Original Article

Preclinical evaluation of antiarthritic activity of ultra-diluted preparations of *capsaicin alkaloids* (CP-10), tumor necrosis factor-alpha, and *Magnesium phosphoricum* in wistar rats

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Abstract

Background: The use of animal models in the development of new medicine and research is common in the conventional medicine. Animal model for new drug discovery and efficacy testing for preclinical research is explored in Homoeopathy only by a few. This study explores the possibility to test in a controlled way the effects of homoeopathic remedies on experimental model of acute inflammation in rats. **Methods:** Wistar rats were divided into seven groups (seventy rats of 6–8 weeks' age); medicines were evaluated by oral administration in the Complete Freund's adjuvant (CFA)-induced arthritis. Two new homoeopathic preparations, a combination of *Capsaicin and Dihydrocapsaicin* (CP-10), tumor necrosis factor-alpha (TNF-α), and one existing homoeopathic medicine, *Magnesium phosphoricum* (0.6 ml oral), were evaluated against vehicle control using *Diclofenac* as a standard. Edema was measured using a water-based plethysmometer, before and at different times after arthritis induction. **Results:** *Magnesium phosphoricum* showed good results, almost similar to *Diclofenac* at days 7 and 21, whereas CP-10 and TNF-α showed nonsignificant results. The body processes reversed the inflammatory condition on day 7 onward indicated by similar paw volume of all the treatments. Arthritic index was higher with negative control, which was decreased by CP-10 although nonsignificantly on days 7 and 21. *Diclofenac* and *Magnesium phosphoricum* showed significant reduction in arthritic index on days 7 and 21. **Conclusion:** Ultra-diluted homoeopathic preparations of *Magnesium phosphoricum* exhibited definite antiarthritic activity. The same could have been confirmed studying the levels of inflammatory biomarkers in a study with longer treatment period.

Keywords: Arthritic index, *Capsaicin*, Complete Freund's adjuvant, *Magnesium Phosphoricum*, Tumor necrosis factor-alpha, Homoeopathy, Ultra-dilute potency

Introduction

Homoeopathy is a system of alternative medicine based on the principle of "like cures like" Homoeopathy rests on the premise of treating diseases with extremely ultra-diluted medicinal agents that, in undiluted doses are deemed to produce similar disease symptoms in healthy individuals. Acceptance in the effectiveness of Homoeopathy in general is widespread and growing among the physicians and public.[1]

The present study was undertaken to investigate the antiarthritic, anti-inflammatory effect of three homoeopathic preparations in ultra-dilute 30C potencies in animal model: (1) a combination of *Capsaicin* and *Dihydrocapsaicin,* (2) tumor necrosis factor-alpha (TNF-α), and (3) *Magnesium phosphoricum*. For blinding, the formulations were coded as formulation I, II, and III, respectively. *Capsaicin* and *Dihydrocapsaicin* are the plant alkaloids, potentized up to 30c potency using a standardized potentizer.[2] TNF-α (cachexin or cachectin) is a cell signaling protein (cytokine) involved in systemic inflammation. This particular preparation is sourced from human TNF-α procured from Sigma Aldrich. Homoeopathic potencies were prepared by a serial dilution of 1:99, where one part of the drug substance is mixed with 99 parts of vehicle (alcohol) and exposed to ten mechanical strokes, to make 1c potency. Again, one part of 1c potency is mixed with 99 parts of vehicle to undergo the same process to arrive at

Access this article online

Quick Response Code:

Website: www.ijrh.org

DOI: 10.4103/0974-7168.200848

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How to cite this article: Shah R, Sathaye S. Preclinical evaluation of antiarthritic activity of ultra-diluted preparations of *capsaicin alkaloids* (CP-10), tumor necrosis factor-alpha, and *Magnesium phosphoricum* in wistar rats. Indian J Res Homoeopathy 2017;11:34-40.

2C potency. Likewise, 30C, 200C, and more potencies were prepared.

In this study, the antiarthritic activity of the three potentized (30C) medicines was compared with *Diclofenac* (10 mg/kg, source- Sigma Aldrich). The selection of medicines was based on the fundamental principle of Homoeopathy "like cures like" and the phenomenon of hormesis, which state that the substance known to produce inflammation or pain may have built-in capacity to relieve the same, if administered in small dose. The author had done anecdotal work using the new homoeopathic preparations used in this study, where he had observed activity against inflammation and arthritis.

