Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL 15, ISSUE 8, 2024

"Pharmacological Evaluation of Anti-arthiritic activity of *Euphorbia hirta* Plant Extract on Rats"

Alok Kumar Shukla[1], Har Govind Garg[2], Ashish Chourasia[3], Mehta Parulben D.[4]

Lakshmi Narain College of Pharmacy, Bhopal, M.P.
Corresponding Author:
Alok Kumar Shukla
Research scholar, Lakshmi Narain College of Pharmacy, Raisen road, Bhopal, M.P. India

Abstract

Euphorbia hirta is of the family Euphorbiaceae, owning various pharmacological properties like anti-inflammatory activity. Rheumatoid Arthritis (RA) is a chronic-autoimmune systemic diseasethat can be characterized by cartilage degradation, synovial hyperplasia, inflammation, and joint damage. Objective of the study is to assess the anti-arthritic activity of methanolic extract of *Euphorbia hirta*. Freund's complete adjuvant induced arthritic Model was followed for the evaluation. 0.1 mL of the FCA by intraplanar injection was used to induce arthritis in Wistar rats. Methanolic extract (100 and 200 mg/kg) of *Euphorbia hirta* were given to Wistar rats. *Euphorbia hirta* extract showed significant anti-arthritic activity in a dose-dependent manner. However, *Euphorbia hirta* extract (200mg/kg) showed potential anti-arthritic activity. From the study, it can be concluded that *Euphorbia hirta* possesses marked anti-arthriticactivity.

Keywords: Rheumatoid Arthritis, *Euphorbia hirta*, Freund's complete adjuvant, anti-arthritic activity

INRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease predominantly affecting the joints and periarticular tissue. RA still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities and cartilage destruction and commonly leads to significant disability, caused by no. of proinflammatory molecules released by macrophages including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines. Generally, a strategic treatment plan is employed for the treatment of the disease which includes four different classes of drugs: non-steroidal anti-inflammatory agents (NSAIDs), corticosteroids, disease modifying anti-rheumatic drugs (DMARDs) and biological agents. As the disease is more prevalent among the females, therefore the treatment strategies for females in the child bearing age need special caution as the treatment employed for curing their arthritic condition can have negative impact on their potential for conceiving and also during pregnancy. Herbal medicines are used for the treatment of various ailments from ancient times and it is not an exaggeration to say that the use of the herbal drugs is as old as mankind. Herbal medicines aresynthesized from the therapeutic experience of generation of practicing physicians of ancient system of medicine for more than hundreds of years. Nowadays, researcher shows a great interest in those medicinal agents that are derived from plants because the currently available drugs are either have certain side effects or are highly expensive. Nature has blessed us with enormous wealth of herbal plants which are widely distributed all over the world as a source of therapeutic agents for the prevention and cure of various diseases. According to WHO, world's 80% population uses herbal medicines for their primary health care needs. Herbal medicines willact as parcels of

human society to combat disease from the dawn of civilization. The medicinallyimportant parts of these herbal plants are chemical constituents that produce a desired physiological action on the body.

Euphorbia hirta belongs to the plant family *Euphorbiaceae* and genus *Euphorbia*. It is a slender- stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color. This group of plants has been a subject of intense phytochemical examination and isolated compounds which include flavonoids, triterpenoids, alkanes, amino acids, and alkaloid. *E. hirta* is used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.), bronchial and respiratory diseases (asthma, bronchitis, hay fever, etc.), and in conjunctivitis.

MATERIAL AND METHOD

Plant collection

The medicinal plant *Euphorbia hirta* (300 gm) was collected. After cleaning, plant part (leaves) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant part (flower) was stored in air tight glass containers in dry and cool place to avoid contamination and deterioration. Medicinal plant *Euphorbia hirta* was authenticated by a plant taxonomist in order to confirm its identity and purity.

Extraction

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Euphorbia hirta* was placed in thimble of soxhlet apparatus. Soxhlation was performing at 60°C using petroleum ether as non-polar solvent.Exhausted plant material (marc) was dried and afterward re-extracted with methanol solvent. Foreach solvent, soxhlation was continued till no visual colour change will observe in siphon tube and completion of extraction were confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporate using rotary vacuum evaporator (Buchitype) at 40°C. The dried extract was weighed, and each extract's % yield was calculated.

