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# Effect of Sustained Released Tablet of Different Herbal Plants on Histone Deacetylase Enzyme-1 Level in Various Tissues of Wistar Rats

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## Keywords

Trapa bispinosa, Cassia uniflora, Bosevillaserrata, and Cissusquadragularis, anti-arthritic,Anti-inflammatory,Freundscompleteadjuvant,Pawedema.

## Abstract

**The aim of this work was to compare sustained-release tablets of different herbal plants. Antiarthritic activity of Trapa bispinosa, Cassia uniflora, Bosevillaserrata, and Cissus quadragularis. One or more joints can get inflamed with arthritis, causing aches, swellings, rigidity, cartilage damage, and erosion of the underlying bone. Researchers are looking for new potential anti anti-inflammatory treatment options because of the adverse side effects of steroidal and non-steroidal anti-inflammatory medications time-release tablets made from various herbal plants. Trapa bispinosa, Cassia uniflora, Bosevillaserrata, and Cissus quadragularis were tested for Histone Deacetylase 1 assay and anti-arthritic activity against Carrageenan, Histamine, and Freund's complete adjuvant-induced rat paw edema. Extended-release tablets showed substantial activity ($p<0.01$) against all inflammatory agents used in adose-dependent manner. Diclofenac sodium (20 mg/kg) was used as reference for comparison.**

## 1. Introduction

In recent times, clinically reported cases of arthritis are increasing. The second most common type of arthritis i.e rheumatoid arthritis is marked by chronic inflammation of the synovial joints and is considered as one of the severe auto immune disease. Subsequently irrevocable damage to the joints tissues and bone degradation are observed which lead to overall compromise in the quality of life and even cause disability. In the early stage, severity of arthritis can be of controlled form but eventually its progress can lead to RA or any other types of chronic arthritis. The etiology of arthritis can vary from hereditary to various environmental factors[1].

Various drugs used to treat arthritis and control the associated inflammation are reported to have multiple side effects. These

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adverse effects can range from hyperacidity to gastric ulcers, bleeding in the g.i.t, renal dysfunction and acute myocardial infarction. Therefore the search for new anti-arthritic drugs is focused towards limiting side effects while enhancing the efficacy.

Traditionally various crudes drugs have been explored and such attempts date back to the prehistoric era. Newer techniques of pharmaceutics are being applied to design drug delivery systems based on these nature derived drugs [2].

Ayurveda, the ancient Indian system of medicine has reported use of single or combination of multiple natural drugs for the management of arthritis. The same approach has been reported in Saldhar Samhita which dates back to 1300 AD. Such polyherbal formulations have been found not only to enhance the efficacy but they also report fewer adverse effects[3-5].

The present work describes a polyherbal formulation containing methanolic extracts of Trapa Bispinosa, Cassia Uniflora, Bosellia Serrata and Cissus Quandrangularis. A sustained release tablet of these extracts was prepared and evaluated.

## 2. Materials And Methods

**Plantmaterial :** Taxonomically identified *Trapa bispinosa*fruits, *Cassia uniflora* leaves, *Bosevilla serrata* leaves, and *Cissus quadrangularis* were obtained from herbal medicine supplier M/s. GU Traders, Pune).The collected plants are identified and certified at Sumatibhai Shah Ayurved Mahavidyalay. Twin 80, Complete Freund's Adjuvant (CFA) was purchased from M/s New Neeta Chemicals, Pune.

**Experimental Animals:**

Wistar rats with body weight ranging between 200-300gm were used for the studies as rats show rapid development of CFA induced arthritis[6]. The rats were kept under standard housing conditions and fed with a pellet animal diet. The Institutional Animal Ethics Committee authorised the experimental protocols.

***In Vivo* Anti-Arthritic Activity of Sustain Release Tablet Formulation:**

**Acute Oral Toxicity Study**

Acute toxicity studies were carried out initially and LD50 cut of dose of the mixture of extracts was found to be 1000 mg per kg body weight. Therefore, therapeutic doses were taken as 100 mg/kg and 200 mg/kg body weight [7,8].