Materials and Methods

Animals

Male and female Albino Wistar rats (180–200 g) were procured from Haffkine Bio Pharmaceutical, Parel, Mumbai, and housed under a controlled environment (room temperature: 23 ± 2°C, relative humidity: 60 ± 5%, 12 h day/night cycle) with balanced diet and water *ad libitum*. The animals were acclimatized for a period of 1 week and were kept under pathogen-free conditions. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Sciences and Technology, ICT, Mumbai (ICT/IAEC/2012/P-39).

Complete Freund's adjuvant-induced arthritis

Arthritis was induced in rats by injecting 0.1 ml of fine emulsion of complete Freund's adjuvant (CFA) in olive oil (1:2, v/v) (at a concentration of 0.25 mg heat-killed *Mycobacterium tuberculosis* per milliliter of emulsion) in the subplantar surface of rats' left hind foot.[3-5] Equal amount of saline was injected at the same site of the right foot. Arthritis was induced in all the animals except normal control. A volume of 0.1 ml saline was injected in both paws of the normal control animals. All rats were randomly divided into seven groups each containing 5 males (M) and 5 females (F). Group I: normal control (distilled water), Group II: vehicle control (dispensing alcohol), Group III: untreated control (CFA), Group IV: homoeopathic formulation I *Capsaicin* and *Dihydrocapsaicin* 30C, Group V: homoeopathic formulation TNF-α 30C, Group VI: homoeopathic formulation *Magnesium phosphoricum* 30C, and Group VII: *Diclofenac*.

Dosage preparation and administration

Homoeopathic formulations *Capsaicin* and *Dihydrocapsaicin* and TNF-α in 30C potency and the vehicle control (Curative Power Lab Ltd., dispensing alcohol 91% v/v, Batch number: HD-18, Mfg. date: February 2013) were supplied by Life Force Homeopathy, Mumbai, India. Formulation *Magnesium phosphoricum* in 30C potency, liquid dilution, was sourced from Dr. Willmar Schwabe India Pvt Ltd. (Batch Number: 2981299, Mfg. date: November 2009) CFA was obtained from Haffkine Biopharmaceuticals, Parel, Mumbai (Lot number 098K8729). The rats received 0.6 ml of homoeopathic formulation or vehicle, in each respective group, and 10 mg/kg of *Diclofenac* every day for 21 days by oral route. Animals were kept nil by mouth for 15 minutes before the drug administration.

Evaluation of the severity of arthritis

Severity of arthritis was evaluated based on the following parameters, namely, body weight, paw volume, arthritic index, and mechanical pain threshold. All animals were treated with CFA except normal control animals, and evaluation parameters were assessed before adjuvant injection and then on days 7, 14, and 21 after adjuvant injection. The body weights of all the rats were recorded, and the paw volumes were measured using a Digital Plethysmometer (UGO Basile [SRL], biological research apparatus, 21025 Comerio VA Italy, Model 7141). For arthritic index, the physical symptoms of arthritis were evaluated by the Arthritic Index Grading System [Table 1]. The animals were sacrificed as per the approved method of euthanasia at the end of study.

Arthritis index[4] for each group was calculated by adding the scores for each rat. Mechanical pain threshold was assessed using external mechanical force. The mechanical pain threshold of hind paw was determined by compressing the paw using a custom-designed balance pressure apparatus consisting of conical tip of 1 mm diameter. The load on the conical tip was gradually increased and the load cell allowed visualization on the digital display of the force applied at each moment of the test in grams. The threshold force of rats squeaking or struggling was expressed in grams.

Statistical analysis

The results were expressed as the mean for the parametric data sets and as the median minimum, maximum for the nonparametric data sets. The significant difference between the means (parametric) was evaluated by one-way ANOVA followed by Dunnett's *post hoc* multiple comparison test for normal data.

