INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

PHARMACOLOGICAL EVALUATION OF DAZZLE COOL CREAM FOR ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

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Article Received on: 10/11/13 Revised on: 21/11/13 Approved for publication: 30/11/13

DOI: 10.7897/2230-8407.041111

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

ABSTRACT

Musculoskeletal injuries are most common risk factors for sport persons. Pain and swelling are the most common associated symptoms of such injuries. A use of non-steroidal anti-inflammatory drugs (NSAIDs) has been routine in the management of musculoskeletal inflammation and pain. Although effective at reducing pain and inflammation, NSAIDs may not be appropriate to use frequently or longer time due to their 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, Dazzle Cool Cream – An Ayurvedic proprietary formulation has been selected for evaluation of its anti-inflammatory and analgesic activity. Anti-inflammatory activity was evaluated by Complete freund's adjuvant (CFA) induced acute and chronic phase inflammation. Analgesic activity was evaluated by Hot plate method and Tail flick method. Total study was performed on 10 groups of animal where each group was containing 6 animals. Marketed gel containing Diclofenac diethylamine BP 1.16 % w/w was considered as standard drug for both activities. Paw volume was significantly lowered at 7th, 14th and 21st day in test drug and standard drug group with respect to disease control. Dazzle Cool Cream showed 28.16 % inhibition in paw volume at 21st day which was comparable to standard marketed gel containing Diclofenac diethylamine BP 1.16 % w/w showing 29.88 % inhibition. Dazzle Cool Cream showed significant effect on inflammatory markers such as ESR, CRP and WBC. It also exhibited significant analgesic activity in both Hot plate and Tail flick nociceptive tests. On the basis of available results, it can be concluded that Dazzle Cool Cream has promising anti-inflammatory and analgesic activity.

Keywords: Dazzle Cool Cream, Anti-inflammatory activity, Analgesic activity, Musculo-skeletal injuries

INTRODUCTION

A moderate amount of physical activity can have substantial health benefits. However, participation in sports and similar physical activities increases the risk of muscular injuries. The most common injuries are at the ankle, which, with an incidence of 1 per 1, 00,000 people a day.[1] The causes of such pains are varied in nature. Muscle tissue can be damaged with the wear and tear of daily activities. Trauma to an area (jerking movements, auto accidents, falls, fractures, sprains, dislocations, and direct blows to the muscle) also can cause musculoskeletal pain. Other causes of pain include postural strain, repetitive movements and prolonged immobilization and disease condition like rheumatoid arthritis.[2] Pathophysiology of musculoskeletal pain is not completely clear, but inflammation, fibrosis, tissue degradation, neurotransmitters and neuro-sensory disturbances have been implicated.[3] The inflammatory process is a reaction of the body against the penetration of an infectious agent, an antigen or cellular damage. Celsius (in 30 A.D.) described the four classical signs of inflammation are redness, heat, pain and swelling.[4] A use of non-steroidal anti-inflammatory drugs (NSAIDs) has been routine in the management of musculoskeletal inflammation and pain. Although effective at reducing pain and inflammation, NSAIDs is not considered appropriate particularly for those who is frequently encounters sport injuries or suffered from musculoskeletal disorders. 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.[5,6] Traditional Indian System of Medicine, mainly consisting of herb based products is nowadays getting global attention and importance in the field of medical research. Herbal products are widely perceived as safe due to their natural origin and long historical clinical use. Dazzle Cool Cream is a proprietary Ayurvedic formulation which contains Mahanarayan oil[7,8] and Nirgundi oil[9,10] (Ayurvedic classical oil formulations); *Ricinus communis* (Erand) seed oil[11,12], *Eucalyptus globules* (Nilgiri) oil[13,14], *Vateria indica* (Sarjras) oleo resin[15], *Aloe vera* (Kumari) leaf juice[16,17], *Mentha sylvestris* (Pudina) satva[18,19] and *Cinnamomum camphora* (Karpoor) satva[20]. Dazzle Cool Cream is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. Majority of ingredients of Dazzle Cool Cream are well reported in Ayurvedic texts and scientific research publications for anti-inflammatory and analgesic activity. However, no such evidence was available which proves efficacy of such combination. Hence in the present study, an attempt was made to evaluate its anti-inflammatory and analgesic activity on experimental animals.

MATERIALS AND METHODS

Experimental animals

The experiment protocol described in present study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: KB/11/242) 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 the experiment. 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' pelleted diet and purified drinking water *ad libitium*. The animals were deprived of food for 24 hour before experimentation but allowed free access to water throughout.

Drugs and chemicals

A proprietary Ayurvedic formulation – Dazzle Cool Cream was provided by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. Complete Freund's adjuvant was procured from Sigma-Aldrich. Standard drug (Volini gel manufactured by Ranbaxy Laboratories Ltd.) was procured from local medical store. It contains Diclofenac diethylamine BP 1.16 % w/w, menthol IP 5 % w/w, linseed oil BP 3 % w/w and methyl salicylate 10 % w/w.

Complete freund's adjuvant (CFA) induced acute and chronic inflammation in wistar rats

The selected animals were divided in to four groups where each group consisted of six animals.

