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Analgesic, Antipyretic and Ulcerogenic Effects of Piperine: An Active Ingredient of Pepper

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Abstract

The aim of the present study was to evaluate the analgesic, antipyretic and ulcerogenic effects of piperine, which is an active ingredient of pepper. Mice were administered piperine (20 and 30 mg/kg) intraperitoneally; hot plate reaction test and acetic acid test were used to determine the analgesic activity of piperine inmice. Antipyretic and ulcerogenic effects of piperine were also evaluated. It was found that piperine exhibits significant analgesic and antipyretic activities without ulcerogenic effects. The results were comparable with indomethacin which was used as standard drug for reference. The results thus obtained prove the analgesic effects of piperine showing its possible use in this regard however further studies are required to study the mechanism of piperine to confirm these activities.

Keywords: Piperine; analgesic; antipyretic; ulcerogenic; pepper

INTRODUCTION

Long term use of non-steroidal anti-inflammatory drugs has various side effects including their ulcerogenic nature. Hence, use of various herbal remedies for their pain relieving and anti-inflammatory action is in current trend these days. *Piper nigrum* (black pepper) and *Piper longum* (long pepper) are known to possess various medicinal properties and are among highly consumed spices worldwide, so are commonly used in Ayurvedic medicine. It has been found that the alkaloid piperine (Fig.1) (trans-trans isomer of 1-piperoyl piperidine) is the main active principle of black pepper, constituting about 5% -9% of it. In India, approximate daily intake of piperine is estimated about 60–110 mM [1, 2]. It is found to be effective against bronchitis, chronic cold, congestion, hemorrhoids, chronic dyspepsia, anorexia, chronic asthma, burning heart, etc. Also, it is known to possess hepatoprotective, anti-tumour, anti-inflammatory and anti-arthritic properties [3-5]. Piperine is known to increase the bioavailability of certain herbal therapeutics [3]. Therefore the present study was conducted to assess the analgesic, antipyretic and ulcerogenic effects of piperine in mice.

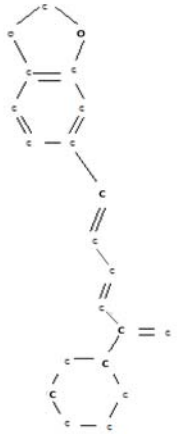

Fig. 1: Chemical structure of Piperine

MATERIALS AND METHODS

Drugs and Chemicals

Piperine (>98% purity by HPLC) was purchased from Natural Remedies Ltd., Bangalore, India and stored at −20°C and indomethacin was purchased from Tamil Nadu, Dadha Pharmaceuticals Ltd., Chennai, India. A homogenous suspension of piperine and indomethacin was made with 0.5% carboxy methyl cellulose in phosphate buffered saline prepared freshly before use. All other reagents used were standard laboratory reagents of analytical grade and were purchased locally. Dose of piperine and indomethacin was fixed according to previous studies [5].

Animals

The study was performed with cross bred swiss albino mice, of both sexes, weighing 25-30 g, obtained from animal house, VIT University, Vellore. Mice were fed with commercial pelleted food and were given water ad libidum. The mice were managed in accordance with the guidelines recommended by the committee for the purpose of control and supervision of experiments on animals, government of India, Ministry of culture, Tamil Nadu, India. Approval for the experimental procedure was taken by departmental ethical committee, Vellore institute of technology, Vellore (Tamil Nadu), India.

Analgesic Activity

Hot plate reaction test

This experiment was done according to the protocol of Williamson et al. [6] by paw lick or jump response. Mice were kept one by one in a beaker which was placed on a temperature-regulated hot plate retained at 50^0C and it was considered as pain threshold when animals lift and lick their paws or try to jump out of the beaker. The rate of the mice reverberation to the hot temperature was counted using stopwatch.

The animals used in the experiment were first tested for paw licks or jump response and those which reacted after 4

sec were used. Animals were tested after 30 minutes of administration of indomethacin (10mg/kg, orally) and piperine (20 and 30mg/kg, i.p.). Control animal were given equal volume of normal saline and the experiment was repeated and the results were noted.

Acetic acid test

Acetic acid is used to produce writhing response in mice using the method by Witkin et al. [7] and mice were treated with intra peritoneal injection of 0.6% solution of acetic acid by muscular contraction. Mice were kept in glass cages and number of stretching per mice was recorded for next half an hour. Piperine (20 and 30 mg/kg, i.p.) and indomethacin (10mg/kg, orally) were suspended in 0.09% saline solution and were administrated 30 minutes before acetic acid injection.