Results

CFA-induced arthritis in rats is used as a model to evaluate the antiarthritic activity of drugs in preclinical research. CFA induces a chronic immune-mediated inflammation similar to arthritis characterized by elevation of pro-inflammatory mediators and development of swelling, pain, and deformity of joints. Inflammation was indicated by increase in paw volume. The reduction in inflammation due to treatment was indicated by decreased paw volume for evaluation of therapeutic efficacy of the drug, administered in the ultra-diluted form.

Table 1: Arthritic index grading system

Redness and nodules in ear	None=0, visible=1
Swelling of connective tissue of nose	None=0, visible=1
Nodules in tail	None=0, visible=1
Forepaw inflammation	None=0, slight=1, moderate=2 and marked=3
Hind paw inflammation	None=0, slight=1, moderate=2 and marked=3

Body weight

No significant difference was observed with respect to negative control in both male and female rats, and a good increase in weight on days 7, 14, and 21 indicated normal growth in all the groups.

Paw volume

In the acute phase of arthritic inflammation on day 1, homoeopathic formulation *Magnesium phosphoricum* indicated a significant reduction in inflammation by reduced paw volume, similar to standard *Diclofenac*. Homoeopathic formulations *Capsaicin* and *Dihydrocapsaicin* and II also showed a nonsignificant reduction in inflammation similar to negative control. The body processes reversed this inflammatory phase as seen by decrease in inflammation by the negative control on day 7. A nonsignificant rise in the inflammation was seen with homoeopathic formulations on day 21, especially with the formulation TNF-α, which was not seen with *Diclofenac*, suggestive of the continued primary action of the formulation. Such an increase is peculiar of ultra-dilute homoeopathic formulation, as per the homoeopathic principle of "like cures like," where initial effect (inflammation index) caused by the medicinal substance gets reduced subsequently by secondary effect[6] (which could be body's defense mechanism), leading to anti-inflammatory effect [Figures 1 and 2]. This study has demonstrated the initial (primary) effect of formulation TNF-α by increasing the inflammation, which could have possibly reversed on further continuation of treatment.

Formulation *Capsaicin and Dihydrocapsaicin* 30C is an alkaloid which is known to produce pain and inflammation, not only in crude form but also in high dilution dose as seen in a Homoeopathic Pathogenetic Trial (HPT).[7] The same compound has also demonstrated anti-pain and anti-inflammatory effects with 30C potency on a range of painful conditions in a clinical trial.[8] The current study has produced similar effects in an animal model. Longer study to explore this phenomenon can be conducted in future.

Arthritic index

Arthritic index was higher with negative control which was decreased by formulation *Capsaicin* and *Dihydrocapsaicin* on days 7 and 21 although not significantly. *Diclofenac* and formulation *Magnesium phosphoricum* showed a significant reduction in arthritic index on days 7 and 21 [Table 2]. Formulation *Magnesium phosphoricum* exhibited similar response as that of standard *Diclofenac* on day 7.

Similar to the paw volume, a trend in nonsignificant rise in the arthritic index was seen in all the groups, probably due to the immunological process systemically. It is extremely important to plan a further study with an increase in the duration of administration of the homoeopathic formulation to evaluate if on a longer administration the symptoms could reverse significantly.

P value was calculated using paired *t*-test for all the three formulations as compared to vehicle control and negative control. It was noted that the statistical significant results ($P < 0.05$) were obtained with formulation *Magnesium phosphoricum* as compared to vehicle control in male rats. In case of *Diclofenac*, statistical significant results ($P < 0.05$) were observed in female rats as compared to vehicle control and negative control.

Mechanical pain

A decreased pain threshold in negative control (CFA) on day 7 indicated induction of arthritic process, i.e., immune-mediated inflammation. No drug treatment could reverse this significantly as compared to negative control on day 7, indicating induction of arthritis and subsequent pain. Reversal of mechanical pain threshold in negative control group on day 14 indicated natural body process toward recovery although none of the drugs could alleviate the decreased pain threshold. On day 21, negative control groups almost reversed back the decreased pain threshold to normal. *Diclofenac*, too, increased the pain threshold, indicating good analgesic, anti-inflammatory activity. None of the homoeopathic formulations could alleviate the pain threshold to normal by day 21 [Figures 3 and 4]. Continuation of treatment further could probably alleviate the pain threshold, relieving the arthritic symptoms. This corresponds to the other parameters as discussed before, where a rise in paw volume and arthritic index was seen in the homoeopathic treatment groups.

