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Anti-arthritic Evaluation of Different Extracts of *Boerhaavia diffusa* Linn. in FCA Induced Arthritis In Rats

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ABSTRACT

The main aim of study is to evaluate the anti-arthritic effect of different extracts of *Boerhaavia diffusa* in arthritic rats. Different extracts were prepared by successive solvent extraction methods by using the various polar and non polar solvents and their % yields were calculated. Arthritis was induced by FCA induced arthritis model in rats and paw volume was measured on different days. Body weights of all animals were also measured simultaneously and at the end of experiment some haematological parameters were measured. On preliminary phytochemical studies extracts showed the presence of alkaloids, fatty acids, terpenoids, flavonoids and phenolic compounds. Among all extracts, methanolic extract significantly decreased the paw volume in all treated groups. Methanolic extracts also restored the body weight significantly. The results of our study revealed that all the extracts treated group's causes significant alterations in the hematological parameters and maximal effects were observed at 400 mg/kg. Since methanolic extract showed best activity in arthritic model and its phytochemical study showed presence of flavonoids and phenolic compounds, so it may be possible that anti-arthritic activity of root extracts may be due to presence flavonoids.

Keywords: Arthritis, FCA induced arthritis, *Boerhaavia diffusa*, haematological parameters, and Body weight

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INTRODUCTION

Clinically, arthritis is characterized by Polyarthritic, swelling and, in many cases, manifests extra-articular involvement. In the early period of the disease, typical signs and symptoms are swelling and pain of the proximal interphalangeal and metacarpophalangeal joints. Later, the better joints become affected, especially those of the arms, feet and knees. In addition, arthritis can affect other systems of the body, and this may range from rheumatoid nodules to life-threatening vasculitis (Smolen & Steiner 2003).

Presently many steroidal, NSAIDs and immunosuppressive drugs are used to change and manage inflammatory reaction and pain; however, they are connected with certain unwanted side effects (gastric ulceration, bleeding and platelets dysfunction) and due to a variety of toxic and adverse effects of synthetic drugs. With these difficulties, the ground of arthritis research has progressed exponentially with the use of herbal drugs (Flower *et al*., 1980).

As per the literature review, it has been observed that *Boerhaavia diffusa* (root) are listed among the various medicinal plants widely been used in the acute and chronic inflammatory conditions. In the absence of any scientific confirmation for their anti-arthritic activity in chronic inflammatory conditions, an attempt is made for establishing the anti-arthritic activity of various extracts of roots of *Boerhaavia diffusa* so that we are able to come up with a more effective extract with fewer side effects in comparison with existing synthetic drugs.

MATERIALS AND METHODS

Procurement of Plant Materials & Authentification

The roots of *Boerhaavia diffusa* were purchased from the local market. The roots were taxonomically identified by Botanist and herbarium sheets were submitted in School of Studies in Botany, Vikram University, Ujjain under voucher specimen number SUI/2018.

Preparation of Extract by Successive Solvent Method

Accurately weighed quantity of roots of *Boerhaavia diffusa* were extracted using petroleum ether, chloroform, methanol and finally water by soxhlet apparatus for 72 h. The extracts were dried completely under reduced pressure. After drying, the respective extracts were weighed and percentage yield was determined (Mukherjee, 2002).

Preliminary Phytochemical Tests

Qualitative chemical tests of ethanolic and Methanolic extracts were subjected to various chemical tests to detect various phytoconstituents (Kokate, 2003; Khandelwal, 2006).

Preliminary *In-Vivo* Anti-Arthritic Activity

Selection of animals

Wistar albino rats of either sex between 2 and 3 months of age weighing 150-200 g were used which were procured from the central animal house of department of pharmacy, Oriental University, Indore. (MP), India. The animals were allowed free to access commercial rat pallet diet (Lipton India Ltd, Mumbai, India) and water *ad libitum.* The study designs were approved by the Institutional Animal Ethical Committee of department of pharmacy, Oriental University Indore. (MP), India.

Acute toxicity studies

The acute oral toxicity studies were carried out according to the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guideline 423 (OECD guidelines, 2001).

Evaluation of anti-arthritic activity

Suspensions of the test extracts were prepared in 1% Tween 80.

The Wistar albino rats were divided into 11 groups of six animals in each. For the induction of chronic inflammatory response, FCA (0.1 ml) was injected in left ankle joint of rats on zero days. Prior to the injection of Freund's Complete Adjuvant (FCA) measured by left paw volume of each animal at zero day for the induction of arthritis in Wistar rats. The treatment with all extracts were given once daily from day of injection to 21st day.

The animal groups are as follows (Arulmozhi *et al*., 2011).

