Original Article

INVESTIGATION OF ANTI-ARTHRITIC ACTIVITY OF SUSTAINED RELEASED TABLET OF DIFFERENT HERBAL PLANTS BY USING IN VIVO AND IN VITRO MODELS

Amita B Dongare[1], Dr. Rakesh Kumar Jatav[2]

[1,2] Department of Pharmaceutical Sciences, Dr APJ Abdul Kalam University, Indore.
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Abstract

Objective: According to statistics, one third of the population is statistically affected by arthritis, making it a very common disorder. The purpose of the current study is to determine the effectiveness of herbal plants in treating arthritis.

Methodology: The carrageenan induced arthritis model was used to evaluate the in vivo anti arthritic activity of sustained-release tablet of herbal plants. The in-vitro protein denaturation method was used to examine the anti-arthritic activity. The result was evaluated spectrophotometrically at 660nm against the reference drug diclofenac sodium. The sustained-release tablet of herbal plants was incubated with bovine serum albumin for complete denaturation.

Result: In contrast to the standard medication, diclofenac sodium, the results showed that the sustained-release tablet of herbal plants had significant anti-arthritic activity and that the extract inhibited protein denaturation at concentrations of 200 g/ml to 1000 g/ml.

Keywords: Anti-arthritic activity, inhibition of protein denaturation, in-vitro studies.

INTRODUCTION

In recent years, arthritis has become a disorder that affects people all over the world. Literally, it means joint inflammation. NSAIDs and other analgesics are primarily used in the management of arthritis because the disease can only be treated with symptomatic relief [1]. Gastric ulcers and other cardio vascular problems can result from using NSAIDs for an extended period of time [2].

Non-steroidal anti-inflammatory drugs (NSAIDS), corticosteroids, immunosuppressants, disease-modifying anti-rheumatic drugs (DMARD), and more recent biological agents like TNF- and monoclonal antibodies can all be used to treat arthritis. But one of their side effects is limitation. Consequently, there is a need to research complementary and alternative medicines that are effective, safe, less toxic, and affordable [3].

Herbal products can be used as a replacement therapy with little to no cost or side effects[4]. Polyherbal sustained released tablet contains four herbal plant extracts Trapa Bispinosa , Cassia Uniflora , Bosevilla Serrata and Cissus Quandragularis . The key ingredients and their scientific names are tabulated in table 1. In a study conducted using carrageenan – induced hind paw model, all ingredients of the formulation – trapa bispinosa [5] , cassia uniflora [6] , bosevilla serrata [7] and cissus quandragularis [8].is also proved to be a potent anti- inflammatory activity. Anti – arthritic activity of sustained released tablet was estimated using carageenan induced arthritis in rats [9].

MATERIAL AND METHODS

Polyherbal sustained released tablet contains four herbal plant extracts Trapa Bispinosa, Cassia Uniflora, Bosevilla Serrata and Cissus Quandragularis.

Table 1: Ingredients of Sustained released tablet

Ingredient	Scientific name
Shingada	Trapa Bispinosa
One leaf senna	Cassia Uniflora
Shallaki	Bosevilla Serrata
Hadsankal	Cissus Quandragularis

EXPERIMENT PROTOCOL

Evaluation of in vivo anti-arthritic activity [10,11]

Carrageenan-Induced Acute Inflammatory Model

Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay. Edema was induced by subplantar injection of 100 μL of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws of each rat of all the groups except the group I. Animals of group II and III were treated with the standard drug Diclofenac sodium and sustained released tablet respectively, 30 minutes prior to carrageenan injection. Paw thickness were measured just before the carrageenan injection, that is, at before injection and then at 1, 3, and 5th hour after carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at "0 hour" and paw thickness at respective hours.

Evaluation of in vitro anti-arthritic activity.

The inhibition of protein denaturation[12,13]. was the adapted methodology for in- vitro anti-arthritic activity. Concentrations chosen for study: 1000 – 200µg/ ml.

Standard: Diclofenac sodium

Chemicals: Phosphate Buffer Saline pH 6.3 0.5% Bovine serum albumin (BSA) – (5% w/v of aq solution).

Instruments required: Incubator spectrophotometer- 660 nm The following 4 solutions were prepared for the test.

TEST SOLUTION: 0.05 ml of test solution of various concentration was added to 0.45 ml of bovine serum albumin making the final volume of 0.5 ml.

TEST CONTROL: 0.05 ml of distilled water was added to 0.45 ml of bovine serum albumin to sum up the final volume to 0.5 ml.

PRODUCT CONTROL: 0.05 ml of test solution was added to 0.45 ml of distilled water to sum up the final volume to 0.5 ml.

STANDARD SOLUTION: 0.05 ml of standard diclofenac sodium of various concentration was added to 0.45 ml of bovine serum albumin to sum up the final volume to 0.5 ml.