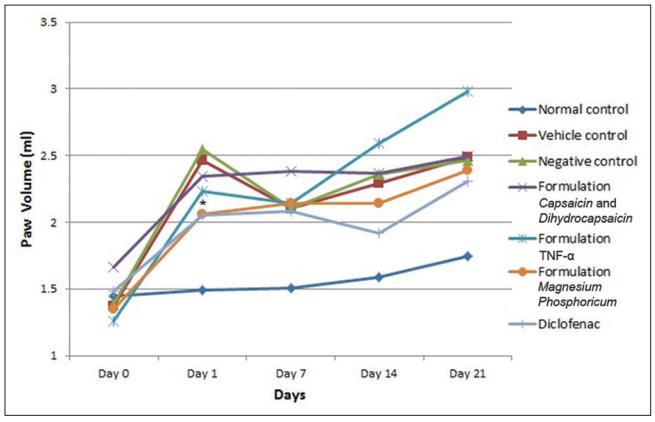

Figure 1: Male rat paw volume

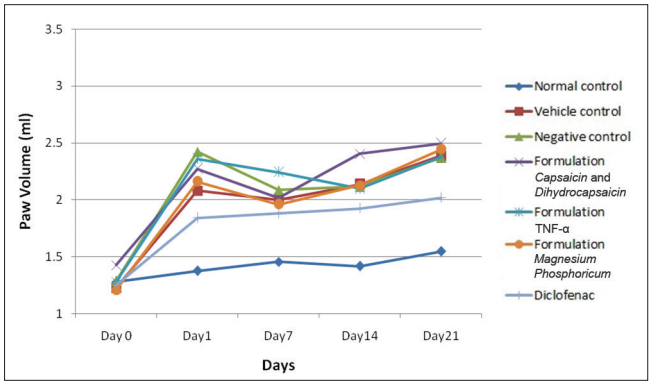

Figure 2: Female rat paw volume

Discussion

The medicinal substances sourced from different materials (herbs, minerals, and biological) are known to act differently, as observed in clinical homoeopathic practice. It is a common observation that medicines from herbs have quicker and shorter span of action; medicines from minerals and metals (depending on the exact ingredient) have slow or fast but longer duration of action; while those from animal source (many subvarieties) may take longer time to act and the action may last longer.[9,10] Of course, there are many other factors that determine the duration of action. Usually, the medicines sourced from biological materials take longer duration to set in action, as compared to the mineral and plant materials. Different formulations having different actions need further exploration.[11]

Our understanding about formulation *Magnesium phosphoricum* (a tissue salt) is based on clinical experience, where it is known to work quickly. It has been in use as an analgesic. It seems that the primary action of this magnesium compound may have been quick and short, quickly followed by the secondary action. Formulation *Magnesium phosphoricum* did not undergo HPT.[12] Interesting to note that magnesium compounds showed efficacy in pain relief when administered in crude form.[13,14] Intravenous $MgSO_4$ infusion has shown analgesic effect. The magnesium compound showed anti-inflammatory efficacy in ultra-diluted form in this study. This calls for further study.

The significant reduction in paw volume by homoeopathic formulation *Magnesium phosphoricum* indicated its anti-inflammatory activity during the acute phase of inflammation. Administration of ultra-dilute potency of TNF-α, which is a pro-inflammatory cytokine, exhibited an increase in paw volume on day 21 [Figure 1]. Although this increase is not statistically significant, a trend toward rise in inflammation is seen as compared to the vehicle control and also to its own activity on days 7 and 14, indicating that the formulation in ultra-dilute potency was capable of producing inflammation showing that it acted and produced biological changes, as compared to the control.

High-dilution medicinal substances are known to induce biological changes and hence symptoms as primary action, which are observed in HPTs.[7] As per the homoeopathic principle and hypothesis, the therapeutic effect of the medicinal substance is through the secondary action of the organism, which is observed once the primary stimulus (formulation) is withdrawn. The constant increase in inflammation in case of TNF-α till day 21, in comparison with the control group, could be the continued primary action of the formulation. It may be hypothesized that on discontinuing the stimulus, there could have been possible reduction in inflammation as a secondary action of the organism. Further studies in this direction alone could bring insight in this phenomenon.

