Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 1(2): 54-59, 2007
ISSN 1819-3455

Analgesic, Antipyretic and Ulcerogenic Effects of Indian Ayurvedic Herbal Formulation Triphala

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Abstract: An Indian ayurvedic herbal formulation, Triphala (500/1000 kg^{-1} $b.wt^{-1}$) was assessed for analgesic, antipyretic and ulcerogenic activities on the experimental models in mice. For comparison purpose, non-steroidal anti-inflammatory drug Indomethacin (10 mg kg^{-1} $b.wt^{-1}$) was used as a standard. It was found that Triphala at both the dose levels produced excellent analgesic and antipyretic effect, with the absence of gastric damage. The results obtained clearly show that Triphala possesses potent analgesic, antipyretic and gastroprotective effect.

Key words: Triphala, analgesic, antipyretic, gastric damage, indomethacin

INTRODUCTION

Triphala, an ancient herbal blend, is one of the most commonly used herbal remedies in Ayurveda, an Indian system of medicine. Triphala, meaning three fruits, is made from the fruits of three trees, Indian goose berry (*Emblica officinalis* Gaertn, family-Euphobiaceae), Belleric myrobalan (*Terminalia belerica* Linn, family-Combretaceae) and Chebulic myrobalan (*Terminalia chebula* Retzr, family-Combretaceae). Triphala has been reported to be a rich source of Vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, β-sitosterol and flavanoids (Jagetia *et al*., 2002). Its components *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula* are reported to possess anti-inflammatory, antimutagenic, antioxidant, cytoprotective, gastroprotective activity, myocardial necrosis, hepatoprotective, antibacterial and anticancer activity (Mukherjee *et al*., 2006). Preliminary studies confirm its anti-inflammatory and lysosomal membrane stabilizing effect on adjuvant-induced arthritis in mice (Rasool and Sabina, 2007). Therefore, the present study was undertaken to identify the analgesic, antipyretic and ulcerogenic properties of Triphala at different doses (500/1000 mg kg^{-1} $b.wt^{-1}$) in standard models in mice. The standard non-steroidal anti-inflammatory drug, indomethacin (10 mg kg^{-1} $b.wt^{-1}$), was used as a reference drug for purposes of comparison.

MATERIALS AND METHODS

Animals

The study was performed with Swiss albino mice, 25-30 g, of either sex. The mice were brought from Tamil Nadu Veterinary College, Chennai, India. The mice were acclimatized for a week in a light and temperature-controlled room with a 12 h dark-light cycle. The mice were fed commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water was made freely available.

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Drug

The commercially available Triphala powder (mixture of dried and powdered fruits of three plants, *T. chebula*, *E. officinalis* and *T. bellerica* in equal proportions (1:1:1)) was obtained from Indian Medical Practitioners Co-operative Stores and Society (IMCOPS), Adyar, Chennai, India and its aqueous suspension in 2% gum acacia was used at a dose 1 g kg^{-1} b.wt^{-1} orally. The Triphala powder used in this study was found to contain approximately 50% polyphenols as investigated by High Performance Thin Layer Chromatography (HPTLC) densitometer analysis. Indomethacin (Tamilnadu Dadha Pharmaceuticals, Chennai, India) was dissolved in 2% gum acacia solution administered orally (Rasool and Sabina, 2007). All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

Analgesic Test

Acetic Acid-induced Writhing Response in Mice

This test was conducted using the method described by Witkin *et al*. (1961). The muscular contractions were induced in mice by intra peritoneal injection of 0.6% solution of acetic acid (10 mL kg^{-1} b.wt.). Immediately after administration of acetic acid, the animals were placed in glass cages and the number of stretchings per animal was recorded during the following 30 min. A significant reduction in the number of writhings by any treatment as compared to control animals was considered a positive analgesic response. Triphala (500/1000 mg kg^{-1} b.wt.) and Indomethacin (10 mg kg^{-1} b.wt.) suspended in 2% gum acacia solution were administered 30 min before the acetic acid injection.

Hot-Plate Reaction Time in Mice

The method of Williamson *et al*. (1996) was used. Mice were placed individually in a 2 l glass beaker placed on a thermostatically controlled hot plate maintained at 55°C. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the beaker. The time taken for the mice to react in this fashion was obtained using a stopwatch. The animals were first tested for the paw-lick or jump response and only those that reacted after 4 sec were used for the experiment. Mice were tested in groups of six per dose 30 min after oral administration of Triphala (500 mg/1000 mg kg^{-1} b.wt^{-1}) or Indomethacin (10 mg kg^{-1} b.wt^{-1}). Control animals received equal volume of normal saline and the experiment was repeated.

Antipyretic Test

The mice were injected subcutaneously with 10 mL kg^{-1} of 20% aqueous suspension of bakers yeast and the rectal temperatures were recorded initially and at 18 h. Triphala (500 and 1000 mg kg^{-1} body weight) and Indomethacin (10 mg kg^{-1} body weight) were administered orally after the 18 h reading. When the increase of temperature was at its peak, it was measured at hourly intervals up to 5 h after administration of drugs (Mukerjee, 1996).

