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Research Article

ANTI-ARTHRITIC POTENTIAL OF *HELIANTHUS ANNUUS* IN LABORATORY ANIMALS

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ABSTRACT

Objective: The present study was carried out to investigate the anti-arthritic potential of ethanolic extract of *Helianthus annuus* leaves (HA).

Methods: The effect of HA was evaluated for chronic inflammation by complete Freud's adjuvant (CFA) induced arthritis in rats.

Results: The paw edema was measured along with biochemical, hematological, histopathological, radiographic parameters, and ulcerogenic potential. In this study, pre-treatment with HA significantly decreased paw volume, arthritic index**,** spleen and thymus weight, ulcerogenic index; and inhibited histopathological changes in joint cavity and inhibited destruction of the knee joints induced by CFA in radiographic examination. Treatment of HA also restored significantly the hematological parameters such as hemoglobin level, total red blood cell, total white blood cell, and erythrocyte sedimentation rate along with antioxidant parameters such as superoxide dismutase, catalase glutathione, and lipid peroxide. The serum marker of arthritis such as C-reactive protein (CRP) and rheumatoid factor were also reduced in the HA-treated arthritic rats.

Conclusion: The results of the present study demonstrate the anti-arthritic potential of HA leaves in the anti-arthritic activity.

Keywords*:* Anti-arthritic, C-reactive protein, Complete Freud's adjuvant, *Helianthus annuus*, Paw volume, Rheumatoid factor.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune chronic inflammatory disease affecting several parts of the joints including cartilage, synovium, tendons, and muscles and characterized by inflammation, pain, swelling and limited movement of joint as well as damage of the cartilage and erosions of the underlying bone [1]. Drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticosteroids or disease modifying drugs such as gold or methotrexate are being used for the treatment of RA with unavoidable side effects. Plants, such as *Ajuga bracteosa* [2], *Sterculia tragacantha* [3], *Vernonia Anthelmintica* [4], and *Palisota hirsuta* [5], are reported to have anti-arthritic potential.

Helianthus annuus (HA), belonging to family Compositae is commonly known as sunflower. Traditionally, it is used for treating painful and inflammatory conditions like arthritis [6]. The extract prepared from different parts of this plant has been reported to have various pharmacological activities such as hyperlipidemic [7], analgesic, and anti-inflammatory activity [8]. However, the anti-arthritic potential for leaves of HA is not yet been reported. Hence, the present study was undertaken to find out the anti-arthritic potential of leaves of HA by using complete Freund's adjuvant (CFA) induced arthritis.

METHODS

Experimental animals

The rats of either sex were purchased from National Toxicology Center, Pune and were housed in a group of five under standard laboratory conditions of temperature (25±2°C) and 12/12 hrs light/dark cycle. Animals had free access to standard pellet diet and water *ad libitum*. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA with IAEC clearance (DYPIPSR/IAEC/12-13/P-12).

Procurement and authentication of drug

The powder of HA leaves was purchased and authenticated from Endeavour exports, Nisha Bhavan, City - Marthandam, Tamil Nadu, India.

Preparation of extract

The leaf powder of HA was defatted with petroleum ether. Defatted course powder extracted with 95% ethanol using 7 days maceration method with occasionally shaking to obtain an ethanolic extract of HA leaves. The extract was dried using a rotary vaccum evaporator under 40°C [7,8]. This extract was reconstituted in distilled water and sodium carboxy methyl cellulose to get the desired concentration for further activity.

Phytochemical screening of the extract

Phytochemical screening of HA was done to find out the presence of phytochemicals such as a steroid, saponin, alkaloid, flavonoid, tannin, phenolic compound, and glycosides [9].

Acute oral toxicity study

Rats of either sex weighing 200-250 g were divided into 4 groups (n=3) and fasted overnight prior to drug administration. On the next day of fasting, the animals were administered with the HA extract at the dose of 5, 50, 300, and 2000 mg/kg body weight p.o. The animals were observed for 5 minutes every 30 minutes until 2 hrs and then at 4, 8, and 24 hrs after treatment for any behavioral changes/mortality and were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the ethanolic extract of HA leaves at the dose of 2000 mg/kg, p.o., and therefore, found safe up to dose of 2000 mg/kg. Thus, the $1/10^{th}$ of the dose, i.e. 200 mg/kg was selected as middle dose for further study [10]. The regime followed for rats is 100, 200, and 400 mg/kg, p.o. for HA extract.