**Antiarthritic Activity**

**Complete Freund' sadjuvant Induced Arthritisin Rats**

Six animals each made up each of the five groups that were created from the wistar rats..Animals received the following oral treatments on day 1: control group received a vehicle; three test groups received two different doses of the test drug (100, 200 mg/kg p.o.); and the standard group received a commercially available medicine for comparison. 12 or 1 hour after receiving the medication, each rat got a 0.1 ml injection of complete Freund's adjuvant in the sub-plantar region of the left hind paw. Body weight and paw volumes on both sides were measured using plethysmography before and

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after medication treatment. Once more, paw volume was measured using a plethysmometer on days 0, 5, and 21. (Orchid Scientific PLM02, Nashik, India). Dosing was discontinued from day 13 to day 21. Day 21's measurements of body mass and the degree of the secondary lesions were recorded [9].

**HDACASSAY; Effect of sustained released tablet of different herbal plants on Histone Deacetylase enzyme-1 level in various tissues of wistar rats.**

The experimental animals were divided into 2 groups for HDAC assay for each tissue. Test group received sustained release polyherbal formulation whereas control group received tween-80 (1%) vehicle for comparison. ELISA kit was used to measure HDAC1 levels[10].

**Preparation of reagent**

Before start of the procedure, all kits and samples were removed from refrigerator and brought to room temperature. The standard solution was suitably diluted to get following concentrations: 50, 25, 12.5, 6.25, 3.12 and 1.56 ng/ml. There was one more test tube that contained only diluent. Distilled water was used in 1:1 proportion to dilute the assay diluent A and B to make 12 ml volume for each. These diluted solutions were further used to dilute the assay diluent A and B about 100 times. 20 ml of wash solution was diluted with distilled water to make 600ml [10].

**HDAC Assay procedure**

Samples were added to wells containing 100 l of prepared standard dilutions (7 wells) and standard diluents (1 well as a blank), and the plate was sealed before being incubated for 2 hours at 37 °C. Each well's liquid was completely drained by cracking a plate onto absorbent paper. Each plate received 100:l of detection reagents A and B, which were then added and incubated for 1 hour and 30 minutes, respectively, before being sealed with plate sealer. Following the addition of each detection reagent A and B, the solution was aspirated and washed with 350 lwash solution for 3-5 min. Thereafter, 90:l of substrate solution was added to each well, and each well was incubated for 15–25 mins under a plate sealer cover. Upon the addition of the substrate solution, the liquid turned blue.

Finally, each well received 50 l of stock solution. After the solution was added, the liquid turned yellow. On the ELISA reader, the absorbance was instantly measured at 450 nm. [10].

**Statistical analysis**

ANOVA was used to check the statistical significance of the data using parametric and non parametric tests.

## 3. Results and Discussion

Sustained released polyherbal tablet formulation showed no mortality till a dose of 2gm/kg. Therefore, this level concentration of the sustained released polyherbal tablet was marked to be safe for chronic administration. Sustained released polyherbal tablet atdoses of 100 and 200 mg/kg significantly reduced edema at 3and4hours after sustained released polyherbal tablet administration compared to

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control group($p<0.001$).Effectswere compared to activity ($p<0.001$) produced by the standard drug diclofenac sodium ondays 3 and 4 after administration (Table 1).

**Table 1 : Rats with arthritis caused by CFA and the effect of a sustained release tablet formulation on paw volume.**

| Group | Treatment (mg/kg) | Paw volume (ml) | | |
|---|---|---|---|---|
| | | Day 0 | Day 5 | Day 21 |
| I | Normal Control | 0.041±0.02 | 0.066±0.03 | 0.041±0.04 |
| II | Arthritic Control | 1.5±1.50**** | 2.7±2.70**** | 3.56±3.56**** |
| III | Standard | 1.64±1.64**** | 0.85±0.85**** | 0.17±0.17**** |
| IV | F1 Formulation (TCBC 100mg) | 1.92±1.92**** | 1.44±1.44**** | 0.81±0.81**** |
| V | F2 Formulation (TCBC 200mg) | 1.72±1.72**** | 1.36±1.36**** | 0.36±0.36**** |

Values are expressed as mean ± SEM (n = 6), **** $p < 0.0001$ compared with arthritic control. Data was analysed using one-way ANOVA followed by Dunnett's multiple comparison test**.**