Ulcerogenic Test

Animals of six groups of six mice in each were kept fasting for 16h and the test compounds were then administered orally. Triphala was administered at dose levels of 1000/2000 mg kg^{-1} body weight and Indomethacin at a dose of 20 mg kg^{-1} body weight. Animals were killed 3 h after the administration of the drugs and the stomachs were removed, cut along the lesser curvature and the gastric mucosa was washed with normal saline and scored according to the scale, 0: no lesion, 0.5: hyperaemia, 1: one or two lesions, 2: severe lesions, 3: very severe lesions, 4: mucosa full of lesions (Cashin *et al*., 1977).

Statistical Analysis

Results were expressed as mean±SD 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

Analgesic Test

In the acetic acid induced writhing method, Triphala treatment similar to Indomethacin, produced a significant reduction in the number of abdominal constrictions in mice. This reduction was dose related and was maximum with 1000 mg kg^{-1} (Fig. 1). In the hot plate method, as shown in Fig. 2, the Triphala treatment increased the reaction time and showed significant analgesic activity at both the doses (500/1000 mg kg^{-1} $b.wt^{-1}$). The analgesic effect shown by Triphala was slightly lesser than that of Indomethacin (10 mg kg^{-1} $b.wt^{-1}$).

Antipyretic Test

Administration of brewer's yeast to rats produced a significant increase in rectal temperature 18 h after yeast injection ($p<0.05$) (Fig. 3). Triphala at the dose of 1000 mg kg^{-1} caused a significant decrease in rectal temperature.

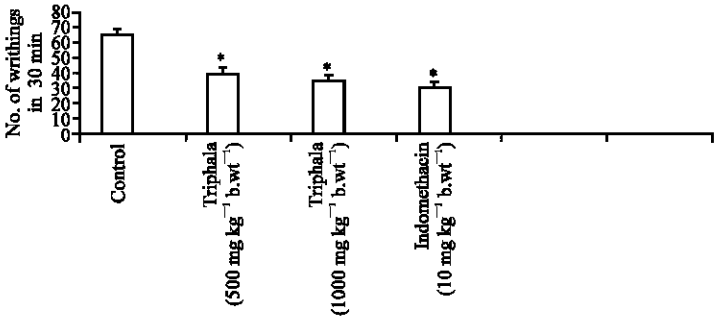

Fig. 1: Effect of Triphala and Indomethacin on acetic acid-induced writhing response in mice. Comparisons are made with control groups. The results are given are mean±SD; number of animals used (n = 6). The symbols represent statistical significance at: *$p<0.05$

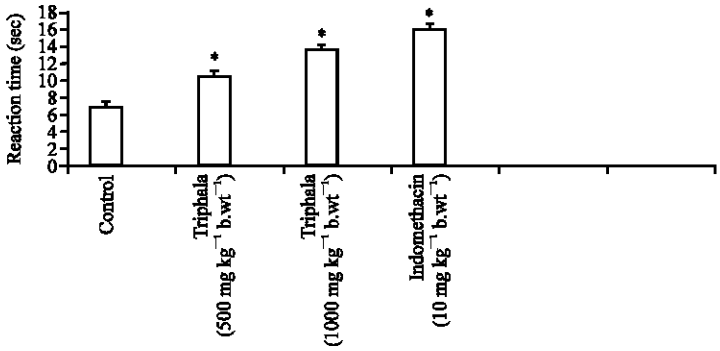

Fig. 2: Effect of Triphala and indomethacin on hot place reaction time in mice. Comparisons are made with control groups. The results are given are mean±SD; number of animals used (n = 6) The symbols represent statistical significance at: *$p<0.05$

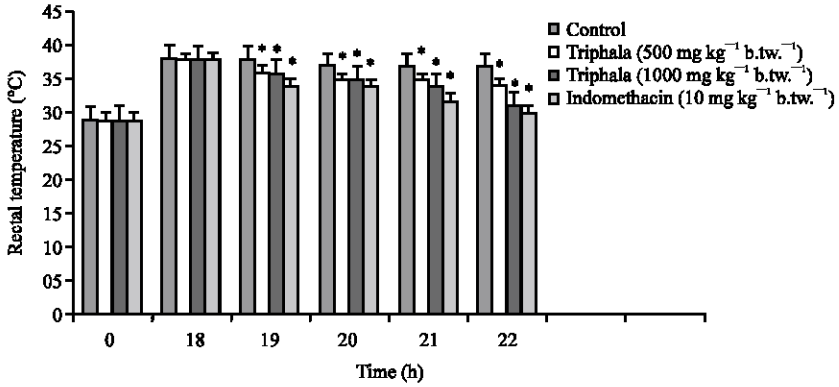

Fig. 3: Effect of Triphala and Indomethacin on yeast-induced pyrexia in mice. Each point and vertical bar represent the mean and SD (n = 6). Comparisons are made with control groups, the symbols represent statistical significance at: *p<0.05

