Annals of Phytomedicine 4(1): 90-93, 2015

Journal homepage: www.ukaazpublications.com

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN : 2393-9885

Analgesic and anti-inflammatory potential of three new topical polyherbal formulations in wistar rats

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Received April 3, 2015: Revised May 10, 2015: Accepted May 15, 2015: Published online June 30, 2015

Abstract

The aim of this study was to evaluate the possible analgesic and anti-inflammatory effects of three new topical formulations; DRDC/AY/8037, DRDC/AY/8039 and DRDC/AY/8040. Activity was carried out on adult male wistar rats of 200-250 g each. Animals were divided into different groups, each group was indicated by the formulation used (DRDC/AY/8037, DRDC/AY/8039 and DRDC/AY/8040), control group for each study and two standards (diclofenac based and herbal based). Further, for both analgesic and anti-inflammatory activity, 300 mg of different formulations and standard were applied to the dorsal surface of the left hind paw by gently rubbing 50 times with the index finger to respective groups. Rats of the control group were applied with vehicle base. For evaluation of analgesic activity, twenty microliter of 5% formalin was injected subcutaneously into the hind paw of animals fifteen minutes after application of formulations. Five minutes later the time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. For evaluation of anti-inflammatory activity, subplantar injection of 100 µL of 1% freshly prepared solution of carrageenan was given for inducing paw edema, 30 min. after application of formulations. Percentage inhibition of paw edema (early and late phase) was recorded with reference to control and was regarded as a measure of anti-inflammatory activity. Results showed that formulations; DRDC/AY/8037, DRDC/AY/8039 and DRDC/AY/8040 showed 38.6%, 64.8% and 55.6% reduction in the pain response compared to control group as measured through reduction in paw edema. Results of analgesic activity of test formulations were comparable to the herbal based standards and better than diclofenac base marketed formulation. Results of anti-inflammatory activity showed that formulations DRDC/AY/8037, DRDC/AY/8039 and DRDC/AY/8040 showed 16.63%, 20.42% and 9.63% early phase paw edema inhibition and 38.35%, 45.54% and 45.85% late phase paw edema inhibition. These results supported analgesic and anti-inflammatory effects of test formulations and their use in local management of pain and inflammation.

Key words: Analgesic, anti-inflammatory, formalin test, paw edema, wistar rats

1. Introduction

Inflamamtion is one of the primary physiologic mechanism that protects body against infection, burn, chemical toxicity and other stimuli. Persistent inflammation is also associated with many other chronic illness (Kumar *et al*., 2004). Currently available analgesic and anti-inflammatory agents include conrticosteroids and non steroidal anti-inflammatory drugs. All these therapies are however, are not free from adverse effects (Ahamed *et al*., 2005).

The use of plant products as medicinal agents has been going on since ages as well as currently increasing in many segments of the population. According to an estimate, 80% of the world's population relies upon herbal medicinal agents for one or many diseases. Most of the presently used synthetic analgesic and antinociceptive drugs cause many side effects. Herbal agents still represent a large untapped source of structurally novel molecules that might serving as lead for the development of novel drugs (Ahmad *et al*., 1992).

Many analgesic and anti-inflammatory medicines of plant origin have been used without any adverse effects for centuries. However, many of these traditionally used plants do not have scientific data on their efficacy. The present study was carried out to evaluate different polyherbal formulations; DRDC/AY/8037, 8039 and 8040 developed by Dabur India Ltd. for its potential analgesic and anti-inflammatory activity.

2. Materials and Methods

2.1 Preparation of test formulations

2.1.1 *Ingredients*

The three test formulations contained methyl salicylate, camphor, eucalypus oil, menthol, caps. oleoresin, salai guggulu, mint oil, til oil. Formulations; 8037 and 8039 were prepared, using ointment base whereas formulation 8040 was prepared using gel base.

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2.1.2 *Method of preparation*

Ointment

For the preparation of ointment, all the active ingredients mentioned above were mixed and dissolved under stirrer. The ingredients were slightly warmed to aid dissolution. All the excipients were separately heated to melt and dissolved under stirring. Followed by this, the active ingredients were slowly mixed with excipients under slow stirring and allowed to cool at room temperature.

Gel

For the preparation of gel, all the active ingredients mentioned above were mixed and dissolved under stirrer. The ingredients were slightly warmed to aid dissolution. All the excipients and preservatives were dissolved in water as per formula. To this, carbomer was slowly added and uniformly dispersed under stirring. Followed by this, the active ingredients were slowly mixed with excipients under stirring and neutralized with a base to form gel structure.

