Research Article

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EVALUATION OF POLYHERBAL FORMULATION FOR ANTI-INFLAMMATORY ACTIVITY IN CARRAGEENAN INDUCED PAW OEDEMA MODEL IN RATS

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ABSTRACT

Dazzle Capsule, a Poly herbal formulation is marketed in management of Arthritis and joint-musculo skeletal pain. In this study, safety and efficacy of this polyherbal formulation was investigated in which it was being proved safe for use via acute toxicity study and efficacy was being evaluated by carrageenan-induced rat paw oedema test in rats in order to explore its anti-inflammatory activity at the dose level of 90 and 180 mg/kg, p.o, compared with Indomethacin (10 mg/kg, p.o.). It showed an highly significant reduction in oedema ($p<0.001$). Indomethacin inhibited oedema by 30.054% and 36.216% at 2 and 3 hr after carrageenan injection, respectively. The inhibitory effect of Dazzle Capsule began at 2 hr or later after carrageenan injection depending upon the administered dose. Low doses of Dazzle Capsule (90 mg/kg) gave highly significant inhibitory effects of 37.894- 39.378%, and higher doses (180 mg/kg) caused highly significant inhibition of 19.125- 30%. The reduction of oedema by Indomethacin and Dazzle Capsule at 2 hr or more after carrageenan injection suggested that both compounds produce anti-inflammatory effects in the second phase of oedema, indicating inhibition of prostaglandin synthesis. Hence, it was concluded that, Dazzle Capsule is having potent anti-inflammatory activity yet safe polyherbal formulation for use in arthritis management.

Keywords: Toxicity study, Anti-inflammatory activity, Paw oedema, Carrageenan, Dazzle Capsule, Poly herbal formulation

INTRODUCTION

Inflammation is an immune response to injury or infection causing pain, redness, heat, and swelling in the affected area.[1] It involves a series of events that can be elicited by numerous stimuli (e.g., infectious agents, ischemia, antigen-antibody interactions and thermal or other physical injury).[2] Presence of Prostaglandin (PGE) and kinins in the inflammatory exudates suggests their role in the genesis of inflammation. COX-2 is clearly associated with inflammation. (Figure 1)[3]

One of the major inflammatory diseases is Arthritis. It is nothing but the "Joint Inflammation" and it involves the breakdown of cartilage.[4] Arthritis is the term often used for diseases that can cause stiffness and swelling in and around the joints, muscles, bones, tendons, ligaments, and some internal organs. Two of the most common forms of arthritis are osteoarthritis and rheumatoid arthritis. Various other types include gout, juvenile arthritis, psoriatic arthritis and ankylosing spondylitis.[5-7]

The goal of treatment of Arthritis is to reduce pain, improve function, and prevent further joint damage. Furthermore many medications such as analgesic, anti-inflammatory, antipyretics are recommended or prescribed by physicians along with lifestyle changes. However, they have many potential risks, especially if used for a long time.[4] Hence, presently available pharmacological treatments in the market are not only causing economical exploitation, but also associated with severe adverse effects.[8, 9] However, to develop a proper medication which will be ecofriendly and having very less side effects that can be used for prophylactic and therapeutic purpose to control this severe disease is still a big challenge to a scientific community working in this area.

Dazzle Capsule, marketed and manufactured by Vasu Healthcare Pvt. Ltd. It is unique combination of traditionally used herbs and bhasma with known pain relieving as well as anti-inflammatory action. The present study on safety and efficacy was carried out with an objective to evaluate the anti-inflammatory activity and safety of Dazzle capsule in management of Arthritis.

MATERIALS AND METHODS

Chemicals and Drugs

Indomethacin, Carrageenan (High Media Laboratories) and all other chemicals were of analytical grade used.

Drug Preparation

Test drug (Dazzle capsule): 90 mg/kg, 180 mg/kg and Standard drug (Indomethacin): 10 mg/kg prepared in 10% CMC (Carboxy methyl cellulose) solution were used for oral administration. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline.

Experimental animals

Healthy Swiss albino mice (20-25g) of either sex were taken for toxicity study and Wistar albino rats (210-260 g) was taken to evaluate anti-inflammatory activity. Both were procured from S.K.Patel College of Pharmaceutical Education And Research, Ganpat University, Kherva, Mehsana, Gujarat, India. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Ethical Clearance Number for same manuscript i.e. IAEC/SKPCPER/2010-02/22, Ministry of Social Justice and Empowerment, Government of India.

