

# **Ventoluft Polyherbal formulation modulates Freund's Complete Adjuvant induced arthritis in experimental rats**

## **Research Article**

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## **Abstract**

**Background:** *Ventoluft* Polyherbal formulation composes multiple traditional medicinal plants composed of various anti-inflammatory agents. Hence, the present study aimed to investigate the effect of *Ventoluft* Polyherbal formulation on Freund's complete adjuvant-induced arthritic male Wistar rats. **Materials and methods:** The study included thirty-six rats, containing six in each. Arthritis was induced by injecting FCA (0.1ml) in joints of the right sub-plantar region of rats in negative control and all test groups i.e. (20, 40, and 80mg/kg p.o) for 28 days except normal group. A positive control, indomethacin (10mg/kg, i.p.) was also tested for 28 days. During the treatment, paw volume was measured weekly once. At the end of the experiment, physical, hematological, and biochemical effects were evaluated. **Results:** Administration of *Ventoluft* at the doses of 20, 40, and 80 mg/kg b.w. p.o exhibited statistically significant ( $p < 0.001$ ) inhibition of the paw edema and joint swelling, along with an increase in body weight, decrease in rheumatoid and spleen index factors. **Conclusion:** The results from hematological and biochemical parameters have shown that *Ventoluft* possesses potent anti-arthritic activity, Results obtained in the present study indicate that *Ventoluft* formulation has exhibited, dose-dependent improvement in FCA induced rheumatoid arthritis.

**Key Words:** Anti-arthritic activity, Freund's Complete Adjuvant, Rheumatoid arthritis, Paw edema, Ventoluft.

## **Introduction**

Arthritis is a chronic autoimmune disorder that causes synovial inflammation by severely affecting synovial joints and destroys the bone. Arthritis primarily attacks the joints rather than muscles and organs which leads to the destruction of cartilage (1). RA affects roughly 0.5 percent to 1.0 percent of people worldwide, and women are more likely than men to be affected. RA has also been associated with a high death rate (2). Immunization adjuvants essentially increase the immunological response. On the other hand, the right selection of adjuvants based on their attributes and side effects is crucial. Among the numerous classifications of adjuvants, Complete Freund's adjuvant is the most preferred adjuvant for testing the immunogenicity of mouse antigen (3). Because it closely resembles human physiological nature, Freund's complete adjuvant model is one of the most commonly rheumatoid arthritis

animal models which causes synovial hyperplasia, leukocyte infiltration, and cartilage deterioration (4, 5).

The most frequent treatments for rheumatoid arthritis include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs), as well as biological. However, these drugs' effectiveness is impeded by significant side effects, encouraging arthritis patients to look for alternatives (6). Indian traditional medicine and Chinese medicine are chosen for arthritis treatment (7-8). Ayurveda literature, on the other hand, has represented polyherbal (9). Blended extracts of individual plants have more activity than individual extracts, according to studies (10).

*Ventoluft* is a polyherbal formulation that includes sixteen herbal compounds (*Pluchea lanceolata*, *Boerhaavia diffusa*, *Tribulus terrestris*, *Tinospora cordifolia*, *Balsamodendron mukul*, *Ricinus communis*, *Withania somnifera*, *Pueraria tuberosa*, *Salmalia malabarica*, *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia belerica*, *Zingiber officinale*, *nPiper nigrum*, *Piper longum* and *Acorus calamus* produced by Progen Research Lab in Belagavi for the treatment of arthritic patients. Its safety and effectiveness have not been scientifically tested using pre-clinical study. Hence, the present study aimed to investigate the effect of *Ventoluft* formulation in FCA-induced arthritis by evaluating paw edema, joint

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diameter, haematological parameters, spleen index, and hepatic enzymes.

## Materials and methods

### Animals and experimental design

Male Wistar rats were procured through In-vivo Bioscience, Bangalore. The animals were housed at cages with stainless steel furnished with paddy husk, at suitable temperature and humidity. The rats were feed with food and water over 7 days. The experimental protocol was previous authorized by the Institutional Animal Ethics Committee, KLE College of Pharmacy, Belagavi. Resolution No. *KLE COP/CPSEA Reg. No. 221/ Po/Re/S/2000/CPSEA, Re.28-12/10/2019*. All the experiments were conducted with firm guidelines as per “Committee for the Purpose of Control and Supervision of Experiments on Animals”.