2.2 Pharmacological activity

2.2.1 *Animals*

Wistar rats weighing 200-250 g were used for the study, procured from the Central Animal House Facility, Hamdard University, New Delhi, India. The animals were maintained under standard laboratory conditions of temperature (25° ± 2°C) and relative humidity (55 ± 5 % Rh) with a 12 h light/dark cycle. The animals were housed in propylene cages (four per cage) with free access to food (Lipton feed, Mumbai, India) and water *ad libitum*. The study protocol was approved by Jamia Hamdard, Institutional Animal Ethics Committee, New Delhi.

2.3 Analgesic activity

The method adopted is as described by Shibata *et al*. (1989). Rats were divided into different groups (n=5). In different treated groups, three hundred mg of different formulations were applied to the dorsal surface of the right hind paw by gently rubbing 50 times with the index finger to other treatment groups. Group 1 served as control and was treated with vehicle base, Groups 2 to 4 were treated with test formulations; 8037, 8039 and 8040, respectively. Two marketed topical analgesic and anti-inflammatory formulations; one diclofenac based and one herbal based were applied to the group 5 and 6 and were designated as standard 1 and standard 2, respectively. After 30 minutes, twenty microliter of 5% formalin was injected subcutaneously into the planta aponeurosis of the right hind paw. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection.

2.4 Anti-inflammatory activity

Anti-inflammatory activity was measured, using carrageenan-induced rat paw edema method (Ambedkar *et al*., 2012). Animals were divided into four groups and intial paw volume of all the animals were measured, using plethysmometer. In different treated groups, three hundred mg of different formulations were applied to the dorsal surface of the left hind paw by gently rubbing 50 times with the index finger to other treatment groups. Group 1 served as control and was treated with vehicle base. Groups 2 to 4 were treated with test formulations; 8037, 8039, 8040, respectively. Groups 5 an 6 were treated with standard 1 and standard 2, respectively. Edema was induced 30 min. after administration of test and standard drugs by subplantar injection of 100 µL of 1% freshly prepared solution of carrageenan in distilled water into the left hind paws of each rat of all the groups. All the animals were injected with 0.1ml of prepared carrageenan solution and change in paw volume was measured as initial response, early phase response (1 h.) and late phase response (4 h). Percentage inhibition of paw edema was calculated as follows:

% inhibition of paw edema = [(Cf-Ci)-(Tf-Ti)]*100/[Cf-Ci]

where, at a particular time, Cf = final paw volume of control group; Ci = initial paw volume of control group; (Cf-Ci) = change in paw volume of control group; Tf = final paw volume of test group; Ti = initial paw volume of test group; (Tf-Ti) = change in paw volume of test group.

2.5 Statistical analysis

The values were expressed as Mean ± SD. The data was analyzed using one-way ANOVA followed by Tukeys test, values with $p<0.01$ was considered significant.

3. Results and Discussion

Results of the formalin test (paw licking activitiy) is shown in Figure 1. All the formulations = reduced paw licking activity significantly ($p<0.001$ vs control). The reduction in paw licking activity was approximately 35.72, 63.18 and 53.54% with formulations; 8037, 8039 and 8040, respectively compared to control group ($p<0.001$ vs. control). Diclofenac based marketed formulation (*i.e.*, standard 1) was found to be ineffective in the experiment, since it produced insignificant reduction ($p > 0.01$ vs control) in paw licking in animals. However, the herbal based formulation (*i.e.*, standard 2) was found to produced significant analgesic activity. Comparison of standard with test formulations showed that the effect of formulations; 8037, 8039 and 8040 were comparable to the herbal based standard 2 formulation. These results suggested that all three formulations possessed promising analgesic activity.

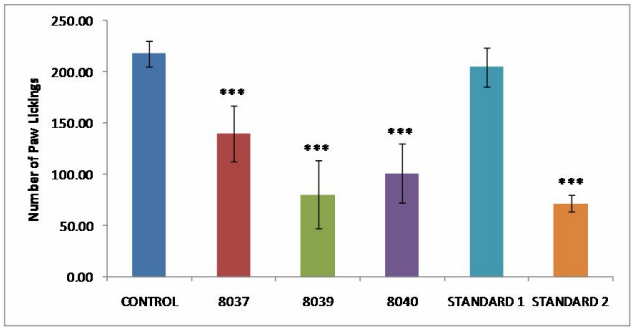

Figure 1: Showing the number of paw lickings observed in various treatment groups in the formalin test. *** $p<0.001$ vs control

Results of anti-inflammatory activity are presented in Table 1. Early phase response was measured 1h after induction of paw edema. Results showed that formulation, 8037 inhibited early phase paw edema by 16.63%. The reduction of paw edema was 20.42% with formulation, 8038 and 9.63% with formulation, 8040, respectively. However, late phase response even higher where 49.10,

52.40 and 44.25% inhibition of paw edema was observed with formulations; 8037, 8039 and 8040, respectively. The inhibition of paw edema in groups treated with standards was better that any of the test formulations. However, the early phase paw edema reduction in diclofenac based formulation (standard 1) was 36.28% which was better than early phase response of herbal based standard 2 (30.73%). Whereas, it was observed that the late phase response on paw edema reduction with herbal based standard 2 was 65.46% and with standard 1 it was 53.20%.

