Original Article

Attenuation of Complete Freund's Adjuvant-induced arthritis by different dilutions of *Eupatorium perfoliatum* and *Crotalus horridus* and their safety evaluation

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Abstract

Objective: The aim of this study was to investigate, the inhibitory effect of the homoeopathic drugs – *Eupatorium perfoliatum* and *Crotalus horridus* in experimental models of inflammation and Complete Freund's Adjuvant (CFA)-induced arthritis with evaluation of their safety aspects by acute and subacute toxicity studies. **Materials and Methods:** Animals were divided into eight groups ($n = 6$). *Eupatorium perfoliatum* and *Crotalus horridus* in different dilutions (6CH, 12CH, 30CH and 200CH) were administered orally, daily during the study period of 21 days, and their effect on joint dysfunction was evaluated by measuring joint diameter in CFA-induced arthritis model. In addition, inflammatory profiles of these homoeopathic drugs were screened in carrageenan-induced paw oedema model. Acute and subacute studies were carried out according to Organization for Economic Cooperation and Development 425, 407 guidelines. The subacute toxicity study was carried out for a duration of 28 days, and all the animals were observed for behavioural abnormalities. At the end of 28th day, animals were sacrificed to carry out the biochemical, haematological and histopathological estimations. **Results:** Findings of the study revealed that on CFA administration, there is a significant ($P < 0.01$) increase in joint diameter in all the tested animals. Maximum increase in joint diameter was observed on day 3 in all the treatment groups. *Eupatorium perfoliatum* 200CH showed significant decrease in joint diameter on day 21. In carrageenan study, the homoeopathic drugs produced a significant reduction in paw oedema at 5-h post-carrageenan administration. Study noted that the anti-inflammatory activity of *Eupatorium perfoliatum* was found to be superior to *Crotalus horridus*. **Conclusion:** Study inferred that *Eupatorium perfoliatum* and *Crotalus horridus* are safe at dilutions 6CH, 12CH, 30CH and 200CH and effective in minimising inflammation and arthritis in CFA-induced model.

Keywords: *Crotalus horridus, Eupatorium perfoliatum,* Histopathology, homoeopathic drugs, Inflammation, Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune-mediated disorder characterised by cellular infiltration and proliferation of the synovial membrane, pannus formation, cartilage and bone erosion, leading to the progressive destruction of the joints through the interaction between infiltrating cells and mediators such as cytokines, prostanoids and proteolytic enzymes.[1] In India, the prevalence of RA is found to be around 0.75% of the adult population.[2] The pathogenesis of RA is associated with accumulation of macrophages, neutrophils and production of free radical-producing enzymes. Conventional treatment options available are disease-modifying antirheumatic drugs, Nonsteroidal Anti-inflammatory (NSAIDs) and glucocorticoids. Although these treatment options are effective in improving disease conditions, chronic and long-term use of these agents may lead to serious adverse effects such as gastric ulceration, cardiovascular abnormalities and emergence of opportunistic infections.[3] Thousands of patients with inflammatory disorders face the problem of gastric ulcers and

Access this article online

Quick Response Code:

Website: www.ijrh.org

DOI: 10.4103/ijrh.ijrh_23_19

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Received: 13.03.2019; **Accepted:** 20.05.2019

How to cite this article: Singh S, Karwasra R, Kalra P, Nayak D, Khurana AK, Manchanda RK. Attenuation of Complete Freund's Adjuvant-induced arthritis by different dilutions of *Eupatorium perfoliatum* and *Crotalus horridus* and their safety evaluation. Indian J Res Homoeopathy 2019;13:107-17.

gastrointestinal bleeding due to prolonged use of NSAIDs,[4] and also considering the chronic nature of these disorders, compliance became an another issue associated with these costly and toxic drugs. It is also seen that the large number of patients with these disorders rely on complementary and alternative medicines either alone or in combination with conventional treatment for better outcome.[5] Hence, discovering new compounds of natural origin in homoeopathic system of medicine with potential therapeutic effect and minimal side effects have been pursued vigorously.[6] Medicinal plants are being used in the form of homoeopathic drugs in the prevention and management of inflammatory disorders in prophylactic way.

Homoeopathic drugs – *Eupatorium perfoliatum* and *Crotalus horridus* are available in different formulations or preparations for the treatment of different ailments. *Eupatorium* and *Crotalus* genera have been studied in some depth and several compounds with varying effects identified. Traditionally, *Eupatorium perfoliatum* was used for the treatment of fever and as a gastrointestinal aid for pains in the stomach.[7] Leaves and flowering parts of the plant 'common boneset' have been extensively used within traditional medicine of North America's native inhabitants against fever and as diaphoretic agent.[8,9] Previous literature on *Eupatorium perfoliatum* depicts its use as mother tincture in homoeopathic system of medicine and used against *in vitro* human monocyte cells for controlling inflammation and oxidative stress. It possesses significant anti-inflammatory, antioxidant, immunomodulatory, cytotoxic and antibacterial activities. *Crotalus horridus* is also known as timber rattlesnake; its venom is used in homoeopathic preparations. It is a venomous pit viper found in eastern parts of United States and produces high venom yield. The venom comprises of Type A-neurotoxic, Type B-haemorrhagic. It possesses antiretroviral action and is potent against Ehrlichiosis infections in different homoeopathic preparations.[10] In this context, the health benefits of *Eupatorium perfoliatum* and *Crotalus horridus* in preventing cancers and infectious diseases[11] have been widely focused. However, very few studies have targeted the eventual benefit of *Eupatorium perfoliatum* and *Crotalus horridus* in attenuation of inflammation.

Materials and Methods

Animals

Adult Wistar albino rats (150–180 g) from our institutional breeding stock were used in the study. Animals were housed at 25°C ± 2°C inside clean polypropylene cages in groups of three. They had access to food and water *ad libitum* throughout the duration of the study. The experimental protocols were duly approved by the Institutional Animal Ethics Committee, All India Institute of Medical Sciences, New Delhi, India (Animal Ethics Approval No-935/IAEC/16). All experiments were carried out in accordance with 'Guidelines for care and use of animals in scientific research (Indian National Science Academy 1998, Revised 2000)'.