Group-I: Normal control, treated with 1 ml of Tween 80.

Group-II: Arthritic control, treated with 0.1 mL of FCA on zero day.

Group-III: Standard control: treated with prednisolone (10 mg/kg, p.o.) + FCA

Group-IV: Treated with pet ether extracts of *B. diffusa* (200 mg/kg, p.o.) + FCA

Group-V: Treated with pet ether extracts of *B. diffusa* (400 mg/kg, p.o.) + FCA

Group-VI: Treated with chloroform extracts of *B. diffusa* (200 mg/kg, p.o.) + FCA

Group-VII: Treated with chloroform extracts of *B. diffusa* (400 mg/kg, p.o.) + FCA

Group-VIII: Treated with methanolic extracts of *B. diffusa* (200 mg/kg, p.o.) + FCA

Group-IX: Treated with methanolic extracts of *B. diffusa* (400 mg/kg, p.o.) + FCA

Group-X: Treated with aqueous extracts of *B. diffusa* (200 mg/kg, p.o.) + FCA

Group-XI: Treated with aqueous extracts of *B. diffusa* (400 mg/kg, p.o.) + FCA

Determination of various parameters

Measurements of paw volume

The strictness of adjuvant arthritis was quantified by measuring the volume of the hind paw using Plethysmograph. Paw volume (ml) was measured at zero days and afterward 4, 8, 12, 16 and 21 days of FCA post-inoculation. Data were uttered as the increase in paw volume with respects to day zero paw volume.

The percentage inhibition of paw volume was calculated by following formula (Arulmozhi *et al*., 2011; Ignacimuthu *et al*., 2011).

$$\% \text{ inhibition} = (Vc - Vt)/Vt \times 100$$

Where,

Vc-Paw volume of control animals

Vt-Paw volume of treated animals

Measurements of body weight

Body weight was measured of all groups at zero days before immunization and at 21st day after treatments over by means of a single pan weighing balance (Jalalpure *et al*., 2011).

Measurements of hematological parameters

On the 21st day after arthritis initiation, rats were anaesthetized with ether and blood samples were collected into Ethylenediamine tetra-acetic acid (EDTA)-coated tubes from retro orbital junction. The number of leukocytes from each rat was determined using a counting chamber (celldyn-1200, Abbott Carepam). Erythrocyte sedimentation rate (ESR) was determined using the Wintrobe method. RBCs and Haemoglobin were determined by routine laboratory method (Jalalpure *et al*., 2011).

Statistical Analysis

The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $p < 0.05$ was chosen as the level of significance.

RESULTS

Extractive Value Determination

Dried roots of *Boerhaavia diffusa* were extracted by various solvents i.e. petroleum ether, chloroform, methanol and finally water. The percentage yields of all dried extracts were as follows.

Table 1: Different extracts with their appearance and % yield (in gm)

S. No.	Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Petroleum ether extract of *Boerhaavia diffusa*	Dark Brown	Sticky	12 %
2	Chloroform extract of *Boerhaavia diffusa*	Dark Brown	Dried powder	7 %
3	Methanolic extract of *Boerhaavia diffusa*	Dark Reddish & Orange	Sticky	18 %
4	Aqueous extract of *Boerhaavia diffusa*	Light Brown	Sticky	5.5 %

Preliminary Phytochemical Screening

Presence of carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds were confirmed on preliminary phytochemical studies.

Anti-Arthritic Activity of Different Root Extracts

Freund's complete adjuvant induced rat paw edema

Paw volume were recorded on 4th, 8th, 12th, 16th, 21st day after injection of FCA/CFA. The CFA-induced arthritic control group showed signs of arthritis development, as seen by the increase in the paw volume and other indications, such as decreased body weight, also showed induction of arthritis in the CFA-treated control group rats. The assessment made on the 21st day showed that the treatment of *Boerhaavia diffusa* at both doses (low and high) had moderately significant and highly significant effect and reduced ($p < 0.01$ & $p<0.001$) the adjuvant-induced lesions in the respective treatment groups as compared with the arthritis control group. The results were expressed in Figure No 1 & 2.