Table 1: Cage side observations of animals (General Behavior)

Sr. No.	Parameters	Observations (2000 mg/kg)
1	Condition of fur	Normal
2	Skin	Normal
3	Subcutaneous swelling	Nil
4	Eyes dullness	Nil
5	Eyes opacities	Nil
6	Color and consistency of faeces	Normal
7	Condition of teeth	Normal
8	Breathing abnormalities	Nil

Table 2: Mean Body weight and percentage body weight gain

Group	Dose (mg/kg body wt)	Body weight		% body wt gain day 1-7	Body weight	% body wt gain day 7-14	% body wt gain day 1-14
		Day 1	Day 7		Day 14		
Control	-	22.47	23.69	5.43%	25.62	8.14%	14.02%
I	2000	22.85	24.44	6.96%	26.25	7.40%	14.87%

Table 3: Mortality Record

Group	Dose(mg/kg body wt)	Mortality	
		Male	Female
I	2000	0/3	0/3

Table 4: Increase in paw volume with time

	Treatment			
	Group 1	Group 2	Group 3	Group 4
	Control (CMC)	Standard (Indomethacin 10 mg/kg)	Dazzle capsule 90 mg/kg	Dazzle capsule 180 mg/kg
0 hr	0.92±0.09*	0.68±0.03*	0.88±0.09	0.92±0.07
1 hr	1.58±0.03***	1.35±0.02***	1.42±0.03	1.58±0.03
2 hr	1.83±0.04***	1.28±0.09***	1.45±0.06	1.48±0.03***
3 hr	1.85±0.05***	1.18±0.12***	1.35±0.06	1.38±0.04***
4 hr	1.9±0.03***	1.15±0.06***	1.18±0.03***	1.33±0.04***
5 hr	1.93±0.02***	1.08±0.03***	1.17±0.04***	1.17±0.04
6 hr	1.93±0.02	0.98±0.03	1.05±0.03	1.08±0.04
***P<0.001 (Highly significant), as compared to control group *p<0.05 (Significant), as compared to control group				

Table 5: % Inhibition of treated groups compared with control group

	% Inhibition of Treated groups		
	Group 2	Group 3	Group 4
	Standard (Indomethacin 10 mg/kg)	Dazzle capsule 90 mg/kg	Dazzle capsule 180 mg/kg
1 hr	15.189	10.126	0
2 hr	30.054	20.765	19.125
3 hr	36.216	27.027	25.405
4 hr	39.473	37.894	30
5 hr	44.041	39.378	39.378
6 hr	49.222	45.595	44.041

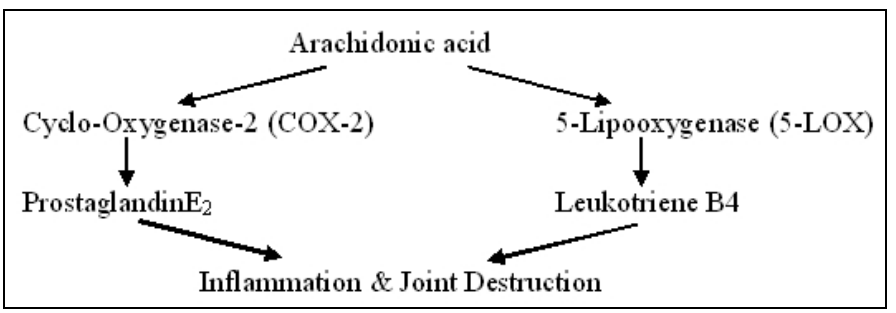

Figure 1: Inflammatory Cycle

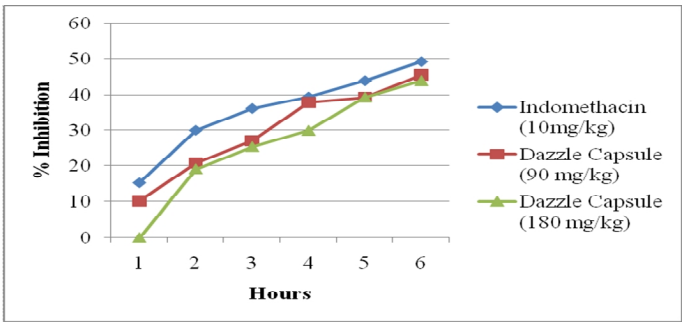

Figure 2: Graphical representation of % inhibition of Dazzle Capsule (90 and 180mg/kg) treated groups

Animals were housed in polypropylene cages, maintained under standardized condition (12-hrs light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to standard palate diet and purified drinking water ad *libitum.* The animals were deprived of food for 24 hrs before experimentation but allowed free access to water throughout.

Acute toxicity Study

For Acute toxicity study on mice, 'Fixed dose' method of the Organization for economic cooperation and Development (OECD) guideline 420 was followed.[10, 11] The formulation was suspended in distilled water and administered by gavages (orally) at single doses of 2000 mg/kg. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the formulation. The general behaviour of the rats was continuously monitored for 3hrs, and then every 30 minute for next 3 hrs till 24 hrs and then daily for a total of the 14 days. Changes in the normal activity of rats, their body weights, sign and symptoms of toxicity and mortality were monitored and recorded.

Efficacy Study

Evaluation of Anti-inflammatory activity by Carrageenan Induced Rat Paw Oedema Model

In carrageenan-induced rat paw oedema method[12], the animals were divided into 4 groups of 6 animals each. Food was withdrawn 16 hrs before drug treatment. The first group received 0.5 ml of 10% CMC solution orally and served as a control. The second group of animals was administered Indomethacin (10 mg/kg) and served as standard. The animals of groups 3 and 4 were treated with the formulation (90 mg/kg and 180 mg/kg, orally). Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the left hind paw of the rats, 1 hr after oral drug treatment.