Chemicals and drugs

Complete Freund's Adjuvant (CFA) was purchased from Difco, CA, USA. Indomethacin was purchased from Sigma Chemical Co., USA. All the other chemicals used were of analytical grade.

Test drug

Homoeopathic preparations of *Eupatorium perfoliatum* and *Crotalus horridus* at different dilutions, i.e., 6CH, 12CH, 30CH and 200CH were procured from Central Council for Research in Homoeopathy (CCRH).

Dose calculation of study drugs for experimental animals

The route of administration is per oral, and the vehicle for administration is deionised water. The standard dose of 20 µl/100 g body weight of rat was administered using a micropipette and solution was mixed with 450 µl of distilled water administered orally with the help of oral catheter and flushed with 500 µl of distilled water.

Experimental design: Toxicity studies

Acute and subacute toxicity studies of these homeopathic drugs were also evaluated according to Organization for Economic Cooperation and Development (OECD) guidelines.

Acute toxicity study

Evaluation of acute oral toxicity of *Eupatorium perfoliatum* and *Crotalus horridus* at different dilutions 6CH, 12CH, 30CH and 200CH was carried out according to the OECD guidelines for testing of chemicals-425.[12] A limit test (2000 µl/kg body weight) was performed using five male Wistar rats (150–180 g) from our breeding stock. All the animals were observed for behavioural changes and mortality till 14 days after administration of the dose [Table 1].

Subacute toxicity studies

Evaluation of 28-day oral toxicity study of *Eupatorium perfoliatum* and *Crotalus horridus* at different dilutions 6CH, 12CH, 30CH and 200CH was carried out according to the OECD guidelines for testing of chemicals-407.[13] Ninety Wistar rats (150–180 g) of both sexes from our breeding stock were allocated into nine groups (n = 5/sex/group). Group I received the vehicle (1 ml/kg body weight, 1% saline) and served as normal control and Group II–V received *Eupatorium perfoliatum* in different dilutions, i.e., 6CH, 12CH, 30CH and 200CH and Group VI–IX *Crotalus horridus* in different dilutions. Drug/vehicle was administered daily for 28 days [Tables 2 and 3].

Experimental protocol: Carrageenan-induced paw oedema model

The experimental protocol and methodologies used were the same as described earlier.[14] Animals were divided into 11 groups (n = 6) and fasted overnight with access to water *ad libitum*. Thereafter, Group I received normal control (1% normal saline, p.o), Group II received carrageenan alone (1% solution of carrageenan dissolved in normal saline), Group III received standard drug indomethacin (3 mg/kg),

Groups IV, V, VI and VII received *Eupatorium perfoliatum* at different dilutions 6CH, 12CH, 30CH and 200CH and Group VIII–XI *Crotalus horridus* in different dilutions. Sixty minutes post-administration of the drug/vehicle, paw oedema was induced by subcutaneous administration of 0.1 mL of 1% λi-carrageenan (constituted in normal saline) into the left hind paw of the animal. Paw oedema was measured using a digital plethysmometer (Orchid Scientific, India) at 1-h, 3-h and 5-h post-carrageenan administration. Increase in paw volume and percentage inhibition of paw oedema of all the treatment groups were calculated.

Experimental protocol: Chronic model of inflammation

Experiment was conducted according to previous standardised methodology.[15] Eleven groups of rats ($n = 6$) were used in the study and fasted overnight with access to water *ad libitum*.

Table 1: Mortality in normal and homoeopathic groups (at different dilutions) for a period of 10 days

Serial number	Groups	Days									
		1	2	3	4	5	6	7	8	9	10
		Number of animals alive/tested									
1	*Eupatorium perfoliatum* 6CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2	*Eupatorium perfoliatum* 12CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
3	*Eupatorium perfoliatum* 30CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4	*Eupatorium perfoliatum* 200CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
5	*Crotalus horridus* 6CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
6	*Crotalus horridus* 12CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
7	*Crotalus horridus* 30CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
8	*Crotalus horridus* 200CH	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Table 2: Biochemical parameters analysed in normal control and homoeopathic medicines *Eupatorium perfoliatum* and *Crotalus horridus* at different dilutions by autoanalyzer

Drug treatment	Change in body weight (g)			
	7th day	14th day	21st day	28th day
Normal control	7.33±0.61	17±0.81	28±0.36	37.33±0.80
Eupatorium perfoliatum 6CH	7.83±0.54	17±0.51	29.33±0.66	37.17±0.47
Eupatorium perfoliatum 12CH	7.0±0.36	16.83±0.65	28.33±0.61	38.0±0.36
Eupatorium perfoliatum 30CH	7.83±0.47	18.33±0.33	28.50±0.71	38.67±0.55
Eupatorium perfoliatum 200CH	7.66±0.49	18.83±0.40	27.67±0.49	38.83±0.30
Crotalus horridus 6CH	7.83±0.30	18.50±0.42	28.83±0.83	38.33±0.33
Crotalus horridus 12CH	7.33±0.33	17.67±0.33	27.33±0.66	37.33±1.05
Crotalus horridus 30CH	7.83±0.47	18.17±0.60	28.50±0.88	37.17±0.70
Crotalus horridus 200CH	8.33±0.33	18.17±0.47	28.33±0.61	38.17±0.60

All values are mean±SEM (n=6). Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparisons. SEM: Standard error of means; ANOVA: Analysis of variance

Table 3: Haematological parameters (red blood cells, white blood cells and platelets) were measured by different methods in all groups