CFA induced arthritis in rats

The Wistar albino rats weighing 200-250 g were divided into five groups (n=6) and fasted overnight with free access to water. Animals from the group I served as arthritic control (AC) were administered with distilled water. Animals belonging to Group II served as standard were administered with methotrexate (0.75 mg/kg, po). Group III to Group V were served as tests were administered with respective doses of ethanolic extract of HA leaves. Animals of all groups were made arthritic by single intra-dermal injection of 0.1 ml of CFA containing 1 mg/ml dry heat killed *Mycobacterium tuberculosis* per ml in sterile

paraffin oil into a foot pad of the left hind paw of rats. Test drug and standard drug were administered orally to a respective group of animals from day 9th to 21st day [3,11].

Paw edema volume evaluation (in ml)

Paw edema volume was measured after a 3-day interval by using plethysmometer (UGO Basile, Italy, 7140). Mean changes in injected and non-injected paw edema with respect to initial paw volume were calculated on respective day and % inhibition of paw edema with respect to the untreated group was calculated using following formula.

$$i=\{1-(\Delta v \text{ treated}/\Delta v \text{ untreated})\} \times 100$$

i=Percentage inhibition of paw edema
Δv treated=Mean changes in paw volume of treated rat
Δv untreated=Mean changes in paw volume of untreated rat

Arthritic index

On 21st day, arthritic index was determined by using following scoring system:

0=Normal paw; 1=Erythema of toe; 2=Erythema and swelling of paws; 3=Swelling of ankle; 4=Complete swelling of the whole leg and inability to bend it.

Body weight (g)

Body weight was measured after the 3-day interval and mean changes in body weight with respect to initial body weight was calculated for respective day.

Hematological and biochemical estimation

On 21st day, blood was withdrawn from all groups of animals and the hematological parameters such as hemoglobin (Hb) content, red blood cell (RBC) and white blood cell (WBC), and erythrocyte sedimentation rate (ESR) were determined by using the standardized laboratory method. The parameters such as C-reactive protein (CRP) and rheumatoid factor (RF) were also analyzed by using kits purchased from Bio lab. The antioxidant parameters, such as lipid peroxide (LPO), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), were evaluated [2].

Radiographic analysis

On 21st day, animals were anesthetized by ether. Radiographs of the adjuvant-injected hind paws were taken with X-ray instrument for radiographic changes.

Histopathological evaluation and determination of organ weight

On the 21st day, followed by radiographic analysis, the rats were sacrificed and the hind paws amputed above the knee joint were fixed in 10% formalin solution for histopathological evaluation. The Spleen and thymus were removed, and weight was determined.

Ulcerogenic index

Animals were further evaluated for ulcerogenic index using following scoring system: 0=No lesions; 0.5=Hyperemia; 1=One or two lesions; 2=Severe lesions; 3=Very severe lesions; 4=Mucosa full of lesions.

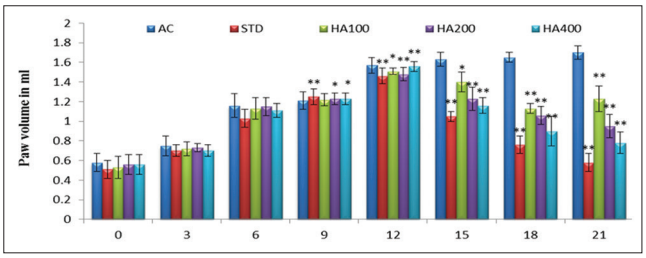
Statistical analysis of data

The statistical significance was assessed by using one-way analysis of variance (ANOVA) followed by Dunnet's comparisons test. All the data are presented as mean±standard error of mean (SEM) and $p<0.05$ and $p<0.01$ was considered as significant.