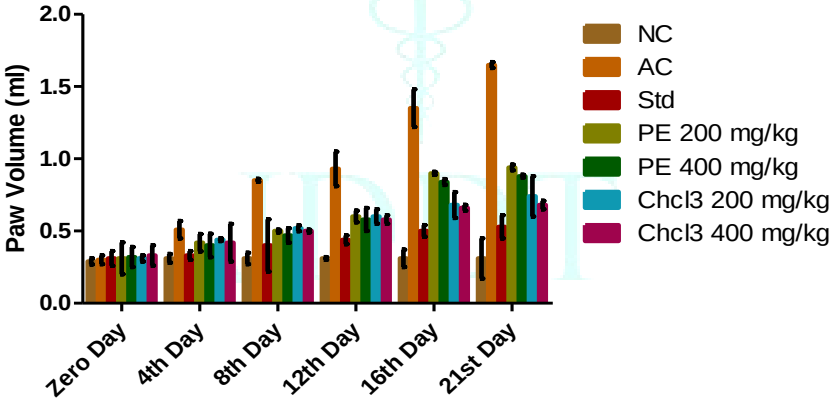

Figure 1: Effect of pet. ether & chloroform extracts on paw volume in arthritic rats

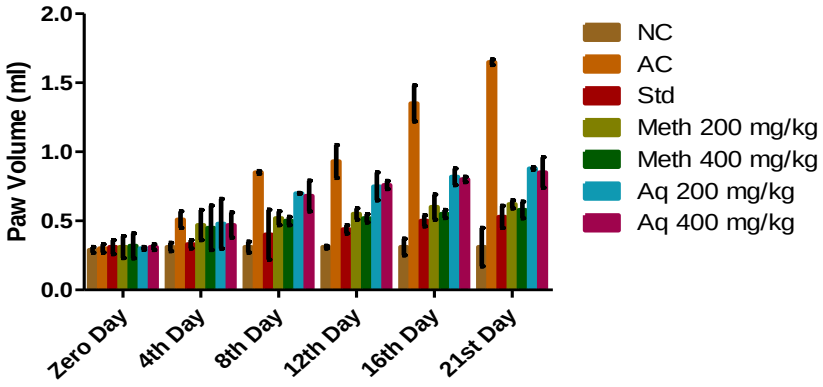

Figure 2: Effect of methanolic & aqueous extracts on paw volume in arthritic rats

Effects on body weight

Although the body weight always declined in arthritic control group from 14th day to 21st day. In arthritic group, decrease in body weight was observed on the subsequent days, whereas groups treated with standard and extracts of *Boerhaavia diffusa* showed improvements in body weight. This effect was observed from 14th day to last day of the experiment as compared to arthritic rats. All the extracts had moderately and highly significant increase in body weight ($p<0.01$ & $p<0.001$) as compared to arthritic rats, but highly significant effect was observed in methanolic extract.

Table 2: Effects of root extracts on body weight in FCA induced arthritis in rat

S. No.	Groups & Treatments	Days	
		Zero	21st
1	Normal Control	190.20±3.18	191.47±0.20
2	Arthritic Control, 1% Tween 80, p.o.	191.40±3.38	160.18±3.24***
3	Prednisolone, 10 mg/kg, p.o.	191.80±4.10	210.30±3.12***
4	Pet. ether, 200 mg/kg, p.o.	191.18±3.40	168.20±3.16
5	Pet. ether, 400 mg/kg, p.o.	190.20±3.50	175.40±2.78*
6	Chloroform, 200 mg/kg, p.o.	190.18±3.12	170.40±3.91
7	Chloroform, 400 mg/kg, p.o.	192.25±1.40	180.60±3.12*
8	Methanolic, 200 mg/kg, p.o.	191.10±2.65	205.35±3.38**
9	Methanolic, 400 mg/kg, p.o.	190.50±2.20	211.63±2.56**
10	Aqueous, 200 mg/kg, p.o.	190.65±2.38	181.44±4.19*
11	Aqueous, 400 mg/kg, p.o.	192.30±1.18	185.81±1.14*

Values are expressed as mean±SEM, n=6 in each group; *p<0.05, compared to disease control ** p<0.01, compared to disease control. ***p<0.001, compared to disease control

Effects on haematological parameters

Arthritis also affects the various haematological parameters i.e. WBCs, RBCs, ESR and Hb. It was found in arthritic rat that on 21st day, elevation in the total WBC counts and reduction in RBC. However, significantly *(p<0.001)* increased ESR while the haemoglobin was significantly *(p<0.001)* reduced in the control group when compared with normal group. However, standard and *Boerhaavia diffusa* had highly significant effects ($p<0.001$) in recovery of RBCs and haemoglobin. They also showed highly significant effects on decrease in WBCs and ESR. Among all the extracts, methanolic extract had highly significant effect on haematological parameters.

