



EVALUATION OF ANTI-INFLAMMATORY EFFECT OF BELLADONNA (A HOMEOPATHIC DRUG) IN EXPERIMENTAL ANIMALS-RATS

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ABSTRACT

Objective: To evaluate the anti-inflammatory effect of Belladonna on acute and chronic inflammatory experimental animal models (rats).

Materials & Methods: The Belladonna, a homeo preparation was tested for its efficacy in acute and chronic inflammatory rats. In acute models: rat paw edema method was used. rats were divided into 4 groups 6 rats each. Group-I (NC), received 2% GA 2ml, Group-II (PC) received Aspirin (200mg/Kg.), Group-III, IV received Low dose (6CH) & High dose (200CH) of Belladonna preparation orally. Edema was developed in injected paw. Immediately the foot edema volume was measured at 0hr and at the end of 3rdhr. The percentage of inhibition of edema by drugs was measured and compared between the Test, Control & Standard groups. In chronic models: granuloma method was used and test drugs were given for 7days. The animals were divided and treated same as in acute model. After 7 days treatment, on 8th day animals were sacrificed each implanted pellet was extracted with adherent granulation tissue. These pellets were dried & weighed. The known weight was deducted from granulation tissue weight. The mean weight of granulation tissue and difference in weight of granulation tissue for each group was calculated and the percentage of inhibition of inflammation was calculated. **Results:** The percentage of inhibition of edema in acute model with 6CH, 200CH after 3 hrs is 76%, 74% respectively and with Aspirin is 78%. In chronic models, the percentage of inhibition of granulation tissue with 6CH, 200CH after 7 days is 31.57%, 29.99% respectively and with Aspirin is 32.77%. The reduction of inflammation was statistically significant in each group is ($P < 0.001$). **Conclusion:** Belladonna, a homeo preparation is a best alternative drug for the treatment of acute inflammatory conditions with no side effects.

KEY WORDS: Inflammation, Belladonna, Carrageenan, Aspirin.



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INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agents. These are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leucocyte infiltration and granuloma formation represent such components of inflammation¹. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability or that increase blood flow². Several experimental models of paw edema have been described. Carrageenan induced paw edema is widely used for determining the acute phase of inflammation. Histamine, 5HT, Bradykinin are the first detectable mediators in the early phase of Carrageenan induced inflammation³, where as prostaglandins are detectable in the late phase of inflammation⁴. Drugs which are in use presently for the management of pain and inflammatory condition are either narcotics e.g. Opioids or non-narcotics e.g. salicylates and corticosteroids e.g. Hydrocortisone. All of these drugs possess well known side and toxic effects. More over synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries, people have been trying to alleviate and treat disease with different plant extracts and formulations⁵. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs⁶. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethanobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. There are two types of plant

chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls etc. The other category of chemicals is called secondary metabolites which include alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system. According to literature available, Belladonna is used for the treatment of various acute and chronic inflammatory conditions by Homeopathic physicians. In the present study, Belladonna was selected to screening of its anti-inflammatory property in experimental animal models.

MATERIALS & METHODS

Institutional Animal Ethics Committee (IAEC) permission was obtained before starting the study. The study was conducted strictly in accordance with the protocol. In the present study, the homeopathic drug Atropa belladonna was screened for its anti-inflammatory activity and compared with that of the standard drug Aspirin in acute and chronic inflammatory animal models.

Atropa belladonna preparation

Belladonna is available in 2 potencies (6CH, 200CH), was taken from homeo stores after approval of homeophysician Dr. Srinivasa Reddy, Kurnool, AP. The drug is supplied by Dr. William Schwab India private limited, in collaboration with DHU-Arzneimittel Germany, A-36, Sector-60, phase III Noida-201301. A pilot study was carried out with the drug, Belladonna's effect on the hind paw edema in rat suggested anti inflammatory effect. The observation made during the pilot study carried beyond four hours did not reveal any significant difference from that of 3 to 4 hours of observation. Hence in the present study, the final observations were made at the end of 3hrs.

Chemicals

All the drugs (1% Carrageenan, Aspirin, Gum acacia, Ether, 5% Povidone Iodine) used in the study were of pharmaceutical grade.

Carrageenan was supplied by Sigma chemical company, Hyderabad, Aspirin was supplied by Dr. Reddy's laboratory, Hyderabad, India.

Experimental animals

Wistar albino rats (150-200g) of either sex were used in this study. The animals were supplied from Sainath Agencies, Hyderabad, AP, India. They were randomly distributed into groups and housed in cages (6/cage) and maintained under standard conditions at $26 \pm 2^{\circ}\text{C}$ and relative humidity 44-56% and 10 hours light: 14 hrs dark cycles each day for 1 week before and during the experiments. All animals were fed the standard rodent pellet diet and water. So this study was cleared by institutional animal ethical committee.

Anti-inflammatory activity

The animals were divided into 4 groups (n=6). Group-I served as control, received the vehicle only (4% gum acacia 1ml. /100g bw p.o). Group-II served as standard, received Aspirin (200mg/Kg p.o). Group-III and Group-IV served as test, received belladonna preparations low dose 6CH (1ml/100g bw), high dose 200CH (1ml/100g bw) respectively.