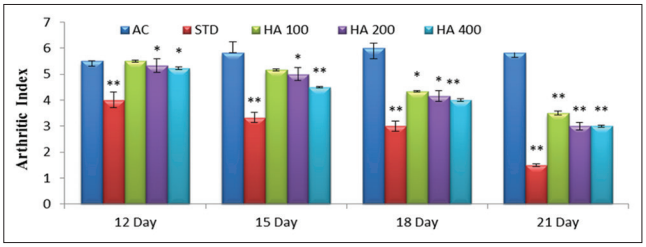
RESULTS AND DISCUSSION

CFA is 1.0 mg dry heat-killed *M. tuberculosis* per milliliter in sterile paraffin oil which in chronic infectious condition creates arthritis in human [12]. CFA-induced arthritis is characterized by increase a chronic swelling with edema of periarticular tissues and joint capsules in the influence of interleukin-1 (IL-1), IL-6, interferon-γ, and TNF-α [13]. These further progresses into erosion of joint cartilage and bone destruction followed by extra cellular activities of lysosomal enzymes. Lysosomal enzymes causes a deficit of structural macromolecules in connective tissue, cartilage proteoglycans and thus mediating tissue injury in rheumatic diseases [14,15,27]. In the present study, Graphs 1 and 2 show that HA extract has significantly decreased arthritic index as well as suppressed paw edema induced by the CFA as compared to arthritic rats. This indicates the possible immunosuppressive and anti-inflammatory activity of HA extract in RA.

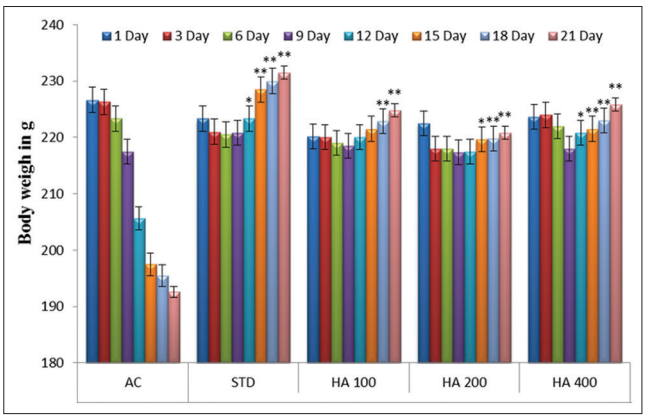
Due to alterations in the metabolic activities of arthritic rats, there may be a possibility to lose the body weight. Hence, change in body weight is being used to measure the cause of the disease and the response to therapy of anti-inflammatory drugs [11]. In previous studies, it has been observed that in inflamed arthritic rats there was reduction in absorption of 14C- glucose and 14C - leucine in intestine. However, with the treatment of anti-inflammatory drugs, a reduction in absorption was abolished and, therefore, the absorption capacity of the intestine during inflammation was normalized [16]. In Graph 3, there was a



Graph 1: Effect of *Helianthus annuus* on injected paw edema volume



Graph 2: Effect of *Helianthus annuus* on arthritic index



Graph 3: Effect of *Helianthus annuus* (HA) on body weight. Arthritic control: Distilled water+0.1 ml of complete Freund's adjuvant (CFA); STD: Methotrexate (0.75 mg/kg, p.o.)+0.1 ml of CFA; HA 100: HA extract 100 mg/kg+0.1 ml of CFA; HA 200: HA extract 200 mg/kg+0.1 ml of CFA; HA 400: HA extract 400 mg/kg+0.1 ml of CFA. The result were expressed as mean±standard error of mean (n=6). The data was analyzed using one-way analysis of variance followed by Dunnett's test. *p<0.05, **p<0.01 when compared with arthritic control group

reduction in body weight in AC rats while in rats treated with HA extract there was a significant increase in body weight. This increase in body weight by the restoration of absorption capacity of intestine may indicate the usefulness of HA extract in the loss of the body weight during arthritic condition.

In CFA-induced inflammatory arthritis, an increase in the production of both granulocyte and macrophages colony stimulating factors due to the release of IL-IB, which further leads to increase in WBC count [17]. Moreover, there is a change in bone marrow that prevents the release of iron for incorporation into RBC. This leads to decrease in erythropoiesis followed by the premature destruction of RBC and reduction in Hb count [18,19]. In the present study, Table 1 shows that there was increase in total WBC, RBC count and decrease in hemoglobin count in AC group of animals; but in rats treated with HA extract, there was significant restoration of total WBC, RBC, and hemoglobin count. This effect might be due to inhibition of migration of leucocytes into the inflamed area HA extract. Similar findings were suggested by Rajendran and Krishnakumar [11].