Drug treatment	Change in organ weight					
	Liver	Kidney	Heart	Brain	Testis	Ovary
Normal control	3.49±0.05	0.71±0.00	0.33±0.01	1.95±0.016	1.120±0.02	0.067±0.002
Eupatorium perfoliatum 6CH	3.33±0.04	0.72±0.01	0.32±0.00	1.90±0.02	1.06±0.03	0.071±0.003
Eupatorium perfoliatum 12CH	3.32±0.03	0.71±0.01	0.34±0.01	1.95±0.02	1.08±0.01	0.069±0.002
Eupatorium perfoliatum 30CH	3.36±0.11	0.71±0.02	0.37±0.00	1.95±0.02	1.07±0.01	0.073±0.001
Eupatorium perfoliatum 200CH	3.46±0.02	0.72±0.01	0.34±0.00	1.91±0.01	1.06±0.02	0.074±0.001
Crotalus horridus 6CH	3.50±0.04	0.68±0.01	0.35±0.01	1.94±0.03	1.08±0.02	0.074±0.001
Crotalus horridus 12CH	3.50±0.01	0.69±0.02	0.32±0.00	1.92±0.01	1.08±0.04	0.071±0.002
Crotalus horridus 30CH	3.44±0.03	0.74±0.01	0.32±0.00	1.91±0.02	1.06±0.02	0.072±0.001
Crotalus horridus 200CH	3.49±0.02	0.74±0.01	0.36±0.00	1.93±0.01	1.07±0.02	0.074±0.001

All values are mean±SEM (n=6). Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparisons. SEM: Standard error of means; ANOVA: Analysis of variance

Group 1 (normal control): normal saline (1 ml/kg/day; p.o.) was administered to rats for a period of 21 days.

Group 2 (CFA control): normal saline was administered to rats for a period of 21 days, and on 0th day, a single subplantar injection of 0.1 ml CFA (Difco: 0.05% [w/v] of *Mycobacterium butyricum* in mineral oil) in the left hind paw was given.

Group 3 (Indomethacin 3 mg/kg + CFA): indomethacin was administered to rats for a period of 21 days, and on day zero, a single subplantar injection of 0.1 ml CFA in the left hind paw was given.

Group 4–7 (*Eupatorium perfoliatum* + CFA): received *Eupatorium perfoliatum* at different dilutions 6CH, 12CH, 30CH and 200CH for a period of 21 days, and on day zero, a single subplantar injection of 0.1 ml CFA in the left hind paw was given.

Group 8–11 (*Crotalus horridus* + CFA): received *Crotalus horridus* at different dilutions 6CH, 12CH, 30CH and 200CH for a period of 21 days, and on day zero, a single subplantar injection of 0.1 ml CFA in the left hind paw was given.

On day 21, the animals were sacrificed, and terminal blood collection was performed by retro-orbital plexus and then centrifuged at 3000 rpm to separate the serum and stored at −20°C.

Measurement of increase in joint diameter

The joint diameter was measured by means of micrometre screw gauge placed on the left ankle joint transversely, and the diameter was measured on day 0 before injecting CFA and then on day 3, 7, 14 and 21 during the study. The increase in joint diameter was obtained by subtracting the day 0 values from the measurements taken on day 3, 7, 14 and 21 and expressed in mm.[16]

Statistical analysis

All data were represented as mean ± standard error of mean (n = 6). Comparison between groups was done by one-way analysis of variance followed by Dunnett's multiple comparison tests using the Graphpad Prism version 5.03, San Diego, CA, USA. P <0.05 was considered as statistically significant.

Results

Effect of different dilutions of *Eupatorium perfoliatum and Crotalus horridus on* acute oral toxicity study

In acute toxicity study, no mortality or toxic signs were observed in any group at different dilutions which indicates that oral LD_{50} of *Eupatorium perfoliatum* and *Crotalus horridus* is >2000 µl All the five Wistar rats were observed at the end of study period in all treatment groups.

Effect of different dilutions of *Eupatorium perfoliatum and Crotalus horridus* on subacute oral toxicity study for 28 days

No significant changes in body weights of treated rats were observed when compared with normal control group. No significant change in mean organ weight of liver, kidney, brain, heart, testes and ovaries were observed at the end of 28th day, when compared to normal control group. The mean organ weight of liver of normal control group is 3.49 ± 0.05; *Eupatorium perfoliatum* at 6CH, 12CH, 30CH and 200CH was found to be 3.33, 3.32, 3.36 and 3.46, respectively. *Crotalus horridus* at 6CH, 12CH, 30CH and 200CH was found to be 3.50, 3.50, 3.44 and 3.49, respectively. The mean organ weight of kidney of normal control group is 0.71 ± 0.00; *Eupatorium perfoliatum* at 6CH, 12CH, 30CH and 200CH was found to be 0.72, 0.71, 0.71 and 0.72, respectively. *Crotalus horridus* at 6CH, 12CH, 30CH and 200CH was found to be 0.68, 0.69, 0.74 and 0.74, respectively.

No signs of gastric ulceration or erosion were observed. Change in mean organ weight of different organs in control and treated groups were presented.

Biochemical and haematological observations also did not produce any significant changes in haematological and biochemical parameters of male [Table 4] and female [Table 5] rats as compared to those in normal control group. All animals survived until the scheduled necropsy, and their physical and behavioural examinations did not reveal any treatment-related adverse effects. No pathological changes were observed in histological section of heart, kidney, liver, testis, ovaries and brain of homoeopathic drugs-treated male and female [Figures 1 and 2] rats as compared to normal control animals.

Effect of different dilutions of *Eupatorium perfoliatum* and *Crotalus horridus* on acute inflammation

The carrageenan-induced paw oedema model was done to evaluate the involvement of anti-inflammatory activity of homoeopathic drugs at the doses tested. Administration of carrageenan produced a significant ($P < 0.05$) increase in paw oedema that was persistent throughout the observation period. Maximum paw oedema was observed at 5-h post-carrageenan administration in all the tested animals [Table 6]. The standard drug, indomethacin, produced a significant reduction in paw oedema at 3-h and 5-h post-carrageenan administration. Percentage inhibition of paw oedema was calculated for all treatment groups [Table 7]. The percentage inhibition found in *Eupatorium perfoliatum* dilutions (6CH, 12CH, 30CH and 200CH) are 8.064%, 8.064%, 16.12% and 27.41%, respectively. In another homoeopathic medicine, i.e., *Crotalus horridus*, the percentage inhibition was found to be 4.838%, 11.29%, 11.29% and 22.58% respectively. However, indomethacin inhibits 43.54% paw oedema in carrageenan-induced paw oedema model.