Table 1: Showing the anti-inflammatory activity of test formulations as a measure of percentage(%) inhibition of paw edema

% Inhibition of paw edema		
Formulations	**Early phase**	**Late phase**
8037	16.63 ± 2.78	38.35 ± 10.87
8039	20.42 ± 1.45	45.54 ± 2.44
8040	9.63 ± 1.66	45.85 ± 1.54
Standard 1	36.28 ± 2.83	53.20 ± 14.04
Standard 2	30.73 ± 7.72	65.46 ± 2.43

Inflammation is result of very complex tissue response to foreign and harmful stimuli which includes irritants, pathogens, trauma, *etc*. (Yadav *et al*., 2012). It is an action by the organism to protect itself through removal of such stimuli and also to heal the damaged tissue. However, inflammation, under chronic condition, can itself result into a number of diseases, *e.g*. hay fever, rheumatoid arthritis and atherosclerosis. A number of natural substances have been reported to possess anti-inflammatory effects. These can either reduce the level of reactive oxygen species by scavenging free radicals (Ostrakhovitch and Afanas, 2001). A number of herbal agents are also reported to modulate expression of proinflammaory genes (Jayakumari *et al*., 2012).

The ingredients of the three formulations have been carefully selected from the previously reported and traditionally established herbal anti-inflammatory agents. The formulations content includes anti-inflammatory principles of natural origin, volatile substances, oleoresins, terpenes and fatty acid containing ingredients. For example, salicylateshave been developed from natural substance salicylic acid and are established molecule possessing anti-inflammatory and analgesic activity. The ingredient methyl salicylate belongs to this category and recently methyl salicylate glycosides have been shown to posses anti-inflammatory and analgesic properties although, without causing associated side effects of currently used analgesic agents (Mao *et al*., 2014).

Similarly, eucalyptus oil has been used as folk medicine for the treatment of different inflammatory and infectious conditions. Its anti-inflammatory effects are shown by inhibition of carrageenan induced rat paw edema, inhibition of carrageenan induced neutrophil migration into rat peritoneal cavities. It has also been shown to reduce carrageenan and histamine induced vascular permeability. All these studies have suggested that it induced analgesic and anti-inflmmatory effects through neutrophil-dependent and independent pathways (Silva *et al*., 2003).

Another constituent of the formulation, sesame oil is a common cooking oil as well as nutritious food. It also has wide pharmaceutical applications. Its active principle sesamin has been shown to possess promising antinociceptive and anti-inflammatory potential (Monteiro *et al*., 2014). Which may have been responsible for its anti-inflammatory and analgesic activity in the test formulations.

Gum-resin extracts of salai guggul (*Boswellia serrata*) have been used as anti-inflammatory agents in folk medicine for centuries. The resinous part of plant contains many terpenoidal molecules having ability to inhibit pro-inflammatory enzymes (Siddiqui, 2011).

The oleoresin constituent of the test formulations include capsicum oleoresin. In a previously reported study, capsicum oleoresin have been shown to suppressed TNF-α and IL-1β secretion from LPS-treated macrophages. It also decreased TGF-β level from macrophages in both absence and presence of LPS (Liu *et al*., 2012).

The *in vitro* anti-inflammatory activity of the monoterpene menthol and mint oil have also been reported using LPS-stimulated monocytes. Both menthol and mint oil have been shown to reduce Leukoriene B4 (LTB4), Prostaglandin (PGE2) and IL-1β level, whereas mint oil exhibited similar effects on LTB4 and IL-1β suggesting preferable anti-inflammatory activity of the two (Juergents *et al*., 1998).

Overall, based on these evidences, the anti-inflammatory and analgesic effect of the test formulations can be attributed to the complex interplay of these ingredients.

4. Conclusion

The test formulations; DRDC/AY8037, 8039 and 8040 possess analgesic and anti-inflammatory activity as shown through preclinical studies. These formulations have promising potential for their clinical development as topical analgesic and anti-inflammatory agents.

Conflict of interest

We declare that we have no conflict of interest.

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