***Fig 1 :*** **Rats with arthritis caused by CFA and the effect of a sustained release tablet formulation on paw volume.**

***Fig 2*: Paw volume on day 0**

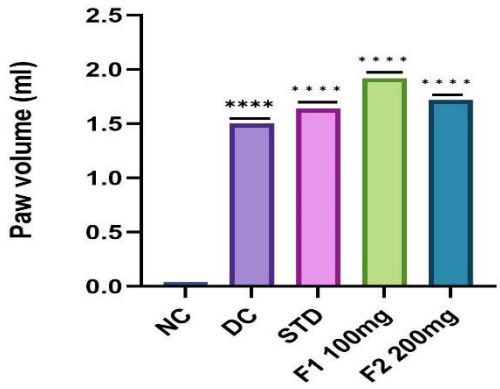


***Fig 3*: Paw volume on day 5**

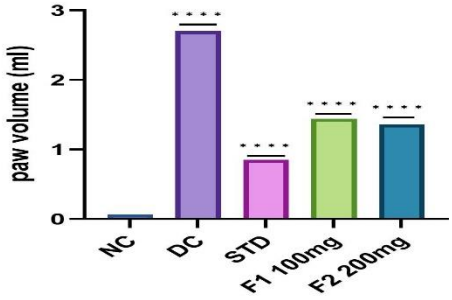


***Fig 4***: Paw volume day 21

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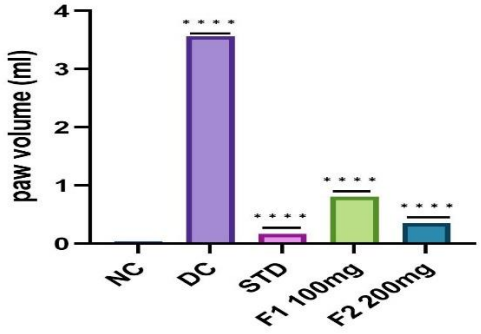


Changesinratpawvolumewererecordedonday s0,5and21afteradministration of the herbal sustained release tablet formulations (100 mg/kg and 200 mg/kg) from days 1 to 12 (Table 1). The arthritic control group showed signs of arthritic progression as seen by increased paw volume. Twenty-one days after CFA induction, a significant ($p<0.0001$) decrease in rat paw volume was observed in the standard diclofenac sodium treatment group and the extended release tablet treatment group. The most significant reduction in paw volume results was seen when compared to the standard extended release tablet formulation (TCBC 200 mg/kg) in the

Diclofenac sodium group containing F2. On the other hand, the F1 formulation (TCBC 100 mg/kg) was found to be less significant compared to the standard diclofenac sodium group. CFA-induced arthritis is the most widely used model, with clinical and pathological changes comparable to those observed in human rheumatoid arthritis.

**ELISA Reader Test Impact on Rats' Histone Deacetylase enzyme 1 Level**

**Table 2 and Figure 1 Impact of Sustained Released Tablet on Rats' ELISA Reader Test Measurement of Histone Deacetylase Enzyme 1 Level**

| Group | Treatment and dose at mg/kg body wt .p.o. | Tissues | | |
|---|---|---|---|---|
| | | Paw | Uterus | Lungs |
| Group I<br>Normal Control | Tween 80 (1%) | 4.1±0.240 | 1.147±0.0 | 0.587±0.03 |
| Group II<br>TCBC 200 mg/kgN=6 | TCBC 200 mg/kg | 2.54±0.03 | 2.45±0.17 | 1.83±0.7 |

All values are expressed as mean ±SEM (n=3) using the ANOVA followed by Dunnet's test. Result considered as significant at * $p \leq 0.05$, ** $p \leq 0.01$compared