CRP is an annular (ring-shaped), pentameric protein synthesized at relatively low rates, retained within the endoplasmic reticulum without being degraded. Inflammatory stimulus leads to IL-6 **sec**retion from macrophages, and T-cells stimulated the release of CRP [17-19]. ESR is an estimation of the suspension stability of RBC's in plasma which is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially, fibrinogen, alpha, and beta globulins [20]. Increase ESR is also an indication of inflammation. Therefore, an increase in ESR and CRP contributes to inflammatory conditions in CFA-induced arthritis. In the present study, an increase in the ESR and CRP count in AC group of animals was counteracted in animals treated with HA justifying its use in the arthritic conditions (Table 1).

RF is autoantibody against the FC portion of immunoglobulin G usually found in RA patients. RF reacts with antigen and form antigen-antibody complex leading to pain and inflammation of synovial membrane [21]. In the present study, there was a significant increase in RF in arthritic rats; HA extract significantly restored levels of RF contributing its use in anti-arthritic activity (Table 1).

Thymus, central lymphoid organ shows it's an important role in cellular immunity by generating circulating T lymphocytes and maturation of thymocytes. An enlargement of thymus gland may be related with RA [22]. The spleen is another an important lymphoid organ involved in immune response. In adjuvant injected rats, there is an increase in cellularity of spleen which leads to antibody formation further supports the hyperimmune status by humoral immunity [23]. Graph 4 indicate that thymus and spleen weight was increased significantly in AC group, whereas treatment with HA extract significantly restored the thymus and spleen weight in arthritic rats as compared to AC group. Similar findings were suggested by Ramprasath *et al.* [24].

Free radicals, such as reactive oxygen species (ROS) trigger arachidonic acid pathway, cytokines production, and the apoptosis process, and, therefore, initiate the production inflammatory process [14]. Overproduction of ROS leads to tissue injury by damaging and LPO of membrane proteins and activate production of other inflammatory mediators through secondary messengers system [25]. ROS interacts with NO leading to the formation of peroxynitrite ($ONOO^-$) or alternatively be rapidly converted by SOD to oxygen and hydrogen peroxide which is eliminated by GSH reductase [26]. Increased level of LPO is an indication of profuse production of free radicals [14]. Anti-oxidant enzymes, such as SOD, CAT, and GSH peroxidase inhibit, the destruction caused by ROS. In the present study, Table 2 shows that the ethanolic extract of HA leaves increased the level of SOD, GSH, and CAT as well as decreased the level of LPO suggesting inhibition of inflammatory process by reducing the production of free radicals. The similar results were observed by Udegbunam *et al.* [3].

In arthritic conditions, bones are exposed directly to cytokines such as TNF- α and IL1. These cytokines then stimulate chondrocytes to produce proteolytic enzymes such as collagenases, glycohydrolases, and neutral proteases which cause degradation, erosion of the cartilage and displacement of bone in arthritic rats. During radiological and histopathological analysis, these changes were reverted back to near normal on *H. annuus* extract (HA) treatment (Figs. 1 and 2). The same observation was revealed by Blake *et al.*[26].

With the use of NSAIDs for the relief of pain and inflammation, there is a risk of gastrointestinal side effects like ulcer. In Fig. 3, HA showed significant inhibition of ulcerogenic index [28]. Thus, ulcer protective activity of HA may contribute further in the treatment of arthritis.

Furthermore, the preliminary phytochemical analysis showed the presence of phytoconstituents such as alkaloids, glycosides, saponins, tannins, and flavonoids which are having antioxidant activity [29-32] which further contributes to the anti-arthritic activity of HA.