### Study design and grouping of animals.

The animals were divided into 6 groups of six animals each as follows:

- Group I: Normal (Received normal saline)
- Group II: FCA group (Injected with 0.1ml with FCA into the sub-plantar region of right hind paw)
- Group III: Drug-treated animals: received *Ventoluft*(20mg/kg, p.o).
- Group IV: Drugs treated animals: received *Ventoluft*(40mg/kg, p.o).
- Group V: Drug-treated animals: received *Ventoluft*(80mg/kg, p.o).
- Group VI: Standard treated animals: received Indomethacin (10mg/kg, i.p).

Each rat was injected with 0.1ml of Freund’s complete adjuvant (FCA) into the sub-plantar region of the right hind paw. Anti-arthritic *Ventoluft* was evaluated on physical parameters such as joint diameter, bodyweight, and paw edema, once a week. On the 29<sup>th</sup> day, the blood was withdrawn from retro-orbital puncture for assessing hematological (WBC, RBC, Hb, and ESR) and biochemical (AST, ALT, RF, and CRP) parameters, later there were studied for joint histology.

### Physical Parameters

Paw volume and joint diameter were performed using Plethysmometer and Vernier Calipers respectively. Further, Spleen Index was calculated using the following formula:

### Estimation of biochemical and hematological parameters

At the end of the study, Rheumatoid Factor level, CRP, SGOT, and SGPT were evaluated. Further, Hematological parameters like RBC, WBC, Hb level, and ESR were also quantified.

### Histopathological studies of joints.

At the end of the study, ankle joints were removed from the hind paw of the animals and were isolated and kept in 10% formalin. The organs were

later stained with hematoxylin-eosin [11]. Later, the sections were examined using a light microscope.

### Statistical analysis

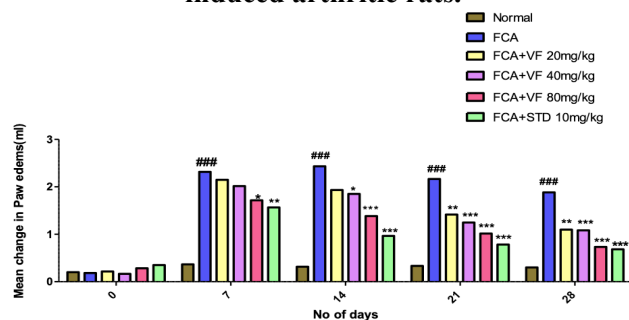
The results were expressed as mean  $\pm$ SEM. Statistical comparison was made between drugs treated groups and arthritic-control groups. Result data such as (Hematological, Biochemical parameters, and spleen index) was calculated using one-way ANOVA followed by Turkey’s comparison test. Data of repeated measures (Paw edema, Joint Diameter, Bodyweight) were analyzed using two-way ANOVA followed by “Bonferroni Post-Test” for multiple comparisons. Statistical analysis was done using “*Graph Pad Prism5*”. P-value  $P < 0.05$  was considered statistically significant.

## Results

### Effect of *Ventoluft* on paw edema and Joint Diameter

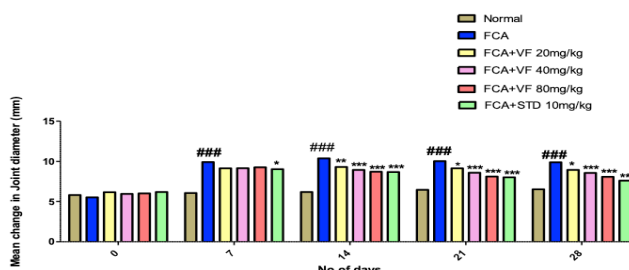
On the 0th day, all groups had their paw volume and joint diameter measured. On the seventh day, the FCA-induced group had a significant increase in paw volume and joint diameter ( $P < 0.001$ ) when compared to the normal. On the third and fourth weeks, the treatment groups, as well as the STD group, demonstrated a reduction in paw edema and joint diameter as compared to the FCA group. When compared to the FCA treated group, treatment groups showed dose-dependent reductions in paw edema and joint swelling. Figures 1 and 2 depict the data.

