & A S Fauci, (Mc Graw Hill, New Delhi) 1987, 1778.

10 Chu P C, Lin M T, Shian L R & Leu S Y, *Diabetes*, 35 (1986) 481.

11 Naga Rani M A, Chittaranjan A & Joy David, *Indian J Physiol Pharmacol*, 36 (2) (1992) 93.

12 Ginawi O T, *Arch Int Pharmacodyn Ther*, 318 (1992) 13.

13 Levine A S, Morley J E, Wilcox G, Brown D M & Handwerger B S, *Physiol Behaviour*, 28 (1982) 39.

14 Morley G K, Mooradian A D, Levine A S & Morley J E, *Am J Med*, 77 (1984) 79.

15 Itmar Ray, David Hasday, Zeev Seltzer & Raphael N M, *Diabetes*, 37 (1988) 1253.

16 Kurt D, Dubowski, *Clinical Chemistry*, 8 (3) (1962) 215.

17 Coderee T J, Abbott F V & Melzack R, *Pain*, 18 (1994) 13.

18 Hiura A, Villalobs E L & Ishizoka H, *Somatosens Res*, 9 (1992) 37.

19 Bennet J & Xie Y K, *Pain*, 33 (1988) 107.

20 Dubussion D & Dennis S G, *Pain*, 4 (1977) 161.

21 Forman L J, Estilow S, Lewis M & Vasilenko P, *Diabetes*, 35 (1986) 1309.

22 Bhakuni D S, Dhar M L, Dhar M M, Dhawan B N & Mehrotra B N, *Indian J Exp Biol*, 7 (1969) 250.

23 Shibata M, Ohkubo T, Takahashi H & Isnoki R, *Pain*, 38 (1989) 347.

24 Goodman W K & Charney D S, *J Clin Psychiat*, 46 (1985) 6.