It was noted that the homoeopathic formulations were administered three times a day, while *Diclofenac* was administered only once a day. Since the handling of animals is known to tire the animals, it might affect the results. In future studies doses may be maintained identical.

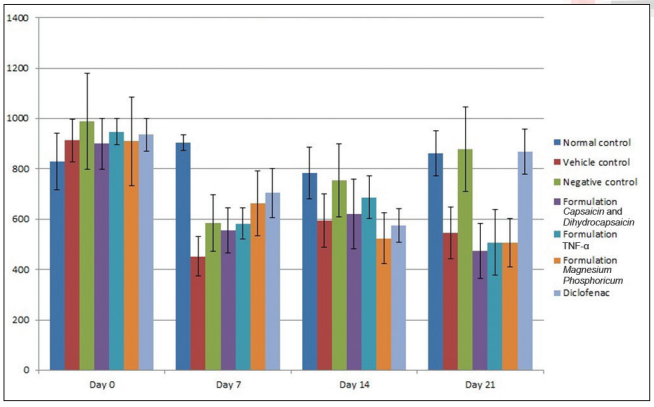

Figure 3: Mechanical pain (male)

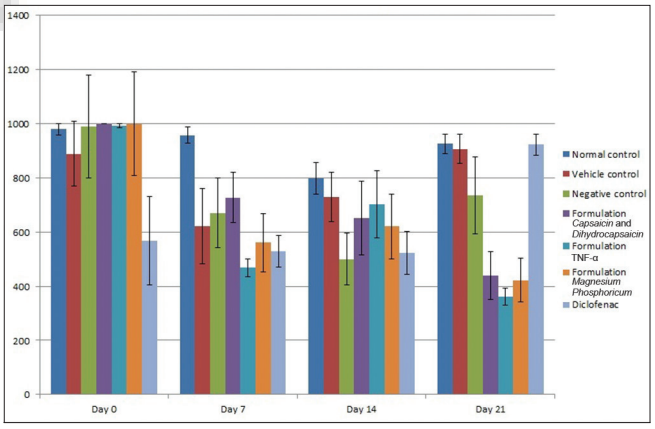

Figure 4: Mechanical pain (female)

Table 2: Arthritic Index for different groups

Days	Normal Control		Vehicle control (Alcohol)		Negative control		(*Caps alkaloids 30C*)		(*TNF-a 30C*)		(*Mag phos 30C*)		*Diclofenac*	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Day 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 7	0	0	2.4	3	3.2	2.6	2.2	2	3.2	3.4	1.5	1.4	1.25	1.4
Day 14	0	0	3	2.25	2.6	2.4	3	2	3.75	2.5	2.25	2.2	2.75	1.5
Day 21	0	0	3.4	3	3.4	2	2.25	2	3.2	2.25	2	2.2	1	1.25

Conclusion

The study was undertaken to evaluate the antiarthritic activity of ultra-dilute potencies of homoeopathic formulations from different origins on CFA-induced arthritic model in rats. A significant decrease in inflammation as well as arthritic index was seen by high-dilution homoeopathic formulation *Magnesium phosphoricum* similar to *Diclofenac*, demonstrating evidence that small dose of substance having a capacity to produce inflammation could work against inflammation, as per like cures like, the fundamental principle of Homoeopathy.

No such effect was seen on the mechanical pain threshold by the homoeopathic formulations.

Further study needs to be designed with longer duration of administration of these formulations to study whether these formulations could reverse the arthritic index and resultant pain and inflammation on long-term administration.

Acknowledgment

We thank the members of IAEC, sponsor, and subject experts for their technical, ethical, legal, and medical inputs; and the department staff for their cooperation.

Financial support and sponsorship

Nil.

Conflict of interest

None declared.