**Fig: 1. Effect of *Ventoluft* on Paw Edema in FCA induced arthritic rats.**



Values are expressed in Mean  $\pm$  SEM; n=6; Analysed by two-way ANOVA “Bonferroni Post Test” for multiple comparisons tests. ### $P < 0.001$  when compared to normal, \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$  when compared to FCA, FCA: Freund’s Complete Adjuvant; VF: *Ventoluft* formulation

**Fig: 2. Effect of *Ventoluft* on Joint Diameter in FCA induced arthritic rats.**

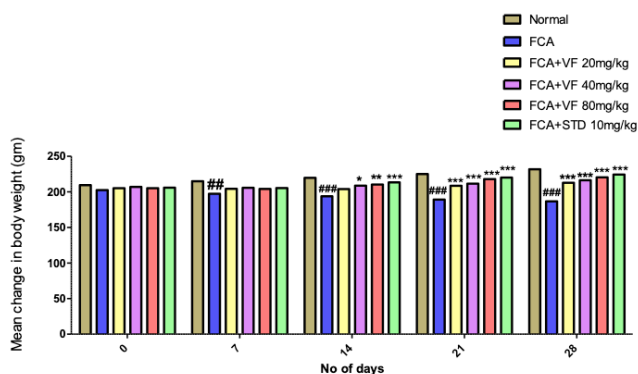


Values are expressed in Mean  $\pm$  SEM; n=6; Analysed by two-way ANOVA “Bonferroni Post Test” for multiple comparisons. ###P<0.001 when compared to normal, \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 when compared to FCA. FCA: Freund’s Complete Adjuvant; VF: *Ventoluft* formulation.

**Effect of *Ventoluft* on Body Weight in rats**

From the 7th day until the end of the study, the FCA-induced group had a lower body weight (P< 0.001) than the control group. Since 14 days, the bodyweight of disease control rats has gradually declined. Treatment with *Ventoluft* (20, 40, and 80 mg/kg p.o) in combination with the STD group resulted in a considerable and dose-dependent weight gain. Figure 3 depicts the data.

**Fig. 3. Effect of *Ventoluft* on Body Weight in FCA induced arthritic rats.**

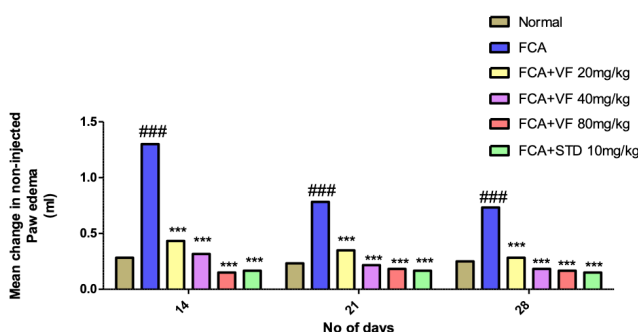


Values are expressed in Mean  $\pm$  SEM; n=6; Analysed by two-way ANOVA “Bonferroni Post Test” for multiple comparisons. ##P<0.01 and ###P<0.001 when compared to the normal group, \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 when compared to FCA, FCA: Freund’s Complete Adjuvant; VF: *Ventoluft* formulation

**Effect of *Ventoluft* on non-injected left Paw Edema**

On the 14th day, the latent secondary swelling that emerges after a few days of joint pain in the Wistar rat’s left paw became visible. When compared to the arthritic control group, the treatment of *Ventoluft*(20, 40, and 80 mg/kg p.o) in combination with STD considerably (P <0.001) diminished joint secondary edema. Figure 4 depicts the data.

**Fig. 4. Effect of *Ventoluft* on non-injected left Paw Edema (secondary response) in FCA induced arthritic rats**



Values are expressed in Mean  $\pm$  SEM; n=6; Analysed by two-way ANOVA “Bonferroni Post Test” for multiple comparisons,###P<0.001 when compared to the normal group, \*\*\*P<0.001 when compared to FCA, FCA: Freund’s Complete Adjuvant; VF: *Ventoluft* formulation

**Effect of *Ventoluft* on hematological parameters**

Table 1 shows that the FCA-induced rats had a lower RBC, Hb level, and a higher ESR and WBC count compared to normal. All of these indicate that anemia is a prevalent occurrence among chronic arthritic patients (12). In comparison to the arthritic control, the selective dosages of *Ventoluft* 80mg/kg p.o treated group, as well as the STD group, showed a significant (P< 0.001) increase in RBC and Hb, as well as a significant drop (P<0.001) in elevated ESR and WBC count levels. When *Ventoluft*(20mg/kg and 40mg/kg p.o) was compared to FCA, no significant differences were found.