Effect of different dilutions of *Eupatorium perfoliatum* and *Crotalus horridus* on chronic inflammation

Injection of CFA into the left hind paw produced an increase in joint swelling, erythema and dysfunction in all the experimental groups. The first signs of arthritis development were visible on day 14, after immunisation with CFA. Treatment with

Table 4: Inhibition of paw volume was measured using plethysmometer in carrageenan-induced paw oedema model in Wistar rats

Drug treatment	Biochemical parameters					
	Blood glucose	Sr. Creatinine	SGOT	SGPT	TG	HDL
Normal control	101.2±2.57	0.85±0.03	110.3±2.25	41.24±1.20	57.33±1.72	31.23±0.67
Eupatorium perfoliatum 6CH	102.3±2.26	0.76±0.04	110.2±5.11	45.26±1.56	57.34±2.16	33.47±1.34
Eupatorium perfoliatum 12CH	103.3±2.41	0.70±0.06	107.3±3.05	42.46±1.11	57.41±2.30	35.41±1.42
Eupatorium perfoliatum 30CH	102.7±2.29	0.73±0.09	103.8±3.91	43.24±1.39	58.32±2.37	35.20±2.30
Eupatorium perfoliatum 200CH	100.7±2.07	0.83±0.08	107.4±3.51	45.28±1.18	56.93±3.45	33.0±1.33
Crotalus horridus 6CH	102.3±1.85	0.81±0.07	112.0±5.26	42.46±2.01	59.77±2.13	33.85±0.85
Crotalus horridus 12CH	99.67±2.64	0.81±0.06	108.4±4.84	45.40±1.56	56.46±2.34	34.58±2.13
Crotalus horridus 30CH	101.3±2.41	0.78±0.10	105.7±2.72	43.63±2.11	55.84±2.91	34.32±2.54
Crotalus horridus 200CH	98.17±2.07	0.81±0.10	110.3±5.49	45.42±1.23	56.91±3.24	33.76±1.21

All values are mean±SEM. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. ANOVA: Analysis of variance; SEM: Standard error of means; HDL: High-density lipoprotein; TG: Triglyceride; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase

Table 5: Percentage inhibition inflammation was measured in carrageenan-induced paw oedema model in Wistar rats

Drug treatment	Haematological parameters			
	RBC	WBC	Platelets	Haemoglobin
Normal control	734.3±15.25	16,767±209.2	35.72±1.10	12.91±0.42
Eupatorium perfoliatum 6CH	716.3±11.58	16,850±168.8	32.95±0.76	12.74±0.31
Eupatorium perfoliatum 12CH	717.2±19.59	16,917±272.5	34.39±0.96	12.79±0.28
Eupatorium perfoliatum 30CH	730.2±15.22	16,817±375.4	34.03±0.65	12.78±0.30
Eupatorium perfoliatum 200CH	748.8±18.70	16,525±430.8	35.32±1.13	12.53±0.27
Crotalus horridus 6CH	751.8±14.83	17,117±227.2	34.80±0.84	12.70±0.44
Crotalus horridus 12CH	725.3±15.07	17,100±291.0	35.56±1.69	12.71±0.21
Crotalus horridus 30CH	720.8±25.59	17,200±240.8	33.19±0.95	12.80±0.40
Crotalus horridus 200CH	702.8±20.33	16,750±304.1	34.52±1.13	12.71±0.35

RBC: Red blood cells; WBC: White blood cells

Table 6: Effect of administering different homoeopathic drugs on paw diameter in complete Freund's adjuvant induced arthritis in Wistar rats

Drug treatment	Increase in paw volume (ml)		
	1 h	3 h	5 h
Normal control (1 ml/kg)	0.11±0.004$^{###}$	0.20±0.006$^{###}$	0.22±0.01$^{###}$
Carrageenan alone	0.43±0.014	0.54±0.010	0.62±0.01
Indomethacin (3 mg/kg)	0.40±0.01	0.33±0.011***	0.35±0.01***
Eupatorium perfoliatum 6CH	0.43±0.009	0.50±0.021	0.57±0.01
Eupatorium perfoliatum 12CH	0.44±0.012	0.47±0.020	0.57±0.01
Eupatorium perfoliatum 30CH	0.41±0.008	0.45±0.037*	0.52±0.02**
Eupatorium perfoliatum 200CH	0.44±0.008	0.42±0.030**	0.45±0.008***
Crotalus horridus 6CH	0.41±0.006	0.50±0.013	0.59±0.01
Crotalus horridus 12CH	0.43±0.006	0.46±0.013	0.55±0.03
Crotalus horridus 30CH	0.42±0.009	0.46±0.020	0.55±0.02
Crotalus horridus 200CH	0.43±0.008	0.43±0.028**	0.48±0.02***

*P<0.05, **P<0.01, ***P<0.001 as compared with CFA-control, $^{###}P$<0.001 as compared with normal control. All values are mean±SEM. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparisons. ANOVA: Analysis of variance; CFA: Complete Freund's adjuvant; SEM: Standard error of means

homoeopathic drugs at different dilutions suppressed hind paw swelling to lesser extent. However, maximum protection was observed in *Eupatorium perfoliatum* 200CH group against CFA-related changes.