Carrageenan induced paw edema

The test was used to determine the anti-inflammatory activity of the Belladonna by the method of Winter et al (1962)⁷. The animals pretreated with Belladonna or Aspirin 1 hour before were injected with 0.1ml of 1% Carrageenan solution into the sub-plantar region of the right hind paw. Paw volume was measured by dislocation of the mercury column in a plethysmometer immediately after Carrageenan application at 0 hr and at the end of 3 hr after the stimulus. Reduction in the paw

volume compared to the control group animals was considered as anti-inflammatory response.

Rexin pellet induced granuloma

The test was performed on the rats using Rexin pellet induced granuloma method. The rats were anaesthetized under light ether anesthesia and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized Rexin pellet ($100 \pm 1\text{mg}$) was inserted in the groin area. All the animals received either Belladonna or Aspirin or vehicle (4% gum acacia) orally depending upon their respective grouping animals for 7 days from the day of rexin pellet insertion (Winter et al 1962)⁷. On the 8th day animals were sacrificed and rexin pellets were removed and dried to constant mass. The weight of the test and standard animal's pellets were compared with control animal pellets.

Statistical analysis

The results were expressed as mean \pm S.E.M. the difference between experimental groups was compared by one-way analysis of variance (ANOVA) followed by Dunnett's test. The results were considered statistically significant when the $p < 0.0001$.

RESULTS

Belladonna a homeopathic preparation is used extensively for pain and inflammatory conditions in homeo practice. so drug was selected to evaluate for anti-inflammatory activity in acute and chronic experimental animal models and the results are summarized in table:1 and 2.

Table-1
Effect of Belladonna on Carrageenan induced rat paw edema at '0' hr and at the end of '3' hr

Groups	Dose (Mg/Kg)	Paw Volume (ml)		% Inhibition
		0 Hr	3hr	
Control	4% Gum acacia	1.025 ± 0.002	0.752 ± 0.095	0
Standard	2% Aspirin(200mg/Kg)	0.952 ± 0.006	0.164 ± 0.012	78
Test-1	200CH Belladonna	1.129 ± 0.072	0.195 ± 0.023	76
Test-2	6CH Belladonna	1.162 ± 0.025	0.174 ± 0.033	74

Standard: Aspirin(200mg/kg.b.w), Test drug: Belladonna: 200CH, 6CH potencies(1ml/100g bw). Each value is the Mean±S.E.M. for 6 rats *P<0.05, **P<0.01, ***P<0.0001 compared with control. The belladonna preparation on Carrageenan induced paw edema in rats is shown in table 1. The results obtained indicates that the drug

found to have significant (p<0.001) anti-inflammatory activity in rats. The belladonna at the test doses 200CH, 6CH 1ml/100g b.w. reduced the edema induced by Carrageenan by 76,74% respectively at the end of 3rd hour, whereas standard drug showed 78% of inhibition as compared to the control group(no inhibition).

Table-2
Effect of Belladonna on Rixin pellet Granuloma in rats on 8th day

Groups	Dose (Mg/Kg)	Granuloma Dry Weight (Mg)	% Inhibition
Control	4% Gum acacia	89.75 ± 2.075	0
Standard	2% Aspirin(200mg/Kg)	60.33 ± 2.589*	32.77
Test-1	200CH Belladonna	62.83 ± 2.142*	29.99
Test-2	6CH Belladonna	61.41 ± 1.987*	31.57

Standard: Aspirin(200mg/kg.b.w), Test drug: Belladonna: 200CH, 6CH potencies(1ml/100g bw). Each value is the Mean±S.E.M. for 6 rats *P<0.05, **P<0.01, ***P<0.0001 compared with control.

The belladonna preparation was screened for rixin pellet-induced granuloma in rats and the results are shown in table 2. The drug inhibited 29.99 and 31.57% inhibition of granuloma formation at the doses 200CH, 6CH 1ml/100g bw respectively. Whereas standard drug aspirin showed 32.77% when compared with control group.(no inhibition).

DISCUSSION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plant products or homeo drugs still hold their own unique place, by the way of having no side effects. Therefore, a systemic approach should be made to find out the efficacy of homeo preparation against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, Phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into

arachidonic acid, which is highly reactive and is rapidly metabolised by cyclooxygenase to prostaglandins, which are major components that induce pain and inflammation⁸.

It is well known that Carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase(during the first 2hr after Carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase(3-4h after Carrageenan injection), kinin and prostaglandins are involved⁹. Our results revealed that administration of belladonna preparation inhibit the edema starting from the first hour and all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation. The rixin pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transudes, the dry weight of the pellet correlate with the amount of granulomatous tissues¹⁰. chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides¹¹. The Belladonna preparation showed less anti inflammatory activity in rixin pellet induced granuloma when

compared with acute study and found to be less effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increasing in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during the granuloma tissue formation.

There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation¹². Most of the anti inflammatory drugs exert their beneficial effects by inhibiting either release of these enzymes or by stabilizing lysosomal membrane, which is one of the major events responsible for the inflammatory process¹³. So we can assume that our drug, Belladonna might be acting by either inhibiting the lysosomal enzymes or

stabilizing the membrane. From above studies it is quite apparent that the Belladonna preparation possesses significant anti inflammatory activity in acute inflammation and less effective in chronic inflammation. The study justifies its use in acute inflammation as suggested in the folklore medicines.

CONCLUSION

Belladonna preparation(Dr. William Schwab India private limited) a homeopathic drug possess anti inflammatory activity more effectively in acute inflammatory conditions than chronic inflammation with low toxicity and better therapeutic index and compared with standard drug aspirin.

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