Phytochemical investigation

An experiment was carried out to determine the presence or absence of several phytoconstituents using thorough qualitative phytochemical analysis.

Quantitative Phytochemical Estimation

TPC

The total phenolic content of *Euphorbia hirta* extract was determined using the Folin-Ciocalteu Assay. The *Euphorbia hirta* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically

TFC

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Euphorbia hirta* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to

stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight.

DPPH

The antioxidant activity of *Euphorbia hirta* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared.

Different concentration of *Euphorbia hirta* extracts /standard (20 – 100µg/ml) were prepared from 1mg/mL stock solution and 2mL of 0.1mM solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using UV spectrophotometer (Shimadzu 1700) at 517 nm. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm.

Acute Toxicity Study

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of thetest substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosedat one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight

Animals

Animals was selected randomly from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal, India and further divided into various treatment groups randomly and kept in propylene cage with sterile husk as bedding. Relative humidity of 30.7 % at 22±2°C and 12:12 light and dark cycle was maintained in the animal house and fed with standard pellets (Golden Feeds, New Delhi, India) and water was available *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. Separate group (n=6) of rats was used for each set of experiments. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal.

Freund's complete adjuvant induced arthritic Model

The rats were injected with 0.1 mL of the FCA by intraplanar injection in the left hind paw. Each mL of the adjuvant contains 1 mg of heat killed and dried *Mycobacterium tuberculosis* (strain H37Ra, ATTC 25177), 0.85 mL paraffin oil and 0.15 mL of mannite monooleate. After the injection of FCA, the paw volume of all the animals was measured using plethysmometer at 0, 7, 14, 21 and 28 days. The percentage inhibition of paw volume was calculated from the mean difference in the paw volume in the drug treated and control group using the following formula:

$$Vc\text{-}Vt/Vc\times100$$

Where Vc is the paw volume of the control group and Vt is the paw volume of the drug-treatedgroup.

The hind legs of the experimental rats were photographed to view the morphology of the arthriticcondition. At the end of the experiment, all the animals were sacrificed by administering high dose of chloroform as anaesthesia and blood was collected by cardiac puncture in plain and EDTA containing tubes, respectively, for serum separation. The serum samples were used for the estimation of marker enzymes like alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), C-reactive protein (CRP) and blood samples were used for analyzing the haematological parameters like red blood cell (RBC) count, white blood cell (WBC) count and hemoglobin (Hb) contents. The proximal interphalangeal joints from the adjuvant-induced arthritic rats were removed and fixed in 10% formalin and used for the histopathological studies.

Analysis of haematological parameters

The haematological parameters like RBC and WBC were counts. The haemoglobin content was determined by the acid haematin method.

Analysis of biochemical parameters

The serum samples obtained were used for the analysis of biochemical parameters like ALP, AST, ALT and CRP using standard kits in the fully automatic biochemical analyzer.

RESULTS

Percentage Yield

In phytochemical extraction was calculated the percentage yield with pet ether 0.55% and methanol 2.04%.

Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total Phenolic content (TPC) estimation

Table 1: Standard table for Gallic acid

S.No.	Concentration (µg/ml)	Absorbance
1.	20	0.150
2.	40	0.183
3.	60	0.194
4.	80	0.231
5.	100	0.275

Total Phenolic Content in extract

Table 2: Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.140	54 mg/gm
2	0.181	
3	0.192	

Table 3: Total Phenolic Content of extract *Euphorbia hirta*

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)

Methanol	54

Total Flavonoids content (TFC) estimation

Table 4: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.179
2.	40	0.203
3.	60	0.278
4.	80	0.321
5.	100	0.331

Total Flavonoid Content in extract

Table 5: Total Flavonoid Content

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.150	16.83 mg/gm
2	0.161	
3	0.195	

Table 6: Total Flavonoid Content of extract *Euphorbia hirta*

Extracts	Total Flavonoid content (mg/gm equivalentof rutin)
Methanol	16.83

In vitro Antioxidant Assays

In the present investigation, the in vitro anti-oxidant activity of extracts of *Euphorbia hirta* was evaluated by DPPH radical scavenging activity. The results are summarized in Tables.

DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 7: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.484	47.505
40	0.436	52.711
60	0.345	62.581
80	0.286	68.980
100	0.145	84.273
Control	0.992	
IC50		30.60

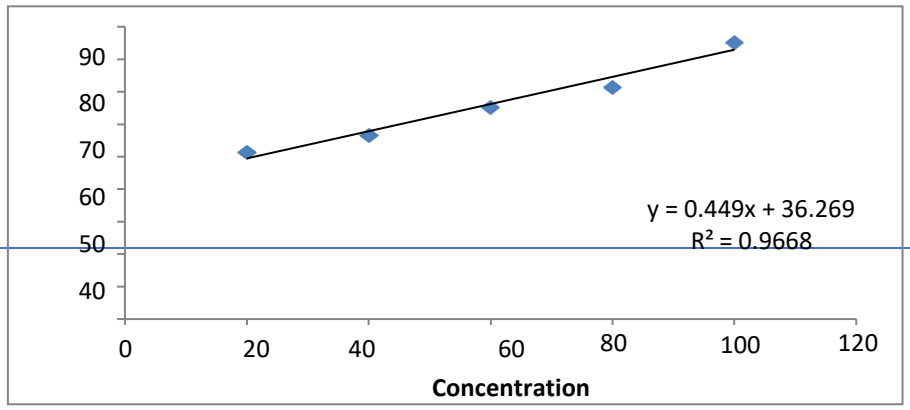


Figure 1: DPPH radical scavenging activity of Std. Ascorbic acid

Table 8: DPPH radical scavenging activity of methanol extract of *Euphorbia hirta*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.520	44.086
40	0.466	49.892
60	0.458	50.752
80	0.414	55.483
100	0.370	60.215
Control	0.930	
IC5		49.04