Group-I (NC): Served as normal control and received distilled water
Group-II (DC): Served as disease control and administered 0.1 mL of CFA into sub-plantar surface of the left hind paw
Group-III (TD): Served as test drug (i.e. Dazzle Cool Cream, topical application) treated group + 0.1 mL CFA
Group-IV (SD): Served as standard drug (i.e. Marketed gel containing Diclofenac diethylamine BP 1.16% w/w, topical application) treated group + 0.1 mL CFA

On the initial day of the experiment, baseline paw volume was recorded by using a plethysmometer. The animals of Group II to IV were injected with 0.1 mL of CFA (0.05 % w/v *Mycobacterium butyricum* in mineral oil) into the sub-plantar surface of left hind paw. Test drug and standard drug were administered topically, once a day, from the day of injection of adjuvant and continued for 21 days. The inflammatory changes were observed on 0, 7th, 14th, 21st day after injection of CFA by using a plethysmometer.[21] Percentage inhibition in paw volume was calculated in comparison to 21st day paw volume of disease control group. On 21st day, 10 mL of blood was collected through retro-orbital route to estimate hematological parameters such as Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood cell (WBC).

Experimental models for assessing analgesic activity

For Hot plate and Tail flick experimental models of analgesic activity, animals were divided in to three groups where each group consisted of six animals.

Group-I (NC): Served as normal control and received distilled water
Group-II (TD): Served as test drug (i.e. Dazzle Cool Cream, topical application) treated group
Group-III (SD): Served as standard drug (i.e. Marketed gel containing Diclofenac diethylamine BP 1.16 % w/w, topical application) treated group

Hot plate test

In this experiment, the central analgesic activity of Dazzle Cool Cream was assessed in male wistar rats, as per the method described by Eddy and Leimbach.[22] 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 ± 0.5°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 and standard drug were applied topically in Group II and III respectively. The increase in reaction time in drug-treated groups was compared with that of the control group.

Tail flick method

The central analgesic activity of Dazzle Cool Cream was studied in tail withdrawal assay, as described by D'Amour and Smith.[23] 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 and standard drug were applied in Group II and III respectively. The tail withdrawal latencies were measured at interval of 30 minutes time for 4 hours.

Statistical analysis

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

Table 1: Effect of Dazzle Cool Cream on mean change and percentage inhibition in paw volume

Groups	Paw volume (mL)				% inhibition in Paw volume on 21st Day
	0 Day	7th Day	14th Day	21st Day	
Normal control (NC)	1.19 ± 0.02	1.21 ± 0.02	1.21 ± 0.02	1.21 ± 0.02	---
Disease Control (DC)	1.21 ± 0.01	1.73 ± 0.03$^{###}$	1.78 ± 0.01$^{###}$	1.74 ± 0.02$^{###}$	---
Dazzle Cool Cream treated (TD)	1.21 ± 0.01	1.58 ± 0.01*	1.51± 0.04*	1.25 ± 0.03***	28.16 %
Standard drug treated (SD)	1.20 ± 0.05	1.52 ± 0.02*	1.49 ± 0.03**	1.22 ± 0.01***	29.88 %

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 disease control group. $^{###}p < 0.001$ when compared to normal control group

Table 2: Effect of Dazzle Cool Cream on hematological parameters on 21st day

Groups	ESR (mm/h)	CRP (mg/L)	WBC (g/dL)
Normal control (NC)	2.50 ± 0.56	1.61 ± 0.21	7.25 ± 0.09
Disease Control (DC)	11.54 ± 0.52$^{###}$	9.67 ± 0.30$^{###}$	12.56 ± 0.69$^{##}$
Dazzle Cool Cream treated (TD)	3.59 ± 0.40***	2.16 ± 0.29***	7.50 ± 0.41**
Standard drug treated (SD)	3.16 ± 0.37***	2.12 ± 0.19***	6.80 ± 0.24**

All the values are expressed as mean ± SEM (n = 6) in each group. $^{**}p < 0.01$ and $^{***}p < 0.001$ when compared to disease control group. $^{##}p < 0.01$ and $^{###}p < 0.001$ when compared to normal control group