**Table 1: Effect of *Ventoluft* on hematological parameters in FCA-induced arthritic rats.**

Groups	WBC (1000/mm <sup>3</sup> )	RBC (millions/mm <sup>3</sup> )	Hb (g/dl)	ESR (mm/hr)
Normal	7.373 $\pm$ 0.323	5.687 $\pm$ 0.3657	13.81 $\pm$ 0.3284	4.207 $\pm$ 0.02728
FCA	11.16 $\pm$ 0.4313####	3.167 $\pm$ 0.04333###	8.203 $\pm$ 0.3353####	7.267 $\pm$ 0.1875###
FCA+VF 20mg/kg	11.11 $\pm$ 0.3564	3.433 $\pm$ 0.2639	9.64 $\pm$ 0.4349	7.37 $\pm$ 0.09609
FCA+VF 40mg/kg	9.84 $\pm$ 0.6857	3.833 $\pm$ 0.2564	10.07 $\pm$ 0.08544*	6.767 $\pm$ 0.2488
FCA+VF 80mg/kg	8.06 $\pm$ 0.4609**	4.577 $\pm$ 0.2307*	12.24 $\pm$ 0.1715***	6.047 $\pm$ 0.4258*
FCA+STD 10mg/kg	8.1 $\pm$ 0.05033**	4.627 $\pm$ 0.309*	12.38 $\pm$ 0.4898***	5.71 $\pm$ 0.2548*

Values are expressed in Mean  $\pm$  SEM; n=6; Analysed by one-way ANOVA “Turkey’s multiple comparison test”, ###P<0.001 when compared to the normal group, \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 compared to FCA, FCA: Freund’s Complete Adjuvant; VF: *Ventoluft* formulation

**Effect of *Ventoluft* on biochemical parameters**

When compared to control rats, all arthritic rats had elevated levels of AST and ALT. In comparison to the STD group, the *Ventoluft* treated animals (at dosages of 40 and 80 mg/kg) demonstrated a significant (P <0.001) reduction in elevated liver enzymes. When arthritic animals were given Freund's adjuvant, there was a statistically significant (P< 0.001) rise in RF and CRP levels in the FCA-induced group compared to the control group. The *Ventoluft* formulation had a similar effect to the STD medication group. When compared to FCA, there were no significant differences in CRP values in the treatment groups. When compared to the FCA-induced group, the *Ventoluft* and STD group demonstrated a statistically significant (P <0.001) drop-in RF. On all of the biochemical markers, no significant



changes were seen in the lower treatment group, which is *Ventoluft*(20mg/kg); Table 2.

**Table 2: Effect of *Ventoluft* on Biochemical parameters in FCA induced arthritic rats**

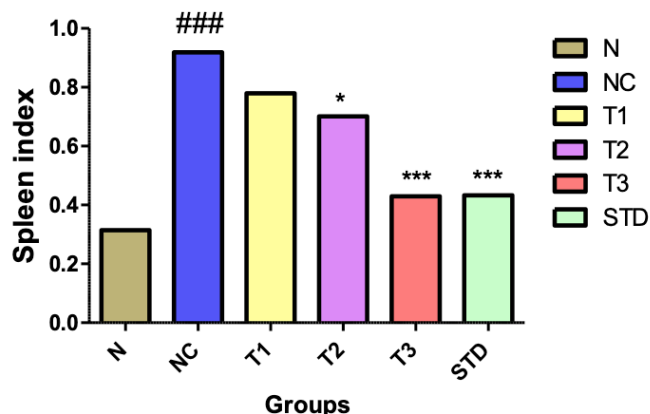
Groups	AST (U/L)	ALT (U/L)	CRP (mg/dl)	RF (IU/L)
Normal	112.9±5.474	54.29±4.229	1.48±0.2337	5.328±0.2981
FCA	159.6±3.71####	85.76±5.454####	3.672±0.1574### #	11.33±0.4347### #
FCA+V F 20mg/kg	137.8±2.825**	79.45±4.18	3.282±0.139	9.778±0.7847
FCA+V F 40mg/kg	136.1±2.31**	75.82±3.794	3.175±0.1763	7.397±0.2697** *
FCA+V F 80mg/kg	130.3±5.053***	67.9±2.562*	2.718±0.266*	7.372±0.3448** *
FCA+S TD 10mg/kg	121.8±3.562***	63.63±2.479**	2.865±0.257	7.083±0.2406** *