Antipyretic Test

The mice were fasted overnight but were provided with water ad libitum before the experiments. This test was performed in mice by subcutaneous treatment with 20% aqueous suspension of baker's yeast to initiate pyrexia. After 18 hours of the treatment, rectal temperature of the animals were recorded. Animals were administered with piperine intraperitoneally (20 and 30 mg/kg) and indomethacin (10mg/kg, orally) and rectal temperature was noted after every 1hr upto 22 hours of the experiment [8].

Ulcerogenic Test

Mice were kept in fasting for 16 hours and then piperine (20 and 30 mg/kg, i.p.) and indomethacin (10mg/kg, orally) were administered to them. The animals were decapitated after 6 hours of the last dose and the stomach was taken out of the body, opened along the great curvature and the severity of the ulcer index was estimated using discretionally scale.

0: no lesions, 0.5: hyperaemia, 1: one or two lesions, 2: severe lesions, 4: mucosa full of lesion [9].

Statistical Analysis

Results were expressed as mean ±SD of six animals and statistical analysis was performed using ANOVA to determine significant differences between groups followed by student's Newman-keul's test; *p<0.05 implied significance.

RESULTS

Pharmacological activities like analgesic, antipyretic, ulcerogenic activities of piperine were determined in mice.

Hot plate reaction test

In this test, mice which were administered with piperine showed significant (p<0.5) dose dependent delayed response time in pain threshold and the results were comparable to standard reference drug indomethacin (Fig.2). It shows the analgesic activities of piperine and indomethacin.

Acetic acid test

In this test, the maximum number of writhings after acetic acid administration were observed in control group of mice. After the treatment of animals with piperine (20 and 30 mg/kg) there was a significant inhibition in the abdominal writhes (Fig.3). Similar results were noted in case of treatment with Indomethacin (10 mg/kg).

Antipyretic test

A significant (p<0.5) increase in the control group of mice was observed in the rectal temperature due to yeast induced pyrexia in mice. There was a dose dependent decrease in rectal temperature in piperine (20 and 30 mg/kg) treated group of mice as compared to the control group. In case of indomethacin treated group of mice, the rectal temperature was more as compared to the piperine treated group (Fig.4).

Ulcerogenic test

The severity of the ulcer index was observed to evaluate the ulcerogenic effects of piperine. It was observed that there was a dose dependent decrease in the ulcer index in the mice treated with piperine (20 and 30 mg/kg). Moreover, indomethacin at a dose of 10 mg/kg produced significant ulcers as compared to the control group of mice (Fig.5).

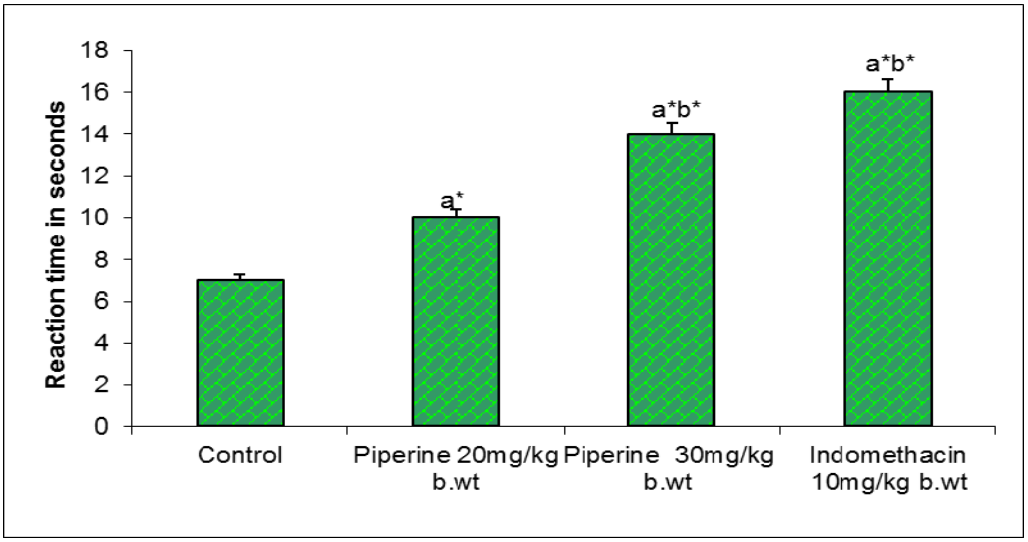

Fig. 2: Hot plate reaction test with or without prior administration of piperine in mice.