Effect of different dilutions of *Eupatorium perfoliatum and Crotalus horridus* on joint diameter

After immunisation with CFA, there was significant increase (P < 0.001) in the joint diameter of injected rats in all

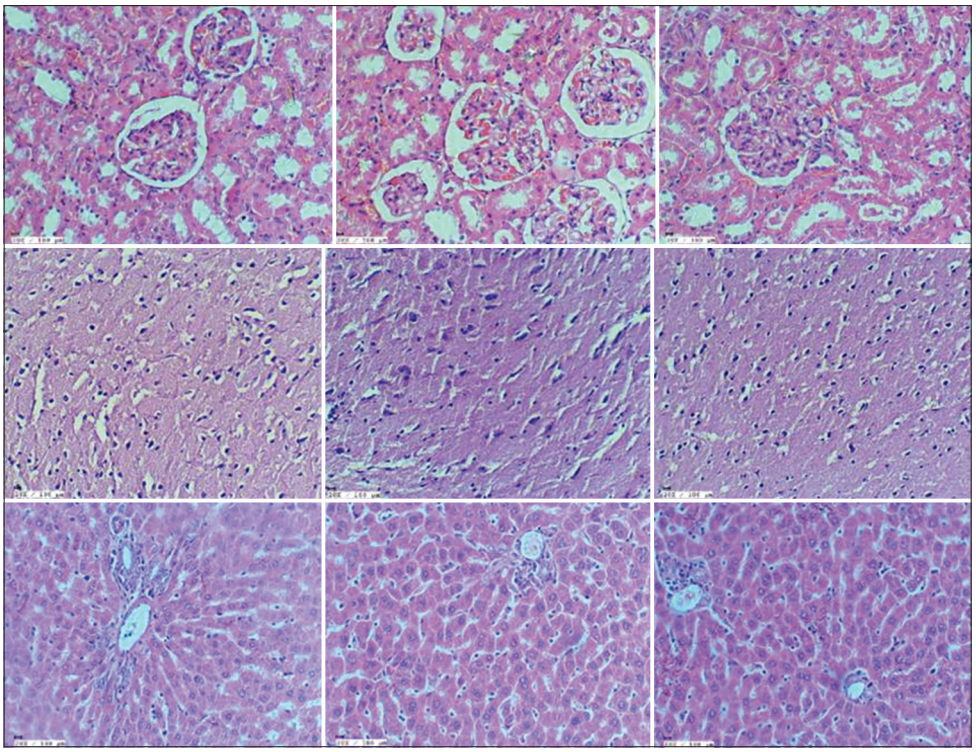

Figure 1: The histological analysis of haematoxylin and eosin in different organs (kidney, brain and liver) control and experimental groups of animals

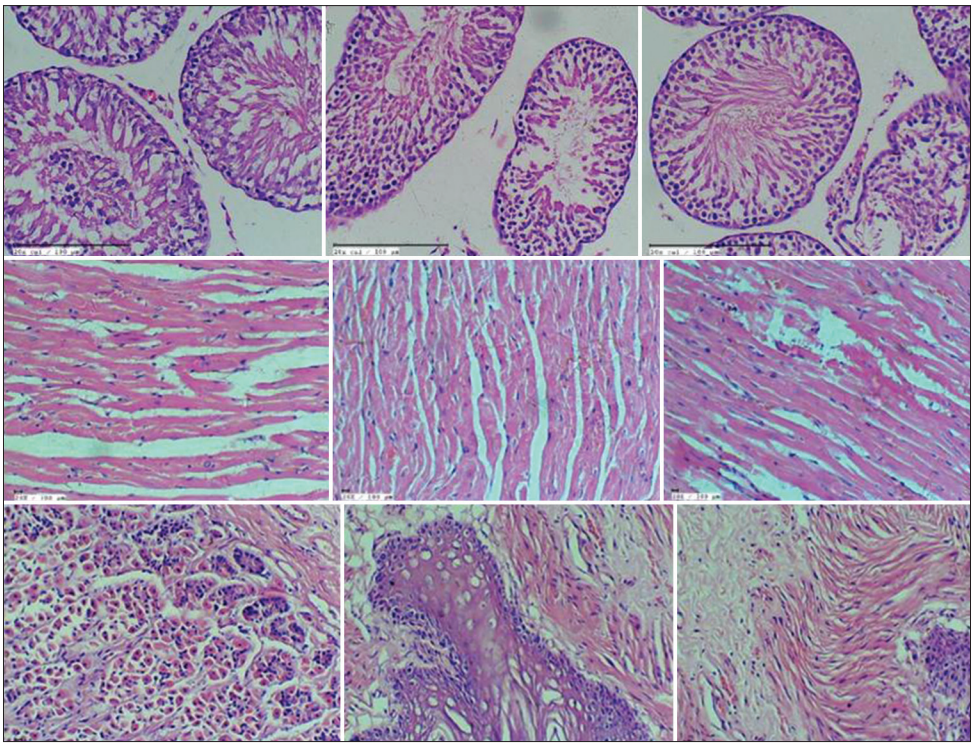

Figure 2: The histological analysis of haematoxylin and eosin in different organs (testis, heart and ovaries) control and experimental groups of animals

experimental groups as compared to normal control. Maximum increase in joint diameter was observed on day 3; after that, there was gradual decrease in joint inflammation in all groups except CFA control animals. There was small increase in ankle joint diameter in CFA control group, after day 14 which is the result of heightened cell-mediated immunity response. Significant inhibition ($P < 0.001$) in joint inflammation was observed by *Eupatorium perfoliatum* 200CH-treated group [Table 8].

Discussion

The present study demonstrated that the homoeopathic drugs' (*Eupatorium perfoliatum* and *Crotalus horridus*) treatment is safe at chronic administration and was effective in controlling inflammation and joint dysfunction in RA.

As mentioned earlier, inflammatory disorders such as RA is a chronic, deforming disease of joints of unknown aetiology. It is characterised by inflammation of synovial membrane, pain, destructive changes in cartilage and bone, restricting mobility of joints. The activated leucocytes and synovial fibroblasts in joint tissue secrete several pro-inflammatory mediators such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8 and interferon- γ (IFN-γ) that cause inflammation and joint damage.[17] Several studies have proposed that the pro-inflammatory cytokines such as TNF-α and IL-1 play a central role in the pathogenesis of RA as they contribute to the synoviocyte self-proliferation and increase the production of tissue enzymes, resulting in cartilage destruction,[5] while numerous other studies suggest that the transcription factor, i.e., NF-κB is also a pivotal regulator of inflammation and joint degradation by upregulating expression of many pro-inflammatory genes, such as, IL-1 β, IL-6, TNF-α, chemokines and matrix metalloproteinases.[18] Some studies also demonstrated the increased NF-κB levels in the synovial joint of human and animal models.[19] In short, we can conclude that in the process of bone erosion, the transcription factor (NF-κB) upregulate the expression of TNF-α, which further triggers the production of other cytokines, and furthermore, it exerts its arthritogenic potency through the induction of IL-1. Therefore, IL-1, NF-κB and TNF-α have been shown to be dominant players in the induction of inflammation and bone erosion. The concomitant presence of chronic pain and associated other symptoms is considered as suggestive underlying immune activity in RA. Thus, the reduction of pain, inflammation and joint damage is the focus of drug treatment of inflammatory disorders such as RA.