All the samples were kept for incubation, for a period of 20 minutes at a temperature of 37ºc and later the temperature was raised to keep the samples at 57ºC for a period of 3 minutes. 2.5 ml of phosphate buffer was added to all the samples after cooling. The absorbance was measured at a wavelength of 660nm using UV- Visible spectrophotometer. The control represent 100% protein denaturation. The result obtained from the study was compared to standard value of diclofenac sodium.

The percentage inhibition was calculated by the formula: Percentage inhibition = 100 – [{(Optical density of test solution – Optical density of product control)/ Optical density of test} × 100].

Effect of Sustained released Tablet (TCBC) on Paw Volume Changes (ml) in Carrageen Induced Paw Edema in Rat

Table 2and Figure: 1, 2, 3, 4: Effect of Sustained released Tablet (TCBC) on Paw Volume Changes (ml) in Carrageen Induced Paw Edema in Rat.

Groups			**Treatment and Dose at mg/kg body wt. p.o**	**Mean Paw edema (ml) ±SEM**			
				Before injection	**1hr**	**3hr**	**5hr**
Group I Normal Control	**Tween 80 (1%)**	**1.15±0.03**	**2.24±0.15**	**2.45±0.09**	**2.06±0.19**		
Group II Std Drug	**Diclofenac Na 20**	**1.24±0.06**	**2.16±0.05**	**2.18±0.05***	**1.65±0.03***		
Test Group III TCBC 100 mg/kg	**TCBC 100 mg/kg**	**1.26±0.05**	**2.24±0.04**	**2.21±0.04***	**1.24±0.02***		
Test Group III TCBC 200 mg/kg	**TCBC 200 mg/kg**	**1.25±0.09**	**1.8±0.12***	**2.25±0.12***	**1.09±0.09***		

Whereas, SEM=Standard Error Mean All values are expressed as mean ±SEM (n=6) using the ANOVA followed by Dunnet's test. Result considered as significant at * *p <0.05 compared with control).*