Results are compared with control groups. Values are expressed as mean ±S.D. (n=6). Comparisons were made as follows: a-group I vs groups II, III and IV and b-group II vs groups III and IV. Symbols represent statistical significance at * p<0.05.

Fig. 3: Acetic acid test with or without administration of piperine in mice.

Results are compared with control groups. Values are expressed as mean ±S.D. (n=6). Comparisons were made as follows: a-group I vs groups II, III and IV and b-group II vs groups III and IV. Symbols represent statistical significance at * p<0.05.

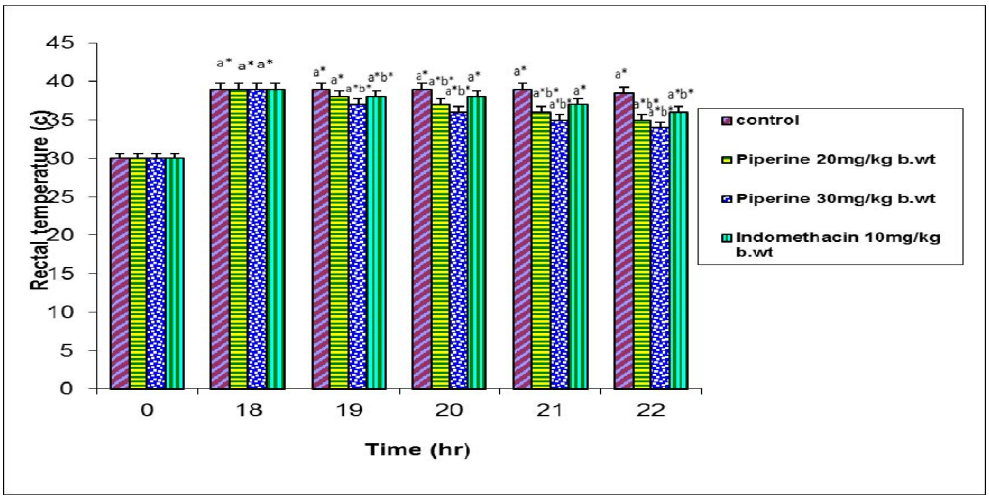

Fig.4: Antipyretic effects of piperine and indomethacin in mice.

Results are compared with control groups. Values are expressed as mean ±S.D. (n=6). Comparisons were made as follows: a-group I vs groups II, III and IV and b-group II vs groups III and IV. Symbols represent statistical significance at * p<0.05.

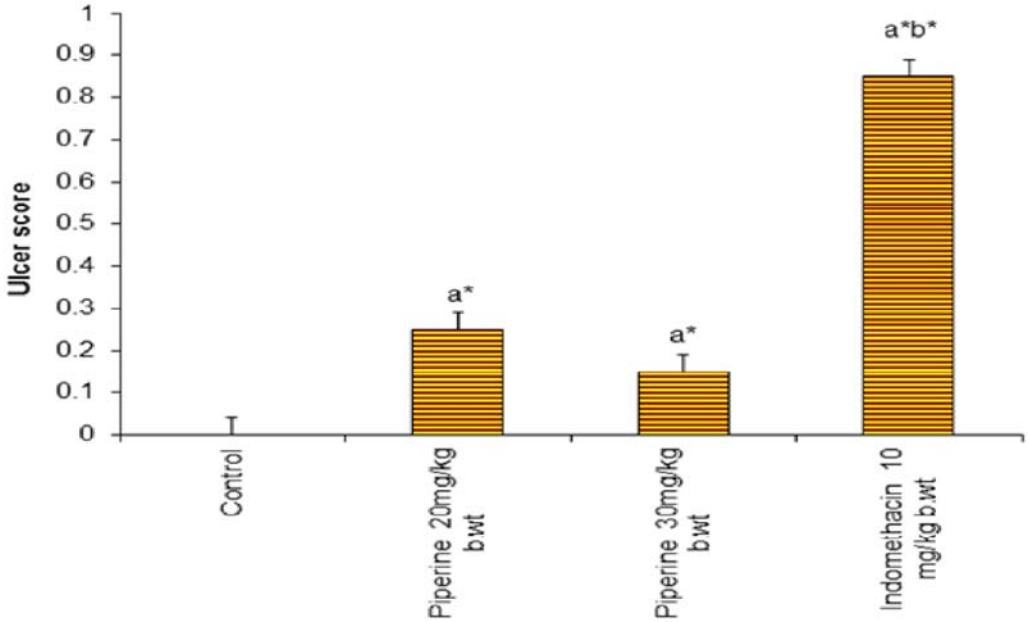

Fig. 5: Ulcerogenic action of piperine and indomethacin in mice.