In our present investigation, we evaluated the effect of homoeopathic drugs on acute and chronic inflammation. These drugs or preparations inhibit the paw oedema, induced by carrageenan injection. Carrageenan-induced paw oedema model is an experimental model used for assessing the anti-inflammatory activity of test substance.[15] Carrageenan has time-dependent triphasic response, in which the first and foremost phase (1–2 h after carrageenan administration) primarily attributed to release of histamine, the second phase (2–3 h) being attributed to release of serotonin and kinins and the third phase (3–5 h) primarily attributed to release of prostaglandins.[20] Different dilutions of homoeopathic drugs act on all the three phases and produced significant reduction in paw oedema. The effect observed may be due to significant changes in the inflammatory response which are comparable with indomethacin. The response to anti-inflammatory activity is directly linked with the antiarthritic activity and to the progression of disease. CFA model is used to screen the newer compounds for its anti-inflammatory or antiarthritic activity. To assess the chronic anti-inflammatory activity of homoeopathic drugs – *Eupatorium perfoliatum* and *Crotalus horridus*, CFA model was used.[15] It has been used in various studies as a model for evaluation of sub-chronic or chronic inflammation in rats and is of considerable relevance to the study of pathophysiological and pharmacological control of

Table 7: The percentage inhibition of paw edema in carrageenan induced rat paw edema model

Drug treatment	Percentage inhibition
Indomethacin (3 mg/kg)	43.54
Eupatorium perfoliatum 6CH	8.064
Eupatorium perfoliatum 12CH	8.064
Eupatorium perfoliatum 30CH	16.12
Eupatorium perfoliatum 200CH	27.41
Crotalus horridus 6CH	4.838
Crotalus horridus 12CH	11.29
Crotalus horridus 30CH	11.29
Crotalus horridus 200CH	22.58

Table 8: The increase in joint diameter in CFA-induced rheumatoid arthritis on day 3, 7, 14 and 21

Drug treatment	Increase in joint diameter			
	Day 3	Day 7	Day 14	Day 21
Normal control (1 ml/kg)	0.13±0.01$^{###}$	0.12±0.01$^{###}$	0.13±0.01$^{###}$	0.13±0.01$^{###}$
CFA-control	1.95±0.01	1.34±0.03	1.28±0.03	1.52±0.02
Indomethacin (3 mg/kg)	1.60±0.02***	1.17±0.02***	0.72±0.03***	0.67±0.02***
Eupatorium perfoliatum 6CH	1.89±0.02	1.39±0.02	1.21±0.01	1.39±0.07
Eupatorium perfoliatum 12CH	1.88±0.02	1.32±0.03	1.22±0.03	1.50±0.09
Eupatorium perfoliatum 30CH	1.85±0.02**	1.19±0.00**	1.14±0.03*	1.28±0.03**
Eupatorium perfoliatum 200CH	1.75±0.02***	1.22±0.02*	1.10±0.04**	1.11±0.04***
Crotalus horridus 6CH	1.92±0.02	1.43±0.02	1.26±0.02	1.36±0.02
Crotalus horridus 12CH	1.89±0.02	1.42±0.01	1.25±0.02	1.34±0.01
Crotalus horridus 30CH	1.90±0.01	1.40±0.017	1.25±0.03	1.35±0.01
Crotalus horridus 200CH	1.83±0.01**	1.22±0.039*	1.14±0.02*	1.28±0.02**

*P<0.1, **P<0.01, ***P<0.001 comparison with CFA-control group. $^{###}P$<0.001 comparison with normal control group. CFA: Complete Freund's adjuvant

inflammatory and analgesic processes.[17] CFA elicits joint swelling, synovial membrane inflammation and cartilage destruction.[5] Treatment of CFA rats with *Eupatorium perfoliatum* 200CH inhibited paw swelling, stiffness and joint inflammation.

In acute toxicity study, there were no mortality or any signs of toxicity observed after oral administration of drugs up to the dose level of 2000 µl/kg in rats. In subacute toxicity study, the body and organ weights of experimental animals did not show any significant changes after administration of the different homoeopathic drugs for 28 days, when compared to the normal control group. Haematological parameters analysed included complete blood count of experimental and control group animals. Analysis of blood parameters in animal studies is relevant to evaluate the risk of alterations of the haematopoietic system in toxicity studies. In this study, administration of the different homoeopathic drugs after 28 days induces no significant change in all haematological parameters as compared to normal control group. Assay of biochemical parameters were performed in order to evaluate the liver, renal, lipid and glycaemic profiles of all experimental animals, In this study, assay of the liver profile parameters (serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase) revealed normal functioning of the liver after 28 days of administration of different homoeopathic drugs, with normal values in experimental animals as compared to normal control group. Histopathological examinations of the liver, kidney, heart, brain, testis and ovaries did not reveal any morphological changes after administration of test drugs. Thus, both homoeopathic drugs, i.e., *Eupatorium perfoliatum* and *Crotalus horridus* at different dilutions were found to be safe with no pathological abnormalities on any organ of treated animals.

Conclusion

Homoeopathic drugs – *Eupatorium perfoliatum* and *Crotalus horridus* are safe and effective in treating inflammation and arthritis in CFA-induced model. However, further studies are required to find the potential mechanism of these homoeopathic drugs and their other possible pharmacological activity.