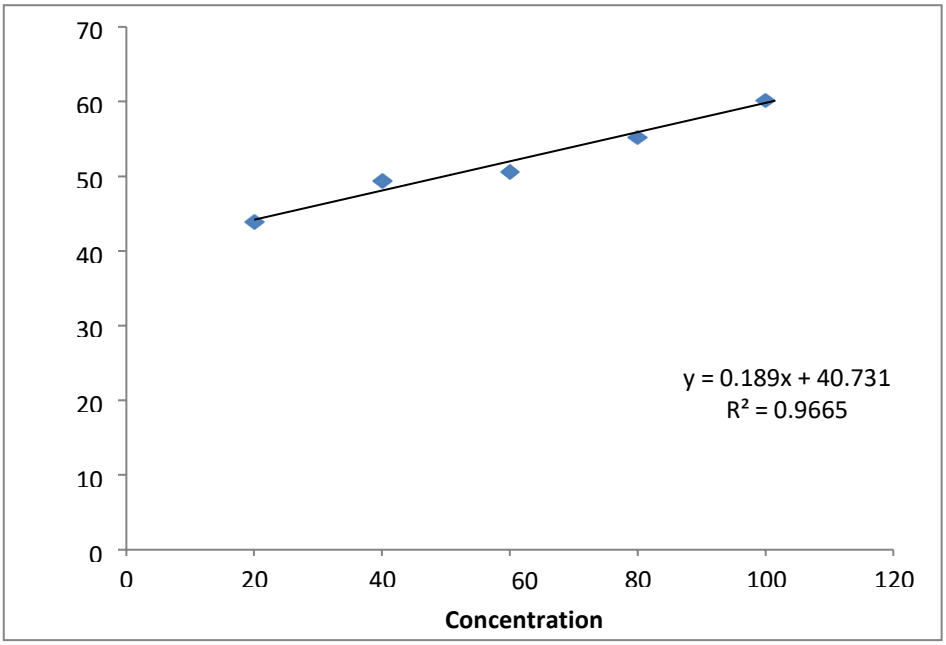


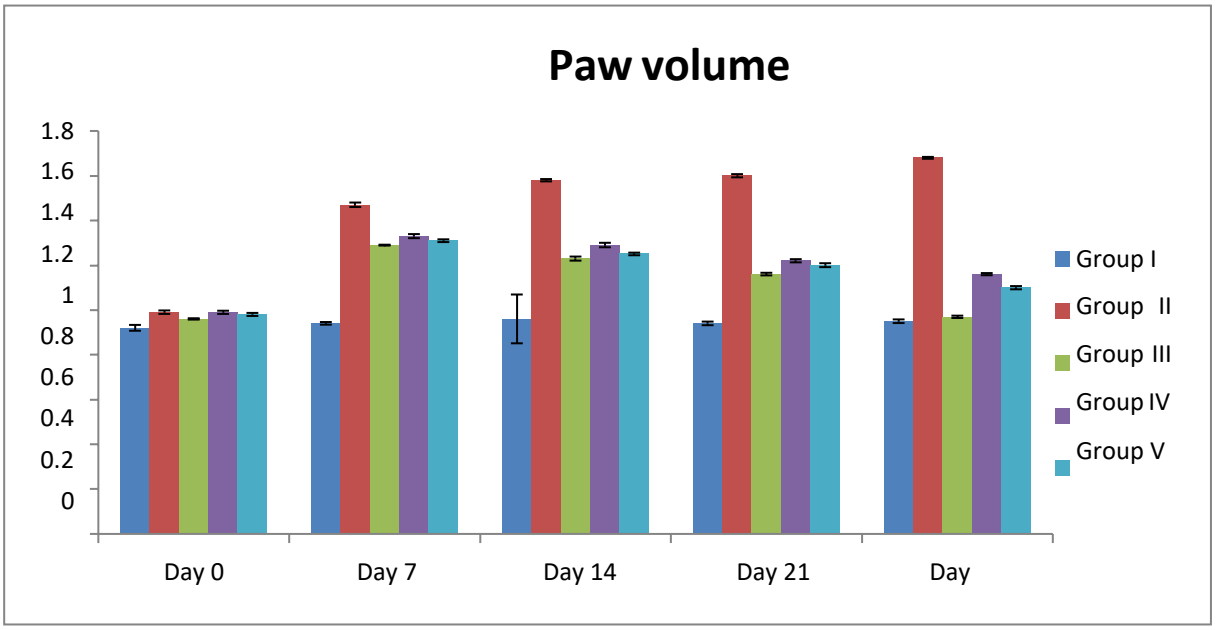
Figure 2: Represents the Percentage Inhibition *Vs* Concentration of extract of *Euphorbia hirta*

Freund's complete adjuvant induced arthritic Model

Table 9: Effect of *Euphorbia hirta* on paw volume of Freund's adjuvant induced arthriticrats.

	Paw volume of the rats in ml Mean ± SEM (% inhibition)				
Groups	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I	0.92 ± 0.013	0.94 ± 0.008	0.96 ± 0.003	0.94 ± 0.007	0.95 ± 0.007
Group II	0.99 ± 0.006	1.47 ± 0.011	1.58 ± 0.002	1.60 ± 0.009	1.68 ± 0.006
Group III	0.96 ± 0.109	1.29 ± 0.005	1.23 ± 0.009	1.16 ± 0.010	0.97 ± 0.006
Group IV	0.99 ± 0.008	1.33 ± 0.007	1.29 ± 0.006	1.22 ± 0.007	1.16 ± 0.009