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Präklinische Bewertung der anti-arthritischen Wirkung hochverdünnter Präparate von Capsaicin-Alkaloiden (CP-10), TNF- α und Magnesium phosphoricum bei Wistar-Ratten

Auszug

Hintergrund: Die Verwendung von Tiermodellen in der Entwicklung neuer Arzneimittel und in Forschung ist in der herkömmlichen Medizin üblich. Die Homöopathie wird seit über zwei Jahrhunderten praktiziert. Tiermodellversuche zur Entdeckung neuer Wirkstoffe und Wirksamkeitstests in der präklinischen Forschung sind in der Homöopathie nur selten angewandt. Diese Studie untersucht die Möglichkeit, die kontrollierte Wirkung homöopathischer Mittel im experimentellen Modell akuter Entzündungen bei Ratten zu testen.

Methoden: Wistar-Ratten wurden in sieben Gruppen (siebzig Ratten im Alter von 6-8 Wochen) aufgeteilt; Arzneimittel wurden evaluiert durch orale Anwendung bei CFA-induzierter Arthritis. Zwei neue homöopathische Präparate, eine Kombination von Capsaicin und Dihydrocapsaicin (CP-10), Tumornekrose-Faktor-a (TNF-&) und ein bekanntes homöopathischen Arzneimittel – Magnesium phosphoricum (0,6 ml oral) - wurden als Standard gegenüber der Lehrprobe unter Verwendung von Diclofenac bewertet. Ödeme wurden mit einem wasserbasierten Plethysmometer vor und während verschiedener Zeitpunkte nach der Arthritisinduktion gemessen.

Ergebnisse: Magnesium phosphoricum zeigte gute Ergebnisse, fast ähnlich dem von Diclofenac am 7. und 21. Tag, während CP-10 und TNF-α nicht signifikante Ergebnisse erbrachten. Der körpereigene Prozess ließ den entzündlichen Zustand vom siebten Tag an abklingen, angezeigt durch ein ähnliches Pfotenvolumen aller Behandlungen. Der arthritische Index war höher mit einer negativen Kontrolle, was durch CP-10, obwohl nicht signifikant, am 7. und 21. Tag abnahm. Diclofenac und Magnesium phosphoricum zeigten eine signifikante Reduktion des arthritischen Index am 7. und 21. Tag.

Fazit: Ein hochverdünntes homöopathisches Magnesium phosphoricum Präparat zeigte definitiv eine anti-arthritische Wirkung. Das gleiche konnte in einer Studie, die über einen längeren Behandlungszeitraum ging und bei der der Gehalt an entzündlichen Biomarkern untersucht wurde, bestätigt werden.

Evaluación preclínica de la actividad antiartrítica de las preparaciones ultradiluidas de los alcaloides de la Capsaicina (CP10), TNF-α y Magnesium phosphoricum en ratas wistar

RESUMEN

Fundamento

El uso de modelos animales en el desarrollo de nuevos fármacos y en la investigación es una práctica habitual en medicina convencional. El sistema de medicina homeopática se practica desde hace más de dos siglos. En homeopatía, únicamente unos pocos investigadores aplican un modelo animal para el descubrimiento de nuevos medicamentos y la comprobación de la eficacia en la investigación preclínica. En este estudio, se explora la posibilidad de examinar de forma controlada los efectos de los remedios homeopáticos en un modelo experimental de inflamación aguda en ratas.

Método: Las ratas Wistar fueron divididas en siete grupos (setenta ratas de 6 a 8 semanas de edad); los medicamentos se evaluaron por administración oral en la artritis inducida por CFA.. Se evaluaron dos preparados homeopáticos nuevos, una combinación de capsaicina y dihidrocapsaicina (CP-10), y el factor de necrosis tumoral α (TNF α), así como un medicamento homeopático existente, *Magnesium phosphoricum* (0,6 ml por vía oral) frente al control con vehículo, utilizando el diclofenac como control. Se midió el edema utilizando un pletismómetro de agua antes y en diferentes momentos tras la inducción de la artritis.

Resultados: *Magnesium phosphoricum* mostró buenos resultados, casi similares al diclofenac en los días 7 y 21, mientras que CP-10 y TNF-α mostraron resultados no significativos. Los procesos corporales invirtieron la inflamación a partir del día 7 en adelante, lo cual quedaba reflejado en un volumen de pata similar en todos los tratamientos. El índice artrítico fue superior en el control negativo, lo que descendió con CP-10, aunque no de forma significativa en los días 7 y 21. Diclofenax y *Magnesium phophoricum* mostraron una reducción significativa del índice artrítico en los días 7 y 21.