Financial support and sponsorship

This research received grant from CCRH, Ministry of AYUSH, Government of India.

Conflicts of interest

None declared.

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यूपोटोरियम परफोलिएटम और क्रोटेलस हॉरिडस के विभिन्न विलयनों द्वारा सीएफए–प्रेरित गठिया का क्षीणन व सुरक्षा मूल्यांकन

उद्देश्यः इस परियोजन का उद्देश्य होम्योपैथिक औषधियों यूपोरियम परफोलिटम और क्रोटेलस हॉरिडस के सूजन और सीएफए–प्रेरित गठिया के प्रायोगिक मॉडल में निरोधात्मक प्रभाव की जांच करना था।

सामग्री और विधिः चूहों को आठ समूहों (एन = 6) में विभाजित किया गया है। 21 दिनों की अध्ययन अवधि के दौरान, अलग–अलग डाईल्युशन्स (6 सीएच, 12सीएच, 30 सीएच और 200 सीएच) में यूपोरिटियम परफोलिएटम और क्रोटेलस हॉरिडस को मौखिक रूप से, दैनिक रूप से दिया गया और कम्पलीट फ्रूड्स एडजुवांत–इनडुस आर्थराइटिस (सीएफए) मॉडल में संयुक्त व्यास को मापने के द्वारा संयुक्त शिथिलता पर उनके प्रभाव का मूल्यांकन किया गया। इसके अलावा, इन होम्योपैथिक दवाओं के सूजन विरोधी प्रोफाइल को कैरेजेनन प्रेरित पॉ एडिमा मॉडल में भी जाँचा गया। ओईसीडी 425, 407 दिशानिर्देशों के अनुसार तीव्र और उप–गहन अध्ययन किए गए। 28 वें दिन, चूहों का बलिदान कर और हिस्टोपैथिक विश्लेषण के लिए जैव रासायनिक और हीमेटोलॉजिकल आकलन किए गए।

परिणामः अध्ययन के निष्कर्षों से पता चला कि सीएफए प्रबंधित, सभी परीक्षण किए गए चूहों में संयुक्त व्यास में एक महत्वपूर्ण (पी <0.01) वृद्धि हुई। सभी उपचार समूहों में संयुक्त व्यास में अधिकतम वृद्धि तीसरे दिन देखी गई। यूपेटोरियम परफोलिएटम 200 सीएच ने 21वे दिन संयुक्त व्यास में उल्लेखनीय कमी दिखाई। कैरेजेनन अध्ययन में, होम्योपैथिक दवाओं ने 5 घंटे के पोस्ट कैरेजेनन प्रबंधन में पॉ एडिमा में महत्वपूर्ण कमी का उत्पादन किया। अध्ययन में पाया गया कि यूटोपोरियम परफोलिटम की सूजन गतिविधि क्रोटालुशोरिडस से बेहतर पाई गई।

निष्कर्षः इस अध्ययन से यह अनुमान लगाया गया है कि यूपेटोरियम परफोलिएटम और क्रोटेलस हॉरिडस 6 सीएच, 12 सीएच, 30 सीएच और 200 सीएच डाईलुशन्स सुरक्षित हैं और सूजन को कम करने में प्रभावी हैं।

Évaluation de l'innocuité et de l'atténuation de l'arthrite induite par l'ACF (adjuvant complet de Freund) en utilisant différentes dilutions d'Eupatorium perfoliatum et de Crotalus horridus

Objectif: Cette étude avait comme objectif d'étudier l'effet inhibiteur des médicaments homéopathiques, *Eupatorium perfoliatum* et *Crotalushorridus* dans des modèles expérimentaux d'inflammation et d'arthrite induite par l'ACF.

Matériels et and méthodes: Les rats ont été divisés en huit groupes ($n = 6$). L'*Eupatorium perfoliatum* et le *Crotalushorridus* ont été administrés quotidiennement par voie orale en différentes dilutions (6CH, 12CH, 30CH et 200CH) pendant la période d'étude de 21 jours et leur effet sur le dysfonctionnement de l'articulation a été évalué en mesurant le diamètre articulaire dans le modèle d'arthrite induite par l'ACF. En outre, les profils inflammatoires de ces médicaments homéopathiques ont été évalués chez les rats dont l'œdème de la patte a été induit par le carraghénane. Des études de toxicité aiguë et subaiguë ont été réalisées d'après les lignes directrices 425, 407 de l'OCDE. Les rats ont été sacrifiés le 28^{e} jour et des estimations biochimiques et hématologiques ont été réalisées pour effectuer l'analyse histopathologique.

Résultats: Les conclusions de l'étude ont révélé que suite à l'administration de l'ACF il y avait une augmentation significative ($P < 0,01$) du diamètre articulaire chez tous les rats testés. Une augmentation maximale du diamètre articulaire a été observée le 3ème jour chez tous les groupes traités. *Eupatorium perfoliatum* 200CH a réduit de manière significative le diamètre articulaire au 21^{ième} jour. Dans l'étude utilisant le carraghénane, les médicaments homéopathiques ont contribué à une réduction importante de l'œdème de la patte 5 heures après l'administration de carraghénane. L'étude a révélé que l'activité anti-inflammatoire de l'*Eupatorium perfoliatum* était supérieure à celle du *Crotalushorridus*.

Conclusion: Cette étude montre que l'*Eupatorium perfoliatum* et le *Crotalushorridus* sont sûrs en dilutions de 6CH, 12CH, 30CH et 200CH et s'avèrent efficaces dans la réduction de l'inflammation.

Evaluación de la seguridad y atenuación de la artritis inducida por CFA por diferentes diluciones de Eupatorium perfoliatum y Crotalus horridus

Resumen

Objetivo: el objetivo de este trabajo fue investigar el efecto inhibitorio de los medicamentos homeopáticos, Eupatorium perfoliatum y Crotalus horridus en modelos experimentales de inflamación y artritis inducida por CFA con evaluación de sus aspectos de seguridad mediante estudios de toxicidad aguda y subaguda.