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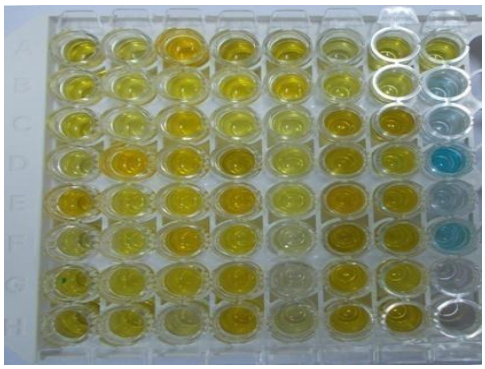
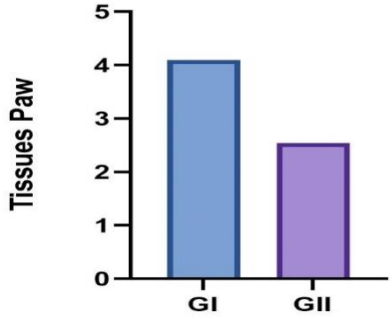
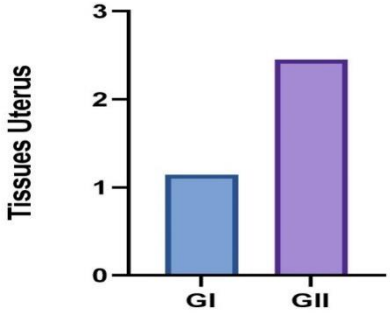
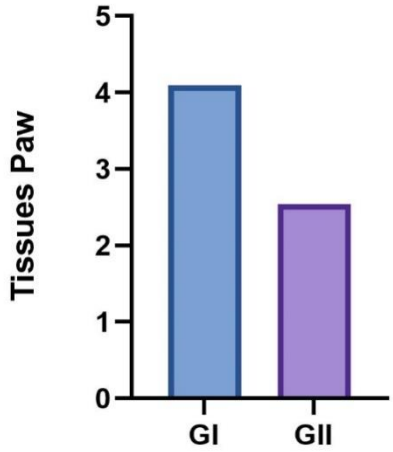
**Fig 5:** ELISA Reader Test Impact on Rats' Histone Deacetylase enzyme 1 Level

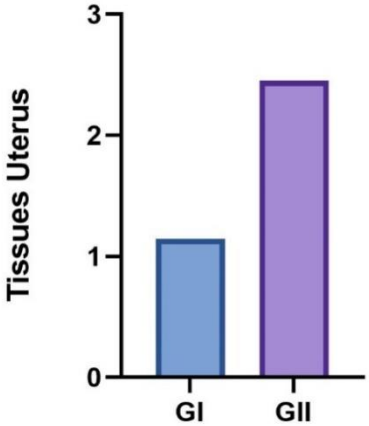
**Fig 6: Effect on HDAC1 Level inFig 7: Effect on HDAC1 Level in**





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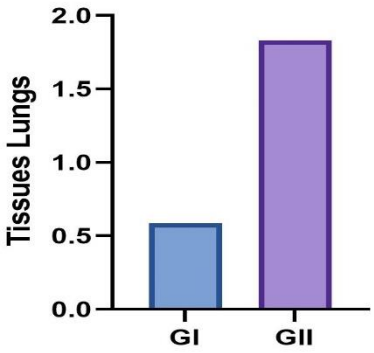


**Paw tissues Rats  Uterus tissues of Rat**

**Fig 8: Effect on HDAC1 Level in Lungs tissues of rat**

After receiving 200 mg/kg of TCBC, there was a considerable drop in HDAC1 levels in the rat paw tissue, suggesting that the medicine may work by inhibiting HDAC1. Although it does not affect inflammatory processes, sustained-release tablet 200 mg/kg dosing caused an increase in HDAC1 levels in the uterine tissue of wistar rats, suggesting the medication's potential to treat infertility. HDAC1 levels are markedly raised by a sustained-release pill at a dose of 200 mg/kg. To better understand the mechanism of HDAC inhibition, additional in-depth chronic investigations are required.

After stimulation with various stimuli, it has been demonstrated that histone deacetylase (HDAC) inhibition controls gene expression and cytokine production. In vitro, pro-inflammatory cytokine synthesis and/or activity is decreased by HDAC inhibitors, and they also have significant impacts on animal models of inflammatory illness.

## 4. Conclusion

The current study on sustained-release tablets of these plants—Trapa bispinosa, Cassia uniflora, Bosevilla serrata, and Cissus quadragularis—showed that these plants have significant anti-inflammatory and antiarthritic properties, which supports their traditional use as a remedy for a variety of pains and inflammation.

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