Conclusiones: Uno de los preparados homeopáticos ultradiluidos of Magnesium phosphoricum mostró una actividad antiartrítica definida. Esto mismo pudo confirmarse al estudiar los niveles de biomarcadores inflamatorios en un estudio con un periodo de tratamiento más prolongado.

विस्टार चूहों में कैप्सीइसीन एल्कलॉइड (सीपी–10), टीएनएफ–α और मेग्नीशियम फोसफोरिकम की अत्यंत–तनुकृत निर्मिति की प्रतिसंधिशोथ गतिविधि का पूर्व नैदानिक मूल्यांकन

सार

पृष्ठभूमि: आधुनिक चिकित्सा और अनुसंधान के विकास के लिए पारंपरिक चिकित्सा में पशु मॉडल का उपयोग आम है। चिकित्सा क्षेत्र में होम्योपैथिक प्रणाली गत दो शताब्दियों से उपयोग में लाई जा रही है। होम्योपैथी जगत में नई औषधियों की खोज और पूर्ण नैदानिक अनुसंधान हेतु प्रभावकारिता परीक्षण, पशु मॉडलों की सहायता से कम ही लोगों द्वारा किया गया है। इस अध्ययन द्वारा चूहों में तीव्र शोथ के प्रयोगात्मक मॉडल पर होम्योपैथिक उपचार के प्रभाव का एक नियंत्रित तरीके से परीक्षण की संभावना की पडताल करना है।

विधि: विस्टार चूहों को सात समूहों में विभाजित किया गया (6–8 सप्ताह उम्र के सत्तर चूहे); सीएफए जिसमें गठिया भी शामिल हैं में मौखिक प्रबंधन द्वारा औषधियों का मूल्यांकन किया गया। दो नई होम्योपैथिक निर्मिति, कैप्सीइसीन और डाइहाइड्रोकेप्सीइसीन (सीपी–10) का एक संयोजन, टयूमर नेक्रोसिस कारक–ए (टीएनएफ–α) तथा एक मौजूदा होम्योपैथिक औषधि, मैग्नेशियम फोसफोरिकम (0.6 मिलीलीटर मौखिक) का मूल्यांकन वाहक नियंत्रण के बनाम डाईक्लोफेनाक का एक मानक के रूप में उपयोग करते हुए किया गया। त्वचा शोथ का मापन एक जल आधारित प्लथिस्मोमीटर का उपयोग कर, गठिया प्रेरण के पहले और बाद के अलग–अलग समय पर किया गया।

परिणाम: मैग्नीशियम फोसफोरिकम ने अच्छे परिणामों का प्रदर्शन किया, लगभग डाईक्लोफेनाक के समान सातवें और इक्कीसवें दिन जबकि सीपी–10 और टीएनएफ–α ने गैर–महत्वपूर्ण परिणामों का प्रदर्शन किया। शरीर प्रक्रिया द्वारा सूजन की स्थिति सातवें दिन के बाद सामान्य हो गई, जो सभी उपचारों में समान पंजा (पा) मात्रा द्वारा इंगित था। गठिया सूचकांक α ऋणात्मक नियंत्रण के साथ उच्च था, जो सीपी–10 द्वारा कम था, हालांकि सातवा तथा इक्कीसवा दिन गैर महत्वपूर्ण था। डाईक्लोफेनाक और मैग्नेशियम फोसफोरिकम द्वारा सातवें तथा इक्कीसवें दिन, गठिया सूचकांक में महत्वपूर्ण कमी का प्रदर्शन किया गया।

निष्कर्ष: एक अत्यंत–तनुकृत मैग्नेशियम फोसफोरिकम द्वारा निश्चित प्रतिसंधिशोथ गतिविधि का प्रदर्शन किया गया। उर्पयुक्त की पुष्टि लंबी उपचार अवधि के साथ सूजन के बायोमार्को के एक अध्ययन विशेष में कर के किया जा सकता है।