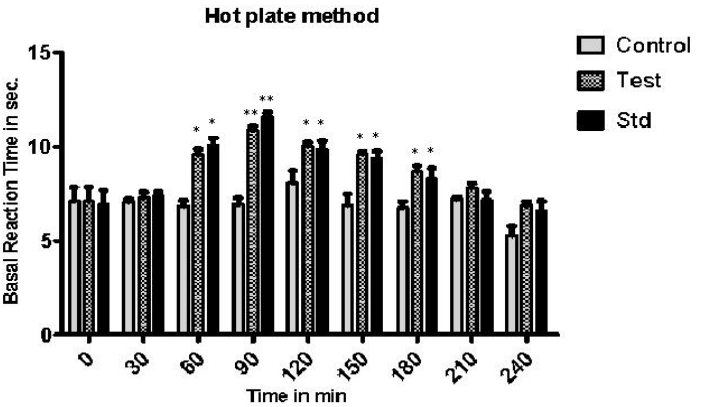

Figure 1: Analgesic activity of Dazzle Cool Cream by Hot plate method

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

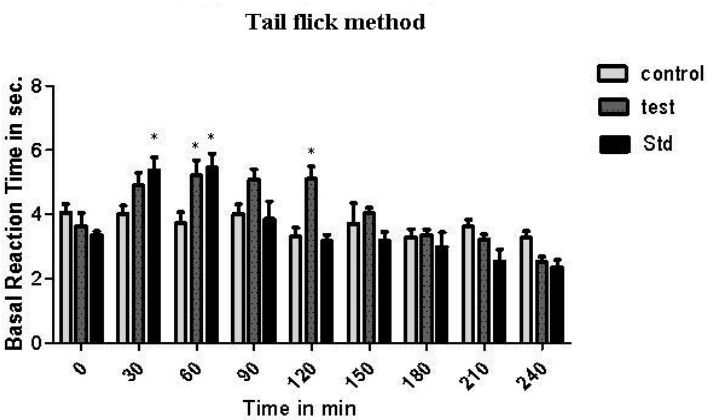

Figure 2: Analgesic activity of Dazzle Cool Cream by Tail flick method

All the values are expressed as mean ± SEM (n = 6) in each group. $^{*}p < 0.05$ when compared to normal control group

RESULTS

Anti-inflammatory activity

The results of anti-inflammatory activity of Dazzle Cool Cream were tabulated in Table 1 and 2. Due to induction of CFA, significant inflammation was developed. Dazzle Cool Cream treatment showed significant reduction in paw volume when compared with DC group. Dazzle Cool Cream showed 28.16 % inhibition in paw volume at 21st day (Table 1). In DC group, there was marked increased in ESR, CRP and WBC which were significantly decreased by Dazzle Cool Cream and Standard drug (Table 2).

Analgesic activity

For the determination of analgesic activity, two methods were used i.e. Hot plate method and Tail flick method. In Hot plate method, the test drug and standard drug showed maximum significant analgesic effect at 90 minutes when compared with normal control (Figure 1). In the Tail flick method test drug and standard drug produced significant analgesic effect at 60 minutes when compared with normal control (Figure 2).

DISCUSSION

In the present study, anti-inflammatory activity and analgesic activity of Dazzle Cool Cream were evaluated on different experimental models. Volini gel, a NSAID base formulation was selected as standard drug which is commonly prescribed to reduce inflammation and pain. It acts by inhibiting the synthesis of prostaglandins and so as COX-2.[24] Test drug was compared with standard drug to evaluate comparative efficacy as anti-inflammatory and analgesic.

Anti-inflammatory activity

The model of adjuvant-induced arthritis in rats has been extensively used in the study of inflammatory processes[25] and validated as a model of acute and chronic pain. This fact is corroborated by evidence of spontaneous pain behaviors in arthritic rats, such as reduced locomotors activity and

increased itching and scratching behaviors in the affected paw.[26] Paw volume was significantly lowered at 7th, 14th and 21st day in TD and SD group with respect to DC. Dazzle Cool Cream and Standard drug showed 28.16 % and 29.88 % inhibition in paw volume at 21st day respectively (Table 1). Erythrocyte sedimentation rates (ESR), C-reactive protein (CRP) and white blood cell (WBC) are known as acute phase proteins, which reflect a measure of the acute-phase response. The term "acute phase" refers to local and systemic events that accompany inflammation.[27,28] Due to induction of CFA, the values of ESR, CRP and WBC were significantly increased in DC when compared to NC. Dazzle Cool Cream showed significant effect on inflammatory markers such as ESR, CRP and WBC (Table 2). Dazzle Cool Cream showed equivalent significant effect like Standard drug.

Analgesic activity

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. 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] Dazzle Cool Cream showed significant analgesic effect in both Hot plate and Tail flick nociceptive tests. The results suggest that Dazzle Cool Cream has a 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 also by the increase in the reaction time of the rats in the Hot plate test (Figure 1 and 2). Analgesic effect of Dazzle Cool Cream was equivalent to standard drug. The results presently discussed demonstrate the peripheral anti-inflammatory effects of Dazzle Cool Cream in the Freund's adjuvant-induced acute as well as chronic phase inflammation. Dazzle Cool Cream also showed central analgesic effect. Dazzle Cool Cream represents an important and promising source of Ayurvedic medicine for the treatment of acute and chronic phase inflammatory and pain conditions.

ACKNOWLEDGEMENT

Authors are sincerely thankful to the management of Vasu Healthcare Pvt. Ltd. for providing test drug samples and K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India for providing the necessary facilities for conducting the study.

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Cite this article as:

Soni Hardik K., Joshi Shefali K., Zaveri Maitreyi N., Patel Sonal S., Patel Ghanashyam R.. Pharmacological evaluation of Dazzle cool cream for anti-inflammatory and analgesic activity. Int. Res. J. Pharm. 2013; 4(11):46-49 http://dx.doi.org/10.7897/2230-8407.041111

Source of support: Nil, Conflict of interest: None Declared