Values are expressed in Mean ± SEM; n=6; Analysed by one-way ANOVA “Turkey’s multiple comparison test”, ###P<0.001 when compared to the normal group, \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 when compared to FCA, FCA: Freund’s Complete Adjuvant; VF: *Ventoluft* formulation

**Effect of *Ventoluft* on Spleen Index**

When compared to the control group, the FCA group had a significant increase (P<0.001) in the spleen index. When compared to FCA, the *Ventoluft* (80mg/kg) and STD groups showed a significant drop in the spleen index. (*Ventoluft* 20 and 40mg/kg) showed no significant differences. Figure 5 depicts the data.

**Fig 5: Effect of *Ventoluft* on Spleen index in FCA induced arthritic rats.**

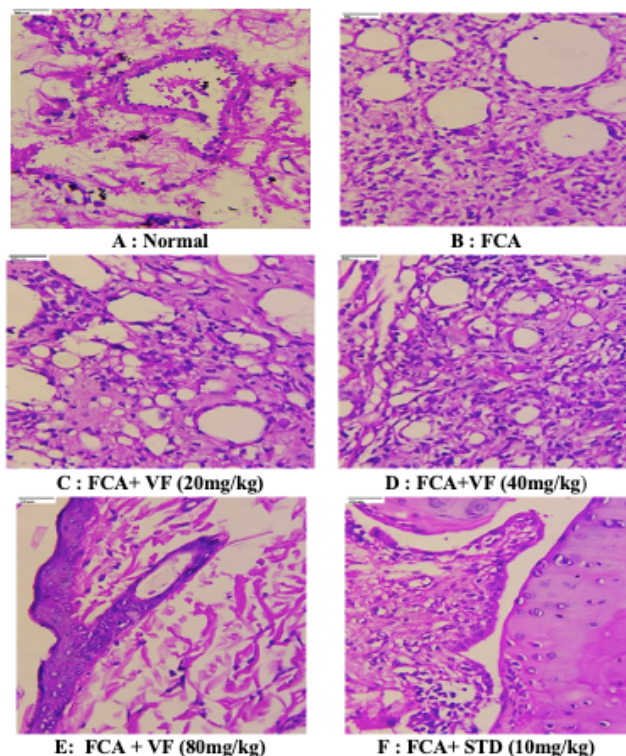


Values are expressed in Mean ± SEM; n=6; Analysed by one-way ANOVA “Turkey’s multiple comparison test”, ###P<0.001 when compared to the normal group, \*P<0.05 and \*\*\*P<0.001 when compared to FCA. FCA: Freund’s Complete Adjuvant; VF: *Ventoluft* formulation

**Effect of *Ventoluft* histopathological changes of ankle joint tissues**

The right hind ankle joints were extracted for histological examination on the 29th day after initial treatment. The rats in the control group exhibited no signs of injury (Fig. 6A). FCA rats’ right ankle joints showed significant structural alterations, including pannus development, synovial hyperplasia, bone erosion, and cartilage degeneration. (See Fig. 6B.) Oral treatment of *Ventoluft*(20, 40, 80 mg/kg) reduced histopathological alterations and joint histological damage scores as compared to the FCA group (Figs. 6C,6D, and 6E). *Ventoluft*-treated rats had considerably Lower joint and histological damage scores, as demonstrated in (Fig.6E). These findings suggested that the *Ventoluft* formulation has an anti-arthritis impact; Figure 6.