Materiales y métodos: Los animales se dividieron en ocho grupos (n = 6). Eupatorium perfoliatum y Crotalus horridus en diferentes diluciones (6CH, 12CH, 30CH y 200CH) se administraron por vía oral, diariamente durante el período de estudio de 21 días y se evaluó su efecto sobre la disfunción articular midiendo el diámetro de la articulación en la artritis inducida por adyuvante de Freund completo (CFA) modelo. Además, los perfiles inflamatorios de estos medicamentos homeopáticos se examinaron en el modelo de edema de la pata inducido por carragenina. Los estudios agudos y subagudos se llevaron a cabo según las directrices de la OCDE 425, 407. El estudio de toxicidad subaguda se llevó a cabo durante 28 días y se observó a todos los animales anormalidades de comportamiento. Al final del día 28, los animales fueron sacrificados para llevar a cabo las estimaciones bioquímicas, hematológicas e histopatológicas.

Resultados: Los hallazgos del estudio revelaron que en la administración de CFA, hay un aumento significativo (P <0.01) en el diámetro de la articulación en todos los animales analizados. El aumento máximo en el diámetro de la articulación se observó el día 3 en todos los grupos de tratamiento. Eupatorium perfoliatum 200CH mostró una disminución significativa en el diámetro de la articulación el día 21. En el estudio con carragenina, los medicamentos homeopáticos produjeron una reducción significativa en el edema de la pata a las 5 horas después de la administración de carragenina. Un estudio observó que la actividad antiinflamatoria de Eupatorium perfoliatum era superior a Crotalus horridus.

Sicherheitsbewertung und Abschwächung von CFA-induzierter Arthritis durch unterschiedliche Verdünnungen von Eupatorium perfoliatum und Crotalus horridus

Ziel: Ziel dieser Arbeit war es, erstmals die Hemmwirkung der homöopathischen Wirkstoffe Eupatorium perfoliatum und Crotalus horridus in experimentellen Modellen bei Entzündungen und CFA-induzierter Arthritis zu untersuchen und deren Sicherheitsaspekte durch akute und subakute Toxizitätsstudien.

Material & Methoden: Die Tiere wurden in acht Gruppen eingeteilt (n = 6). Eupatorium perfoliatum und Crotalus horridus wurden in verschiedenen Verdünnungen (C 6, C 12, C30 und C200) täglich während des Untersuchungszeitraums von 21 Tagen oral verabreicht. Ihre Wirkung auf die Gelenkfunktionsstörung wurde durch Messungen des Gelenkdurchmessers bei derFreund's adjuvans (CFA)-Pfotenentzündung bewertet. Darüber hinaus wurden Entzündungsprofile der verwendeten homöopathischen Arzneimittel im Carrageenan-induzierten Pfotenödem-Modell untersucht. Akute und subakute Studien wurden gemäß der Richtlinien der OECD 425, 407 durchgeführt. Die subakute Toxizitätsstudie wurde über eine Dauer von 28 Tagen durchgeführt, und alle Tiere wurden auf Verhaltensstörungen hin beobachtet. Am Ende des 28. Tages wurden die Tiere getötet, um die biochemischen, hämatologischen und histopathologischen Schätzungen durchzuführen.

Ergebnisse: Die Ergebnisse der Studie zeigten, dass durch CFA-Verabreichung bei allen getesteten Tieren eine signifikante (P < 0,01) Zunahme des Gelenkdurchmessers zu verzeichnen war. Die maximale Zunahme des Gelenkdurchmessers wurde am dritten Tag in allen Behandlungsgruppen beobachtet. Eupatorium perfoliatum C 200brachte am 21. Tag eine signifikante Abnahme des Gelenkdurchmessers. In einer Carrageenan-Studie zeigten die homöopathischen Arzneimittel eine signifikante Reduktion des Pfotenödems fünfStunden nach der Verabreichung von Carrageenan. Die Studie zeigte, dass die entzündungshemmende Aktivität von Eupatorium perfoliatum der vonCrotalus horridus überlegen war.

Schlussfolgerung: Die Studie ergab, dass Eupatorium perfoliatum und Crotalus horridus in den Verdünnungen C 6, C 12,C 30 und C 200 sicher und wirksambei der Minimierung von Entzündungen und Arthritis im CFA-induzierten Modell sind.

Safety evaluation and attenuation of CFA-induced arthritis by different dilutions of *Eupatorium perfoliatum* and *Crotalus horridus*

不同稀釋層級的蘭草（*Eupatorium perfoliatum*）和森林響尾蛇（*Crotalus horridus*）對CFA誘導關節炎的安全性評價及抑制作用

摘要

目的：本研究的目的是研究順勢療法藥物蘭草（*Eupatorium perfoliatum*）和森林響尾蛇（*Crotalus horridus*）在炎症和CFA誘導關節炎的實驗模型中的抑制作用，並通過急性和亞急性毒性研究評估其安全性。

材料與方法：將動物分為8組（$n = 6$）。在21天的研究期間，每天口服不同稀釋層級（6CH，12CH，30CH和200CH）的蘭草和森林響尾蛇，通過以完全弗氏佐劑誘導的關節炎（CFA）模型量度的關節直徑，來評估它們對關節功能障礙的影響。此外，在角叉菜誘發的腳掌腫模型中篩選這些順勢療法藥物的炎症特徵。根據經合組織（OECD）425,407指南進行急性和亞急性研究。亞急性毒性研究持續28天，觀察所有動物的行為異常。在第28天結束時，處死動物以進行生物化學、血液學和組織病理學評估。

結果：該研究的結果顯示，在注入CFA後，所有測試動物的關節直徑均顯著增加（$P<0.01$）。第3天，在所有治療組均觀察到關節直徑增加到最大。在第21天，蘭草200 CH的關節直徑顯著減小。在角叉菜膠研究中，順勢療法藥物可在角叉菜膠給藥後5小時顯著減少足腫脹。研究指出，發現蘭草的抗炎活性優勝於森林響尾蛇。