



Research Article

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EVALUATION OF ACUTE TOXICITY AND ANALGESIC ACTIVITY OF PLUGIT CAPSULE: AN AYURVEDIC FORMULATION

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ABSTRACT

Noiceptive pain arises from the stimulation of specific pain receptors. These receptors respond to heat, cold, vibration, stretch and chemical stimuli released from damaged cells. A use of non-steroidal anti-inflammatory drugs (NSAIDs) is routine in the management of pain. Although effective at reducing pain and inflammation, long term use of NSAIDs is associated with known side effects. Herbal and Ayurvedic products are widely perceived as safe due to their natural origin and long historical clinical use. Hence in the present study, an attempt was made to evaluate acute toxicity and analgesic activity of an Ayurvedic proprietary formulation "Plugit Capsule" on experimental animals. Acute toxicity study was carried out as per OECD guideline 423. Analgesic activity was evaluated by hot plate and tail flick method. Selected animals were divided in to four groups in both experimental models. Test drug was evaluated for two different dose levels and administered orally at 260 mg/kg (TED-I) and 520 mg/kg (TED-II) in Group 2 and 3 respectively. Diclofenac sodium was used as reference standard. The animals did not manifest any signs of toxicity and mortality at both dose level 2000 mg/kg and 5000 mg/kg. Plugit Capsule at TED-II showed more significant analgesic effect in both hot plate and tail flick nociceptive tests with respect to TED-I. On the basis of study data it can be concluded that Plugit Capsule produces central analgesic effect without any side effect hence can be employed as a safe and effective alternative to NSAIDs for the treatment of painful conditions.

Keywords: Ayurvedic formulation, Plugit Capsule, acute toxicity, analgesic activity, hot plate, tail flick

INTRODUCTION

Noiceptive pain is the is the afferent activity in the peripheral and central nervous systems produced by stimulation of specialized free nerve endings called "nociceptors" or "pain receptors".¹ The causes of such pain is varied in nature. It includes trauma to an area (jerking movements, auto accidents, falls, fractures, sprains, dislocations, and direct blows to the muscle), postural strain, repetitive movements and prolonged immobilization and disease condition like rheumatoid arthritis.² A use of non-steroidal anti-inflammatory drugs (NSAIDs) is routine in the management of pain. Although effective at reducing pain and inflammation, long term use of NSAIDs is associated with known side effects like gastric ulcer, intestinal bleeding, an increased risk of myocardial infarctions, hypertension, heart failure, liver damage, blood dyscrasias, rashes and vision impairment.^{3,4} Natural products have long been recognized as an important source of therapeutically effective medicine. Different approaches used to analyze the analgesic and anti-inflammatory potential of plant and plant derived compounds in past years.⁵ Selected test drug for this study, Plugit Capsule is a proprietary Ayurvedic formulation which contains extract of Maharasnadi kwath^{5,6}, Pathyadi kwath⁷, *Zingiber officinale* (Shunthi) rhizome^{8,9}, *Ocimum sanctum* (Tulsi) aerial^{10,11}, *Vitex*

negundo (Nirgundi) leaves^{12,13}, *Commiphora mukul* (Guggulu) gum resin^{14,15} and powder of Narsinh churna¹⁶, Shulgajkesari gutti¹⁷, Godanti bhasma¹⁸, Shankh bhasma¹⁹ and Swarnamakshik bhasma²⁰. It is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. Majority of ingredients of Plugit Capsule are well reported in Ayurvedic texts and scientific research publications for analgesic activity. However, no such evidences were available which proves safety and efficacy of their combination. Hence in the present study, an attempt was made to evaluate acute toxicity and analgesic activity of Plugit Capsule on experimental animals.

MATERIALS AND METHODS

Test drug and experimental dose

Test drug (Plugit capsules) were emptied to receive powder which was then mixed with distilled water to make suspension. For acute toxicity study 2000 mg/kg and 5000 mg/kg single dose was administered orally. For analgesic activity, dose of the test drug was fixed by extrapolating the human therapeutic dose to laboratory animals based on body surface area ratio as per the table of Paget and Barnes.²¹ It was administered at two different dose level 260 mg/kg (Therapeutic Effective Dose: TED-I) and 520 mg/kg (Double of Therapeutic

Effective Dose: TED-II), orally. Standard drug, diclofenac sodium was dissolved in distilled water and administered at 25 mg/kg, orally.

Experimental animals

The experiment protocol described in present study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: KB/11/241) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.: 238/CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy adult wistar rats weighing 180-230 g were used for acute toxicity study and analgesic activity. Rats were housed in polypropylene cages, maintained under standardized condition (12-hour light/dark cycle, 24°C, 35 to 60 % humidity) and provided free access to 'Sabardan' pellet diet and purified drinking water *ad libitum*. The animals were deprived of food for 24 hour before experimentation but allowed free access to water throughout.