Group V	0.98 ± 0.008	1.31 ± 0.004	1.25 ± 0.005	1.20 ± 0.005	1.10 ± 0.001



Graph 01: Effect of *Euphorbia hirta* on Paw volume

Haematological parameters

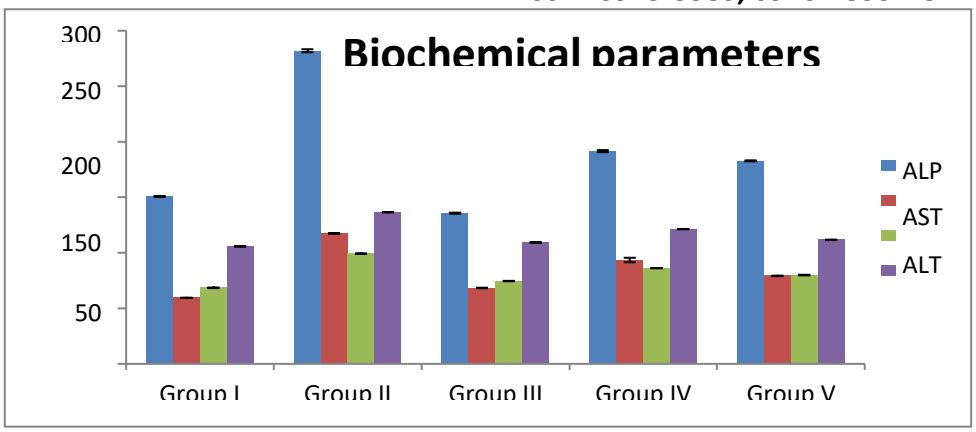
Table 10: Effect of *Euphorbia hirta* on haematological parameters of control and adjuvant induced arthritic rats

Groups	RBC (millions/mm3)	WBC (thousands/mm3)	Hb (g/dL)
Group I	7.21 ± 0.044	9.84 ± 0.018	14.15 ± 0.039
Group II	3.39 ± 0.038	19.89 ± 0.151	9.03 ± 0.037
Group III	6.06 ± 0.016	9.86 ± 0.009	13.12 ± 0.068
Group IV	5.43 ± 0.681	11.37 ± 0.062	10.99 ± 0.145
Group V	5.95 ± 0.021	9.99 ± 0.209	11.11 ± 0.047

Biochemical parameters

Table 11: Effect of *Euphorbia hirta* on biochemical parameters of control and adjuvantinduced arthritic rats.

Groups	ALP (Alkaline phosphatase)	AST (Aspartate transaminase)	ALT (Alanine transaminase)	CRP (C-reactive protein)
Group I	150.8 ± 0.013	59.42 ± 0.177	68.56 ± 0.245	105.8 ± 0.186
Group II	281.9 ± 1.379	117.4 ± 0.109	99.23 ± 0.074	136.6 ± 0.141
Group III	135.7 ± 0.471	68.43 ± 0.199	74.63 ± 0.158	109.3 ± 0.297
Group IV	191.6 ± 0.886	93.49 ± 2.057	86.23 ± 0.142	121.3 ± 0.142
Group V	182.8 ± 0.409	79.31 ± 0.193	80.01 ± 0.258	111.7 ± 0.209



Graph 02: Effect of *Euphorbia hirta* on biochemical parameters

Body weight

Table 12: Body weight

	Body weight	
	Initial weight	**Final weight**
Group I	210±1.62	216±2.89
Group II	215±1.66	224±2.49
Group III	212±0.18	218±1.30
Group IV	214±1.05	221±0.35
Group V	213±1.52	219±7.39

DISCUSSION

Phytochemical analysis of methanolic extract of *Euphorbia hirta* showed the presence of alkaloids, carbohydrate, saponins, flavonoids and saponin. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) andtotal flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. Results shown in Table 7 & table10. DPPH radical scavenging activity of *Euphorbia hirta* extract exhibited percent inhibition 60.21% and its IC 50value was found to be 49.04µg/ml. Ascorbic was used as a reference compound which exhibited percent inhibition 84.27% and showed IC 50 value of 30.60µg/ml.

In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 100 mg/kg and 200 mg/kg have been fixed for study. Changes in the paw volume of the adjuvant-induced arthritic rats were measured using digital plethysmometer. From the results obtained, it was observed that *Euphorbia hirta* (200 mg/kg) was effective in equivalent to the standard drug Diclofenac sodium on reducing the increase in paw volume. In the present study, the histopathological studies of hind paw joints in arthritic control rats showed the prominent abnormalities like destruction of the bone marrow and extensive infiltration of the cells in the articular surface. *Euphorbia hirta* treatment has shown marked reduction in all the above-mentioned pathological conditions, indicating its effective antiarthritic activity by protecting the bone

from degeneration.

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