Table 3: Effects of root extract on haematological parameters in arthritis in rat

S. No.	Groups & Treatments	Haematological Parameters			
		Total WBCs count×10^3 cells/mm^3	RBCs (Million/$mm)^2$	Haemoglobin (g/dl)	ESR (mm/h)
1	Normal Control, 1% Tween 80, p.o.	8.45±0.90	8.88±1.11	15.82±2.33	10.40±2.42
2	Arthritic Control, 1% Tween 80, p.o.	15.34±1.22***	5.23±1.16**	9.46±2.12**	15.83±2.18***
3	Prednisolone 10 mg/kg, p.o.	8.66±1.75***	8.10±1.28**	14.24±2.46***	11.90±2.12***
4	Pet. ether, 200 mg/kg, p.o.	14.18±1.16	5.24±1.18	9.78±2.16	14.40±2.16
5	Pet. ether, 400 mg/kg, p.o.	14.26±1.31	5.96±1.24	9.48±2.38	14.10±2.19
6	Chloroform, 200 mg/kg, p.o.	11.38±2.26	6.44±1.09	10.32±2.31	13.85±2.10
7	Chloroform, 400 mg/kg, p.o.	10.86±2.21*	6.58±1.19	11.64±2.82	13.12±2.32
8	Methanolic, 200 mg/kg, p.o.	10.18±1.16**	7.24±1.18**	12.78±2.55**	12.49±2.16**
9	Methanolic, 400 mg/kg, p.o.	8.46±1.31***	8.96±1.24***	14.48±2.89***	11.56±2.19***
10	Aqueous, 200 mg/kg, p.o.	14.10±2.12	5.72±1.36	11.31±2.21*	13.89±2.26
11	Aqueous, 400 mg/kg, p.o.	13.52±2.51	5.84±1.57	12.65±2.34*	13.14±2.14

Values are expressed as mean±SEM, n=6 in each group; *p<0.05, compared to disease control ** p<0.01, compared to disease control. ***p<0.001, compared to disease control

DISCUSSION

CFA-induced experimental model for arthritis is well thought-out neighboring to simulating human rheumatoid arthritis and therefore it is the most widely used chronic test model in which the linked clinical and Histopathological changes are comparable to those seen in human form (Billingham & Davies, 1979; Butler *et al.*, 1992). In this model, dead tubercle bacilli in liquid paraffin initiate an immune-mediated inflammatory reaction which ultimately culminates in chronic inflammation (Cai *et al*., 2006). Initially the injection of CFA into the right hind-foot produces an inflamed swelling in the paw which reaches to its maximum during the first 3 days. After the 13th day additional swelling of the feet or joints occurs and by the 21st day the inflammation starts to subside leaving pale granulomatous swellings around the joints (Newbould *et al.*, 1963).

Treatment with all test extracts of roots showed dose dependent suppression in edema of the injected paw (primary lesions). Maximal effects were observed at the dose of 400 mg/kg body weight of test extracts. (Pearson & Wood, 1963). Our study results reveal that extracts of *Boerhaavia diffusa* treated rats significantly reduced the paw volume as compared to control animals.

During the expansion of arthritic syndrome, the body weight of rats used as a meandering index in restitution of health. The body weight was significantly decreased in arthritic rat as compared to normal rat, but in the test extracts and standard drug treated groups, the body weights of the rats did not decline. (Winder *et al*., 1969).

In our study, the body weight was significantly increased in the groups treated with prednisolone and all the extracts treated groups and this may be due to the restitution of absorption capacity of intestine.

With the development of arthritic conditions, there was a significant change in haematological parameters i.e. red blood cells (RBCs), white blood cells (WBCs), Haemoglobin (Hb) and erythrocyte sedimentation rate (ESR). As the disease progressed, RBCs and haemoglobin were decreased while; WBCs and ESR were increased in arthritic control group.

It was planned that the reduction in the Hb and RBC count during arthritis results from premature destruction of red blood cells, reduced erythropoietin levels (Mowat *et al.*, 1971). Further information of a significant rise of WBC count, in arthritic control group is possibly due to the stimulation of immune system. In addition an increase in ESR is a common feature in rheumatoid arthritis (Glenn *et al.*,1971) and this increase in the ESR is attributed to the accelerated formation of endogenous proteins (Hu *et al.*, 2005).

The results of our study revealed that all the extracts treated group's causes significant alterations in the hematological parameters and maximal effects were observed at 400 mg/kg.

Since methanolic extract showed best activity in arthritic model and its phytochemical study showed presence of flavonoids and phenolic compounds, so it may be possible that anti-arthritic activity of root extracts may be due to presence flavonoids.

CONCLUSION

This study was designed to evaluate the anti-arthritic activity of various extracts of *Boerhaavia diffusa* in suitable arthritic models in rats.

In conclusion, the mechanism may be mediated via the hang-up of prostaglandin synthesis in acute inflammatory reaction as well as reserve of various lysosomal enzymes in chronic inflammatory responses this, justifies the claim made by Siddha and Ayurveda.

Flavonoids present in methanolic extract could serve as lead molecules for development of prospective anti-arthritic agents.

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