Research Article

Evaluation of anti-arthritic activity of Dazzle ointment - A polyherbal formulation

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ABSTRACT

Background: Rheumatoid arthritis is a chronic multi-system disease of unknown cause. It affects people in their prime of life, predominantly between the ages of 20-50 years with unpredictable course. Dazzle ointment is a polyherbal formulation, used for the treatment of inflammation and rheumatoid arthritis.

Objective: The present study was planned to evaluate efficacy of Dazzle ointment using complete Freund's adjuvant-induced arthritic model.

Materials and Methods: The animals were divided into three groups of 6 animals each as CFA treated, standard drug and test drug treated groups. The animals were injected with 0.2 ml of complete Freund's adjuvant into the sub-plantar surface of left hind paw. Drugs were administered topically, once a day, commenced on the day of injection of adjuvant and continued for 21 days. The assessment of the change in the inflammatory reaction was made by measuring the paw volume plethysmographically on 0, 4th, 14th, 17th, 21st day after injection of complete Freund's adjuvant (CFA). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's comparison test. A p value of < 0.05 was considered as statistically significant.

Results: It was observed that Dazzle ointment produced significant anti-arthritic effect on 21st day. In CFA treated group, there was marked increase in the ESR and WBC count which was significantly decreased by test drug Dazzle ointment and standard drug diclofenac gel (Omni gel).

Conclusion: The results indicate that Dazzle ointment possesses anti-arthritic activity in the experimental animal model.

Key words: Dazzle ointment, anti-arthritic, complete Freund's adjuvant, plethysmometer

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by the chronic inflammation of synovial joints which results in severe bone destruction.[1] It affects approximately 5 million people worldwide of which 50% are unable to work beyond 10 years of diagnosis.[2] A number of anti-inflammatory and anti-rheumatic drugs used in treatment of RA have been developed over the past few decades, but still there is an urgent need for more effective drugs with lower side effects.[3] Non-steroidal anti-inflammatory drugs (NSAIDs) play vital role along with disease modifying anti-rheumatoid drugs (DMARDs) in the management of RA. It is now recognized that NSAIDs are effective in relieving the symptoms of the disease. However, they do little to overcome the main underlying cause, and in some instances may contribute to its progression. In the same line, though various drugs have been used to control RA there are numerous reports regarding the side effects of these drugs. This further suggests a need of effective as well as safe alternative therapy for the radical treatment of RA.[4]

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Recent studies show that many medicinal plants and herbal preparations are practiced for treatment of RA.[5] Dazzle ointment is one such combination which contains traditionally used oils and scientifically proven natural oils with known mascular and joint pain relieving action like *Gaultheria fragrantissima* (Gandhapuro) oil,[6] *Cedrus deodara* (Devdaru) oil,[7] *Mentha piperata* (Pippermint),[8] Narayan oil,[7] Mahamansh oil,[7] Vishgarbh oil[7] and *Capsicum annuum* (Mirch) oil.[8]

MATERIALS AND METHODS

Experimental animals

The experiment protocol described in present study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.:VBT/IAEC/10/12/36) and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.: 35/1999/CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy adult male Wistar rats weighing 200 - 250 g were used. 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 pelleted 'Sabardan' 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

The poly-herbal formulation "Dazzle ointment" was manufactured and supplied by Vasu Healthcare Pvt. Ltd., Baroda, Gujarat, India. The formulation was administered topically. Complete Freund's adjuvant was procured from Sigma-Aldrich. Standard drug diclofenac gel (Omni gel) was procured from Cipla Pharmaceuticals. It contains diclofenac diethylammonium, menthol 5%, linseed oil 3% and methyl salicylate 10%.

Groups and treatment

Albino Wistar rats were divided into three groups of 6 animals each. Group I served as CFA treated (complete Freund's adjuvant induced arthritis). Group II served as standard (arthritis treated with Omni gel). Group III served as Test drug (arthritis treated with Dazzle ointment).

Complete Freund's adjuvant induced arthritis in Wistar rats

The animals were injected with 0.2 ml of complete Freund's adjuvant into the sub-plantar surface of left hind paw. Drugs were administered topically, once a day, commenced on the day of injection of adjuvant and continued for 21 days. The assessment of the change in the inflammatory reaction was made by measuring the paw volume plethysmographically on 0, 4^{th}, 14^{th}, 17^{th}, 21^{st} day after injection of Complete Freund's adjuvant (CFA).[9]

Paw volume displacement

On 0 day, the left hind paw volume of all rats as a volume displacement was measured using digital plethysmometer and on 1^{st} day arthritis was induced in all rats using CFA. The drugs were applied topically on the affected joint, once daily on 1^{st} day and continued for 21 days. The assessment of anti-arthritic activity was carried out by measuring change in paw volume edema on 4^{th}, 14^{th}, 17^{th} and 21^{st} day after induction. The percent inhibition of paw volume of treated rats was evaluated by using following formula;

$$\text{\% inhibition in paw volume} = \frac{\text{Paw volume of disease control} - \text{Paw volume of treatment control}}{\text{Paw volume of disease control}} \times 100$$

On day 21, 8 ml of blood was collected through retro orbital route to estimate hematological parameters such as ESR, WBC and Hb.

Statistical analysis

Results were presented as Mean ± SEM of six animals. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's comparison test. The significance difference if any among the groups at $p < 0.05$ was considered statistically significant, $p < 0.01$ was considered statistically highly significant.