Results are compared with control groups. Values are expressed as mean ±S.D. (n=6). Comparisons were made as follows: a-group I vs groups II, III and IV and b-group II vs groups III and IV. Symbols represent statistical significance at * p<0.05

DISCUSSION

Various drugs which are currently in use for treatment of anti-inflammatory disorders are always accompanied with several pyretic and ulcerogenic effects causing gastric damage. Piperine was the first amide isolated from piper species and has anti-inflammatory properties [10]. Piperine blocks the mixed function oxygenase system, inhibits P450 isoenzymes non-specifically [11] and restrains prostaglandin and leukotriene biosynthesis in vitro [12]. This study was conducted to assess the analgesic effect of piperine combined with evaluation of its antipyretic and ulcerogenic effects. Analgesic effect of piperine was determined using acetic acid writhing test and hot-plate method. The acetic acid writhing test is a non-discriminatory antinociceptive model. Intraperitoneal injection of acetic acid was given to mice, nerve endings were excited due to the painful response and acute inflammation, because of release of endogenous substances in the peritoneal area [13]. Aspirin and other non-steroidal anti-inflammatory drugs can reduce number of writhes in this model by blocking cyclooxygenase enzyme in peripheral tissues, obstructing the transduction in primary afferent nonciceptors. Thus the analgesic effect of piperine may be due to blockage of the local level of prostaglandins. However, the determination of this writhing test alone does not confirm that this effect is related with central analgesic substances. Furthermore, the hot plate test is extensively applied method in the analgesic investigations for several decades in determining the action of drugs though central nervous system. This test along with the writhing test, usually differentiates between central and peripheral effects [14]. A significant analgesic action was shown by piperine in hot plate method after 30 minutes administration. The results showed significant analgesic effect in acetic acid writhing response and hot plate reaction test by piperine. This confirms that analgesic effects of piperine are resultant of both peripheral and central acting mechanisms.

Fever is for natural nonspecific immune response against various ailments. Antipyretic are drugs, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature [15]. The anti-pyretic effect was investigated by yeast inducing pyrexia test in mice. In yeast induced fever or pathogenic fever, production of prostaglandins occur which set the thermoregulatory centre at a higher temperature and is regulated by hypothalamus. Antipyretic activity is mainly due to inhibiting effect on prostaglandin-formation [16, 17]. Administration of antipyretic compounds after 15 to 18 hours of yeast injection is a common method used by many researchers to investigate their pyretic effects. Mice were administered with piperine (20 and 30 mg/kg) and indomethacin (10mg/kg) after yeast injection. The results obtained using piperine showed a significant ($p<0.05$) reduction in rectal pyrexia, similar to standard drug indomethacin (Fig.4). Thus it can be concluded that piperine possesses significant antipyretic effect in yeast-induced elevation of body temperature in mice and this can also be due to its anti-inflammatory effects.

Since it is well known that anti-inflammatory agents that exhibit their activity through the inhibition of prostaglandin biosynthesis may induce gastric ulceration. Appearance of gastric lesions and thus ulcers is a common side effect associated with nonsteroidal anti-inflammatory compounds [18]. Indomethacin is observed to possess ulcerogenic effects as proved by previous studies [19]. In this study, after fasting appearance of ulcers was observed in control group of rats but when mice were administered with piperine there was significant ($p<0.05$) dose dependent reduction in the ulcer index as compared to the control group of mice (Fig. 5). Moreover, gastric lesions were seen in indomethacin treated mice.

CONCLUSION

Various analgesic i.e. pain relieving therapeutics are available in market but they have several side effects so there is a need to evaluate the potential of natural compounds in this regard. The result of the study shows piperine has antipyretic, analgesic and anti ulcerogenic properties, however further studies are required to elucidate the mechanism of piperine to confirm these activities. Hence, our research contributes towards traditional use of piperine with scientific support.

Conflict of Interest Statement

There is no conflict of interest between the authors.

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