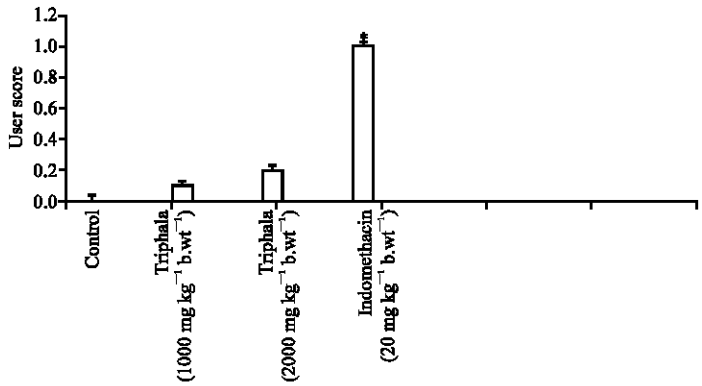

Fig. 4: Ulcerogenic action of Triphala and Indomethacin in mice. Comparisons are made with control groups. The results are given are mean±SD; No. of animals used (n = 6). The symbol represents statistical significance at: *p<0.05

Ulcerogenic Effect

The mice administered with Triphala at both doses (1000/2000 mg kg^{-1} $b.wt^{-1}$) were found to be devoid of gastric lesions (Fig. 4), whereas standard anti-inflammatory agent Indomethacin (20 mg kg^{-1} $b.wt^{-1}$) administration produced gastric lesions associated with pinpoint haemorrhagic spots and few ulcers in the stomach.

DISCUSSION

Our previous preliminary studies confirm that Triphala possesses anti-inflammatory and lysosomal membrane stabilizing effect on adjuvant-induced arthritis in mice (Rasool and Sabina, 2007). Anti-inflammatory drugs, presently available for the treatment of various inflammatory disorders, usually found to show analgesic, antipyretic effect associated with gastric damage. Therefore, an attempt was made to search whether Triphala exhibit analgesic and antipyretic activities with the absence of gastric damage.

Increased body temperature and pain are known as the main symptoms of the body against an inflammatory stimulation. Therefore, it is generally essential to possess analgesic and antipyretic activities for an anti-inflammatory compound (Kasahara *et al*., 1985). The writhing response of the mouse to an intraperitoneal injection of noxious chemical is used to screen for both peripherally and centrally acting analgesic activity. Acetic acid causes analgesic by liberating endogenous substances and many others that excite pain nerve endings. In order to evaluate the analgesic activity, Triphala were studied against acetic acid induced writhings in mice. Triphala showed analgesic activity at both the dose levels (500/1000 mg kg^{-1} b.wt) without inducing any gastric toxicity (Fig. 1). Induction of the acetic acid writhing in mice is an effect of the acute inflammatory reaction related to the increase in levels of prostaglandins E2 and $F2_{\alpha}$ in the peritoneal fluid (Deraedt *et al*., 1976). Collier *et al*. (1968) postulated that acetic acid acts indirectly by inducing the release of endogenous mediators of pain sensitive to non-steroidal anti-inflammatory drugs and opioids. In our study, the mechanism of analgesic action of the Triphala could probably be due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similarly to non-steroidal anti-inflammatory drugs.

The hot plate is a specific central antinociceptive test in which opioid agents exert their analgesic effects via supra spinal and spinal receptors (Nemirovsky *et al*., 2001). In the hot plate test, Triphala (500/1000 kg^{-1} b.wt) showed a significant analgesic action 30 min after its administration. From the results it is evident that the Triphala showed a significant analgesic effect in both hot plate test and acetic acid induced writhing response. Analgesic effect of Triphala in both models confirms that it has been acting through both peripheral and central mechanism.

Antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis (Vane, 1987). The yeast-induced pyrexia in mice was employed to investigate the antipyretic activity of Triphala. Using this method, several investigators recorded pyrexia 18 or 15 h, after yeast injection and then they administrated the antipyretic drugs to be studied (Asha and Pushpangadan, 1999). It was found that Triphala caused a significant reduction in rectal pyrexia similar to Indomethacin. This result seems to support the view that the Triphala has same sequence on prostaglandin biosynthesis because prostaglandin is believed to be regulatory of body temperature.

The main side effect of non-steroidal anti-inflammatory drugs is their ability to produce gastric lesions (Pagella *et al*., 1983). Indomethacin is an established ulcerogen especially in an empty stomach, the evidence of indomethacin-induced ulceration is mostly on the glandular (mucosal) part of the stomach (Nwafor *et al*., 1996). The ulcerogenic activity of non-steroidal anti-inflammatory drugs is due to inhibition of cyclo-oxygenase enzyme responsible for the production of prostaglandins involved in general house keeping activities, e.g., maintenance of gastric mucosal integrity (Rasool and Varalakshmi, 2006). In this study, Triphala did not induce any adverse effect on gastric mucosa, indicating non ulcerogenic activity; whereas Indomethacin treated mice produced small erosions.

In conclusion, the present study clearly showed that Triphala possessed good analgesic, antipyretic activity with absence of gastric damage and also scientifically validated the use of this Indian ayurvedic herbal formulation for treating inflammatory disorders in the folk medicine.

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