RESULTS

The effect of drugs on paw volume is reported in Table 1 and Table 2. It was observed that Dazzle ointment produced maximum effect on 21st day. It showed significant decrease in paw volume on 21st day in comparison to CFA treated group (Table 1). In CFA treated group, there was marked increase in the ESR and WBC count which was significantly decreased by test drug Dazzle ointment and standard drug diclofenac gel (Omni gel) (Table 2). There was no significant change observed in hemoglobin level (Table 2).

Table 2: Haematological parameters in various animal groups on 21st day

Parame-ters	Control group	Standard group	Test Drug group
ESR (mm/h)	5.00 ± 0.57	1.33 ± 0.33#	2.33 ± 0.33#
WBC count (cells/mm^3)	7833.33 ± 233.33	6433.33 ± 753.51#	7266 ± 88.19*
Hb (g/dL)	9.90 ± 1.23	10.53 ± 0.08	10.13 ± 0.26

Data represent in Mean ± SEM, where n=6
* P<0.05, # P<0.01 vs. control group

Table 1: Comparison of effects of drugs on mean change in paw volume

Groups	Paw volume (ml) (Mean ± SEM)					% inhibition in paw volume on 21st day
	0 Day	4th Day	14th Day	17th Day	21st Day	
Control group	2.94 ± 0.01	2.92 ± 0.01	2.94 ± 0.01	2.95 ± 0.00	2.94 ± 0.01	0 %
Standard group (Omni gel)	2.78 ± 0.01	2.73 ± 0.01	2.63 ± 0.01*	2.63 ± 0.02*	2.49 ± 0.01#	15.02 %
Test drug group (Dazzle ointment)	2.94 ± 0.04	2.86 ± 0.04	2.72 ± 0.07	2.70 ± 0.07*	2.65 ± 0.07*	9.55 %

Values are expressed in Mean ± SEM, where n = 6, *P < 0.05, #P < 0.01 vs. control group

DISCUSSION

Polyherbal formulation used in this study i.e. Dazzle ointment contains herb oil which has potent anti-inflammatory, analgesic activity and is used for arthritic condition.[6-8] Combine anti-arthritic effect of these oils in form of polyherbal formulation is measured through this study.

The purpose of Omni gel for use as a standard drug is that it contains diclofenac which is a NSAID. It reduces inflammation and as an analgesic, it reduces pain in conditions such as arthritis or acute injury. It acts by inhibiting the synthesis of prostaglandins by inhibiting COX-2.[10]

To conduct this study, Complete Freund's adjuvant (CFA) induced arthritis model in rats was used which is probably the best and most widely used model and satisfies mostly the allied conditions of arthritis in rat which resembles human conditions. In this model, bacterial peptidoglycan and muramyl dipeptide are responsible for induction of arthritic changes.[11] They mediate cell autoimmunity through structural mimicry between mycobacteria and peptidoglycan in rats.[12]

Findings of rat paw volume by standard drug and test drug in CFA treated group is given in Table 1. The assessment made on 21st day showed that, standard drug treated group

(Group II) showed 15.02% paw volume inhibition and test drug treated group (Group III) showed 9.55% paw volume inhibition. From 17th day, both drugs significantly lowered paw volume. Hence, it was observed that treatment with test drug Dazzle ointment significantly reduced the rat paw volume as compared to control group. There was no significant difference between the effects of the two drugs.

The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. Chronic inflammation involves the release of number of mediators like cytokines (IL-1β and TNF-α) and interferons. These mediators are responsible for the pain, destruction of bone and cartilage that lead to severe disability.[13] TNF-α induced free radical generation like H_2O_2 activates inflammatory signaling pathway, including NF-KB in vascular cells,[14] and regulates the expression of cell adhesion molecules on endothelial cells, hence plays an important role in various inflammatory diseases. [15]

In adjuvant-induced arthritis model, rats developed a chronic swelling in hind paw with influx of inflammatory cells, erosion of joint cartilage, bone destruction and remodeling which have close similarities to human rheumatoid arthritis.[16] These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal. The CFA administered rats showed soft tissue swelling around the ankle joints during the development of arthritis, which was considered as oedema of the particular tissues.[17]

In present study, standard drug diclofenac gel (Omni gel) and the test drug Dazzle ointment significantly suppressed the swelling of the paws. Reduction of paw swelling in the Dazzle ointment treated rats observed from the 3rd week onwards may be due to immunological protection rendered by the formulation effect. In the study, there was an increase in erythrocyte sedimentation rate (ESR) level in CFA treated group which is a common diagnostic feature in patient in chronic arthritis.[18] Increase in the ESR is an indication of active but obscure disease process which gets elevated during response to stress, inflammation and cell necrosis.[19] WBC count which plays a major role in body defense mechanism is mild to moderately increased in arthritic condition; An increase in WBC count may be due to the release of interleukins, responsible for production of both granulocytes and macrophages colony stimulating factor.[20]

In the present study, increased ESR in the CFA treated group was significantly reduced by standard and test drug. Also increased level of WBC count was significantly reduced by standard and test drug which signifies relativity of ESR and WBC with inflammation. The significant decrease in paw swelling, ESR and WBC count by polyherbal formulation, Dazzle ointment, and lack of adverse effects indicates its potential for use as an antiarthritic agent.

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