The paw volume was measured plethysmometrically at 0, 1, 2, 3, 4, 5 and 6 hrs after the carrageenan injection. The difference in initial and the subsequent reading gave the actual oedema volume and the percent inhibition which was calculated by difference between the initial volume in control (Vc) and in groups treated with test compounds (Vt).

$$\% \text{ inhibition} = \frac{(V_c - V_t)}{V_c} \times 100$$

Statistics

All values are shown as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $p<0.05$ was considered statistically significant and $P<0.001$ was considered statistically highly significant, as compared to control group.[13]

RESULT

Acute toxicity Study

Clinical Signs of Intoxication, Body weight and Mortality

In the preliminary acute toxicity study, formulation seems to be safe at 2000 mg/kg. There was no toxic or deleterious effects (Table 1) observed immediately in 24 hrs and up to 14 days of observation period. There was no major change in body weight (Table 2) and no mortality (Table 3) was found in any animals.

Efficacy Study

Evaluation of Anti-inflammatory activity by Carrageenan Induced Rat Paw Oedema

The results indicated that the formulation, Dazzle capsule possessed good anti-inflammatory activity compared to standard (Indomethacin) group. (Table 4)

Percentage inhibition for all the treatment groups at every 1 hr interval was calculated. Results Showed good efficacy of the capsule inhibiting increasing paw oedema volume at every hour. (Table 4) Inhibition at 4th hour was found 37.89 % of Dazzle Capsule (90 mg/kg) as compared to 39.47 % of standard Indomethacin. (Table 5) It is also presented by graphical representation (Figure 2) which also shows significant % inhibition of Dazzle capsules (90 and 180mg/kg) treated groups on Carrageenan Induced Rat Paw Oedema

DISCUSSION

In the preliminary acute toxicity study, Dazzle Capsule seems to be safe at 2000 mg/kg. There was no toxic or deleterious effects observed immediately in 24 hrs and up to 14 days of observation period. There was no major change in body weight and no mortality found in any animal.

The preliminary phytochemical screening of poly herbal formulation Dazzle capsules showed the presence of alkaloids, flavonoids, and tannins. These compounds have well known anti-inflammatory effects[14-17]. The effects observed with Dazzle capsules could possibly be due to

the synergistic actions of these compounds. In the present study, Dazzle capsules demonstrated a highly significant ($P<0.001$) anti-inflammatory activity at different dose levels in rat model of inflammation.

To assess the anti-inflammatory activity, Dazzle capsules was evaluated by popular screening model widely used for NSAID namely Carrageenan induced rat paw oedema.[18] It shows a biphasic effect.[19] The first phase is due to release of histamine and serotonin (5-HT) (0-2 hrs), plateau phase is maintained by kinin like substance (3 hrs) and second accelerating phase of swelling is attributed to Prostaglandin release (4 hrs).[20]

To demonstrate the validity of the carrageenan-induced paw oedema test, rats were administered Indomethacin orally as a positive control at a dosage of 10 mg/kg 1 hr before carrageenan injection. As expected, Indomethacin was highly significantly ($p<0.001$) decreased paw oedema at 2 and 3 hrs after carrageenan injection compared to control CMC group, with inhibition levels of 30.054% and 36.216%, respectively (Table 5). These results demonstrate that Indomethacin, a cyclooxygenase inhibitor, exerts an anti-oedematous effect during the second phase of paw oedema due to the reduction of prostaglandins, which are second phase inflammatory mediators.[19] Our results are also consistent with a previous study showing that Indomethacin strongly inhibits the second phase of oedema without affecting the first phase.

The administration of Dazzle Capsule to rats resulted in a dose dependant decrease in the paw oedema volume at 2, 3, 4 and 5 hrs. Dazzle Capsule at a dose of 90 mg/kg p.o. highly significantly inhibited paw oedema at 4 hrs ($p<0.001$) and 5 hrs ($p<0.001$) respectively and at 180 mg/kg p.o. highly significantly inhibited paw oedema at 2 hrs ($p<0.001$), 3 and 4 hrs ($p<0.001$) respectively as shown in Table 4. Hence, in our study, Dazzle capsules (90 and 180 mg/kg) highly significantly ($P < 0.001$) reduced the oedema induced by carrageenan majorly in second phase i.e. Prostaglandin release.

CONCLUSION

The result of the acute toxicity test, for oral preparation of capsule formulation indicates that it is relatively safe and non-toxic to rats.

The result of evaluation of Anti-inflammatory study of Dazzle Capsule was demonstrated to inhibit inflammation in the carrageenan-induced rat paw oedema model. The onset and duration of action suggest that the anti-inflammatory mechanism of formulation occurs through probably by inhibition of prostaglandin synthesis via cyclooxygenase pathway. Hence, it was concluded that Dazzle Capsule possesses anti-inflammatory property which may have a potential benefit for the management of pain and inflammatory disorders such as Arthritis.

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