Acute toxicity study

Healthy Wistar albino rats (180 - 230 g) were divided into 2 groups of 3 animals each. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of test drug. The suspension of Plugit Capsule powder was administered orally by gavage at single dose of 2000 mg/kg to 1st group and single dose of 5000 mg/kg to 2nd group. The general behavior and mortality of the rats were continuously monitored for 1 h after dosing periodically during first 24 h (with special attention given during the first 4 h.) and then daily for total 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity and mortality were monitored and recorded. Acute toxicity study was carried out as per OECD Guidelines 423.²²

Experimental models for assessing analgesic activity

Hot plate and tail flick experimental models were used to evaluate analgesic. Selected animals were divided in to four groups where each group consisted of six animals.
Group 1: Normal control (NC) - Received distilled water
Group 2: Therapeutic Effective Dose (TED-I) - Rats treated with Plugit Capsule at 260 mg/kg, orally
Group 3: Double of Therapeutic Effective Dose (TED-II) - Rats treated with Plugit Capsule at 520 mg/kg, orally
Group 4: Standard drug (SD) - Rats treated with Diclofenac sodium 25 mg/kg, orally

Hot plate test

In this experiment, the central analgesic activity of Plugit Capsule was assessed in male wistar rats, as per the method described by Eddy and Leimbach.²³⁻²⁵ Overnight fasted animals were placed individually on a thermostatically controlled heated metal plate and the reaction time of each rat was recorded. The temperature of the hot plate was maintained at $55 \pm 0.5^\circ\text{C}$. The reaction time was considered as the time elapsed between placing of the rat on the hot plate and appearance of signs of acute discomfort, characterized by flicking or licking of the hind paw, forepaw or jumping in an attempt to

escape from the pain. The rats showing initial reaction time of 10 sec or less were selected for this study. The reaction time of each rat was recorded at interval of 30 minutes time for 4 hours with a cut-off time 30 seconds. Test drug was administered orally at 260 mg/kg and 520 mg/kg in Group 2 and 3 respectively. Standard drug - Diclofenac sodium was administered orally in Group 4 at 25 mg/kg. The increase in reaction time in drug-treated groups was compared with that of the normal control group.

Tail flick method

The central analgesic activity of Plugit Capsule was studied in tail withdrawal method, as described by D'Amour and Smith.²⁵⁻²⁷ Radiant heat was applied to the base of the tail using a tail flick unit and the latency time for removal of the tail from the stimulus was recorded. The intensity of the heat stimulus was set to elicit a tail flick within 10-12 sec. A cut-off time of 20 sec was used to prevent tissue damage. After recording the baseline latency (at 0 h), test drug was administered orally at 260 mg/kg and 520 mg/kg in Group 2 and 3 respectively; standard drug - Diclofenac sodium was administered orally in Group 4 at 25 mg/kg. The tail withdrawal latencies were measured at interval of 30 minutes time for 4 hours.

Statistical analysis

Results were presented as Mean \pm SEM (n = 6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test.

RESULTS

Acute toxicity study

The animals did not manifest any signs of toxicity during cage side observation and mortality at both dose level 2000 mg/kg and 5000 mg/kg. (Table 1 and 2)

Analgesic activity

For evaluation of analgesic activity, two methods were used i.e. hot plate method and tail flick method. In hot plate method, TED-II group showed more significant effect then TED-I at different time interval in comparison to normal control group. TED-II showed significant analgesic activity starting from 60 minutes to 180 minutes after drug administration (Figure 1). In the tail flick method, TED-II showed similar type of prominent significant results in comparison to TED-I (Figure 2).

DISCUSSION

In the present study, analgesic activity of Plugit Capsule was evaluated on different experimental models. Diclofenac sodium, a NSAID formulation was selected as standard drug which is commonly prescribed to overcome painful conditions.²⁸ Efficacy of test drug was evaluated at two different dose levels. To assess the central mechanism of the compound in producing analgesia, hot plate and tail-flick tests were employed. These methods differ from each other in their tendency to respond to nociceptive stimuli conducted through neuronal pathways.