**Figure 6: Histopathology of joints**



**Discussion**

The purpose of this study was to see how the *Ventoluft* formulation affected FCA-induced arthritis in male Wistar rats. FCA-induced arthritis is associated with increased paw edema and joint diameter, according to the data provided in this study. FCA-induced arthritis causes paw edema and increases joint width in rats, which is consistent with previous studies (13-15). Secondary swelling on the Wistar rat’s non-injected left paw which was observed on the 14<sup>th</sup> day was decreased in *Ventoluft* treated rats. These observations suggest that *Ventoluft* has a synergistic effect in reducing paw volume due to secondary reaction. Decreased cellularity of synovial tissue is the major cause of decreased paw edema and joint diameter in *Ventoluft* treated rats (16). Furthermore, multiple experimental studies on rats, as well as our research, show that the arthritic group had a

lower body weight. It has previously been established that decreased body weight during inflammation is caused by inadequate nutrient absorption through the colon. Chronic *Ventoluft* therapy of arthritic mice promoted body weight loss (13). Hepatic involvement is shown in arthritic patients with increased aminotransferases like AST and ALT, indicating the presence of inflammation, as shown in the present rat studies (17). The current study findings show that arthritic groups had greater levels of AST and ALT. However, in rats, the *Ventoluft* treated groups, as well as the STD group, showed a significant drop in AST and ALT levels, indicating that the illness status had improved. Rheumatoid factors (RF) possess a key role in the etiology of rheumatoid arthritis, according to several clinical investigations (18).

Researchers believe that RF is important for the severity of RA in people who have been exposed to FCA (19). According to current research, FCA-induced groups have a lower RBC count, a lower Hb level, and a higher ESR. Furthermore, these characteristics point to anemia, which is common in RA patients (20). When administered with the *Ventoluft* formulation, anemia and arthritis symptoms improved, indicating that the test medication has anti-arthritic properties. In clinical investigations, the erythrocyte sedimentation rate (ESR) is a critical diagnostic tool for detecting the progression of inflammation (21). ESR levels are higher in FCA-induced mice. Reduced levels in the *Ventoluft* treated and STD groups, on the other hand, suggest anti-inflammatory capabilities. Several additional important indicators indicate the severity of inflammation. CRP is one of them, and it contributes to the diagnosis of rheumatoid arthritis disease. CRP levels are increased in RA patients, which is consistent with current rat research. Increased CRP levels cause bone injury, according to clinical data (22). CRP levels were recovered in the *Ventoluft*-treated groups. Arthritis patients, on the other hand, might develop a variety of problems, including Felty's syndrome, which causes an enlarged spleen. Furthermore, we have had to estimate the spleen index in the current investigations. The spleen index was found to be lower in the treated groups, indicating that it is effective in reversing splenomegaly (23).

Necrosis was not present in normal rats, and there was no evidence of lymphocytic infiltration. However, FCA-induced rat necrosis was validated by a massive inflow of inflammatory cells, synovial hyperplasia, and greater levels of necrosis. The regular connective tissue of the tibiotarsal joint was present in the treatment groups, combined with decreased levels of edema.

Even though the *Ventoluft* formulation has the potential to have a greater effect than individual plant extracts, it has a positive and constructive way, including a lack of knowledge about which chemical constituents are responsible for the rheumatic activity. However, the findings of this study show that the *Ventoluft* formulation improves FCA-induced rheumatoid arthritis in a dose-dependent manner.

It has been demonstrated that traditional medicinal plants act by targeting multiple proteins and regulating various pathways concerned with infectious and non-infectious pathogenesis (24-28) based on the principle of the regulation of multiple proteins by a single compound (29,30). Hence, it would be required to identify the interaction of the multiple bioactives with their respective targets of the *Ventoluft* formulation to propose the mechanism of action for anti-arthritic activity.

## Conclusion

Remarkably, *Ventoluft* like Indomethacin significantly slowed the progression of arthritic illness in our current investigation. The capacity of *Ventoluft* to considerably diminish arthritic edema in injured tissues is determined by its therapeutic activity. Even though *Ventoluft* is effective in arthritis patients, the relevance of the model utilized in this study reflects pathological changes in humans, indicating that *Ventoluft* might be classified as a novel therapeutic agent in the treatment of arthritis.

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## Conflict of interest

The authors declare no conflict of interest

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Nil

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