Fig: 1Mean paw edema before injection

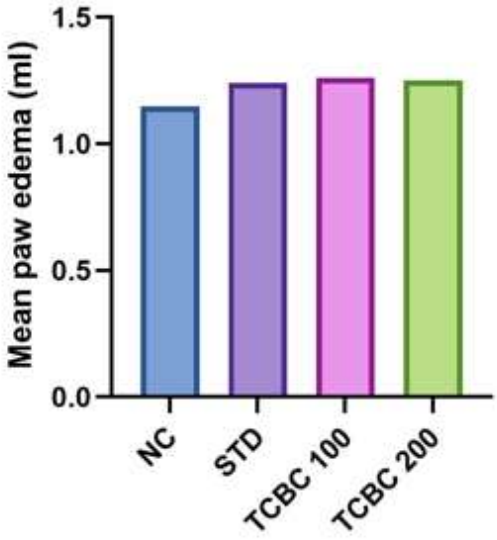


Fig: 2 Mean paw edema after 1 hour

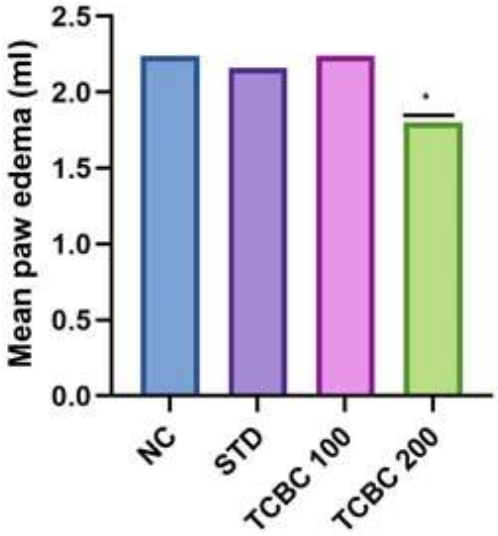


Fig: 3 Mean paw edema after 3 hours

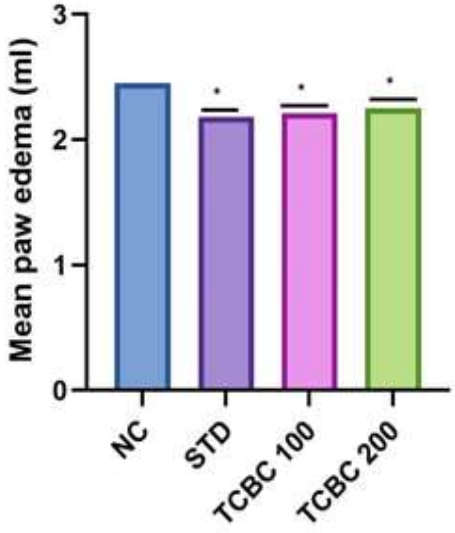
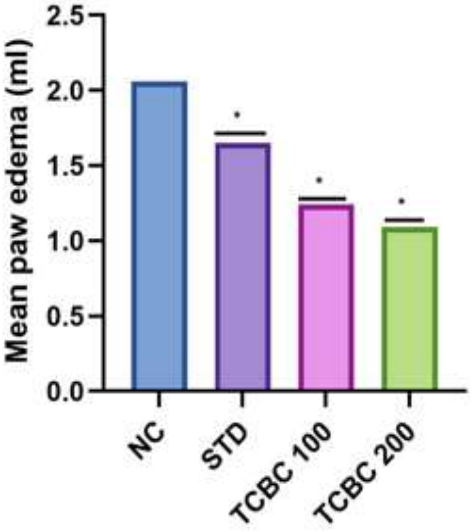


Fig: 4 Mean paw edema after 5 hours



Percentage inhibition of Protein Denaturation

Table 3 Percentage inhibition of Protein Denaturation exhibited by Sustained released tablet on comparison with standard

Sr no	Concentration µg/ml	Percentage inhibition of protein denaturation by diclofenac	Percentage inhibition of protein denaturation by TCBC Tablet
1	200	98.89 ±0.42	90.32±0.62
2	400	97.28 ±0.12	91.37±0.16
3	800	98.90±0.84	94.05±0.64
4	1000	99.95±0.43	93.20±0.46

The values are expressed as mean ± SD. N=3 All the values are expressed as Mean+ SEM (n=3), All the values are significant when compared to control $p < 0.05$

Fig: 5 % inhibition by diclofenac

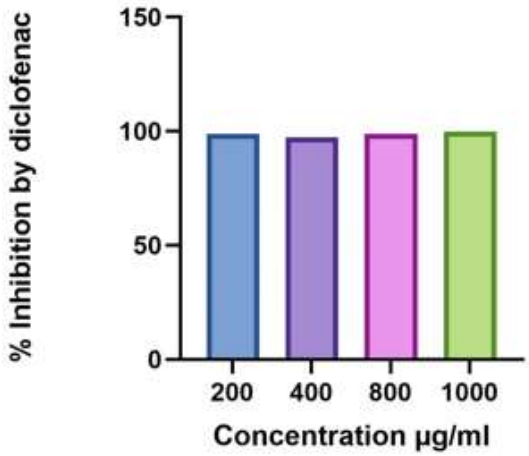
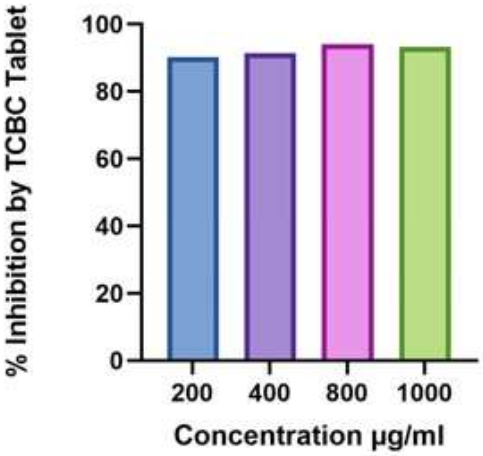


Fig: 6 % inhibition by TCBC tablet



RESULT AND DISCUSSION

The Sustained released tablet formulation has been shown to be anti-inflammatory in this study. A test that has significant predictive value for anti-inflammatory agents that act by inhibiting the mediators of acute inflammation reveals that the Sustained released tablet formulation significantly inhibits the rat paw edema that is induced by carrageenan. Inflammation caused by carrageenan can be used to identify oral anti-inflammatory agents.

The Sustained released tablet at the dose level of 200 mg/kg decreased the edema significantly (***p <0.001***) at 3 rd and 4thhrs after administration of the extract when compared to the control group. The effect was compared to the activity (***p <0.001***) produced by standard drug Diclofenac sodium at 3rd and 4th hr after administration.

By inhibiting protein denaturation, the sustained release tablet displayed significant anti-arthritic activity at 200-1000 g/ml. Comparing the sustained release tablet to the standard diclofenac sodium was used to investigate its effect. A number of studies indicate that protein denaturation is one of the causes of rheumatoid arthritis, which results in the production of autoantigens. At a concentration of 800 g/ml, the sustained release tablet has the greatest activity. The findings of the study suggest that sustained release tablets can be used to treat arthritis. The results are shown and tabulated.in Table 3and fig 5,6

CONCLUSION

The in vivo and vitro anti-arthritic study conducted on the polyherbal sustained released tablet concluded that the sustained released tablet exhibited significant anti- inflammatory activity and hence can be used effectively in the management of arthritis. The constituent responsible for the action need to be identified.

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