Table 1: Cage side observations of animals during acute toxicity study of Plugit Capsule

Parameters	Observations	
	2000 mg/kg	5000 mg/kg
Condition of fur	Normal	Normal
Skin	Normal	Normal
Subcutaneous swelling	Nil	Nil
Eyes dullness	Nil	Nil
Eyes opacities	Nil	Nil
Color and consistency of faeces	Normal	Normal
Abdominal distention	Nil	Nil
Breathing abnormalities	Nil	Nil

Table 2: Mortality record during acute toxicity study of Plugit Capsule

Group	Dose (mg/kg body wt)	Mortality
I	2000	0/3
II	5000	0/3

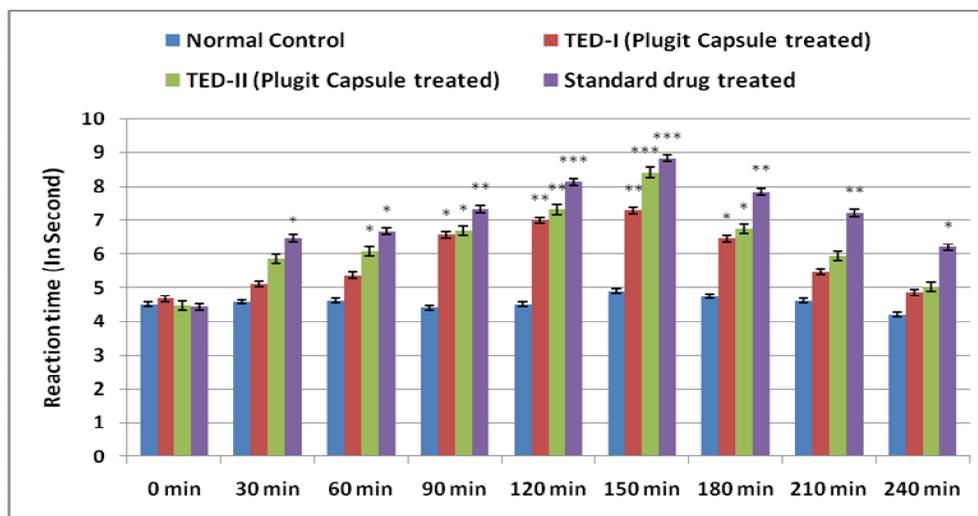


Figure 1: Analgesic activity of Plugit Capsule by hot plate method

All the values are expressed as mean ± SEM (n = 6) in each group. *p < 0.05, **p < 0.01 and ***p < 0.001 when compared to normal control group

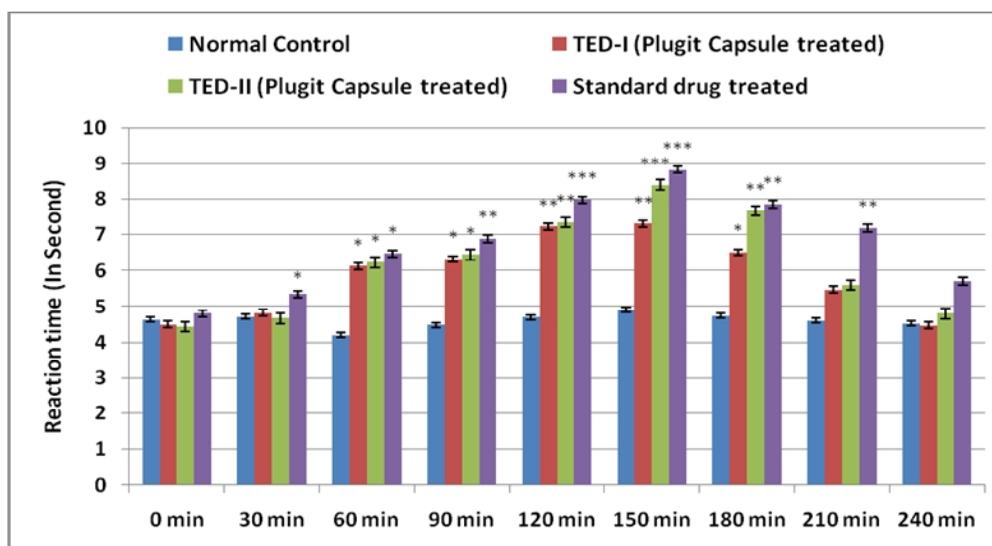


Figure 2: Analgesic activity of Plugit Capsule by tail flick method

All the values are expressed as mean ± SEM (n = 6) in each group. *p < 0.05, **p < 0.01 and ***p < 0.001 when compared to normal control group

Tail flick mediates spinal reflex to a painful stimuli, whereas Hot plate test involves higher brain functions and is considered to be a supra-spinally organized response.²⁹ Plugit Capsule at TED-II showed more significant analgesic effect in both hot plate and tail flick nociceptive tests with respect to TED-I. The results suggest that Plugit Capsule produced significant central analgesic effect, as evidenced by the prolonged delay in response when rats were subjected to a nociceptive stimulus in the tail flick test and hot plate test (Figure 1 and 2).

CONCLUSION

A use of non-steroidal anti-inflammatory drugs (NSAIDs) are routine in the management of pain, but long term uses of them are associated with known side effects. Hence, Plugit capsule can be a safe and effective alternative to NSAIDs for the treatment of painful conditions. On the basis of data it can be concluded that Plugit capsule possesses central analgesic effect without any side effect.

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