CONCLUSION

The present study concludes the use of HA leaves in the treatment of arthritis by showing significant reduction in local paw edema,

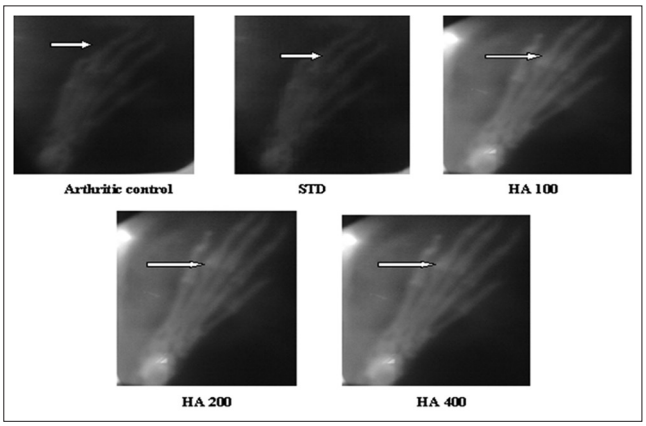


Fig. 1: Effect of HA on radio logical analysis (arrow - showing damage/displacement of joint tissue).

Table 1: Effect of HA on hematological parameters

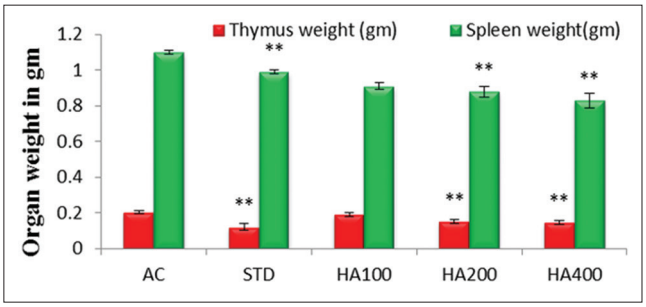
Group n=6	AC	STD	HA 100	HA 200	HA 400
Hb% (g/dL)	8.37±0.33	12.25±0.17**	11.10±0.32**	11.25±0.28**	12.25±0.31**
Total RBC ($\times10^3$/L)	3.4±0.25	7.2±0.16**	6.6±0.188**	6.9±0.18**	6.2±0.32**
Total WBC ($\times10^6$/L)	11172±1863	4655±221.27**	6546.66±463.23*	5963.33±759.10**	5043±3.0.51**
ESR mm/hr	5.01±0.18	3.22±0.17**	4.46±0.21	3.76±0.18**	3.55±0.12**
CRP mg/L	12.06±0.60	6.33±0.18**	9.45±0.71*	10.15±0.26**	9.7±0.38**
RF (IU/L)	13.1±0.51	6.1±0.48**	10.28±0.34	9.8±0.42**	9.11±0.22**

The result were expressed as mean±SEM (n=6). The data was analyzed using one-way ANOVA followed by Dunnett's test. *p<0.05, **p<0.01 when compared with AC group. AC: Arthritic control, WBC: White blood cell, RBC: Red blood cell, HA: Helianthus annuus, ESR: Erythrocyte sedimentation rate, RF: Rheumatoid factor, Hb: Hemoglobin, CRP: C-reactive protein, ANOVA: Analysis of variance, SEM: Standard error of mean

Table 2: Effect of HA on antioxidant enzymes

Group n=6	SOD (units/mg of wet tissue)	GSH (µg of GSH/g of wet tissue)	CAT (µM of H_2O_2/g of wet tissue/minute)	LPO (nM of MDA/g of wet tissue)
AC	66.66±6.0	16.17±0.5	16.08±0.38	20.41±0.09
STD	73.33±7.0**	21.83±0.66**	22.75±0.67**	16.95±0.66**
HA 100	68.33±7.14	18.21±0.59	16.18±0.75*	18.78±0.64*
HA 200	70.66±3.8*	21.48±0.5**	18.25±0.34**	17.85±0.77**
HA 400	70.83±3.7**	22.41±0.53**	20.25±0.31**	17.41±0.60**

The result were expressed as mean±SEM (n=6). The data was analyzed using one-way ANOVA followed by Dunnett's test. *p<0.05, **p<0.01 when compared with AC group. GSH: Glutathione, CAT: Catalase, SOD: Superoxide dismutase, LPO: Lipid peroxide, MDA: Malondialdehyde, HA: Helianthus annuus, AC: Arthritic control, SEM: Standard error of mean



Graph 4: Effect of *Helianthus annuus* on organ weight (thymus weight and spleen weight)

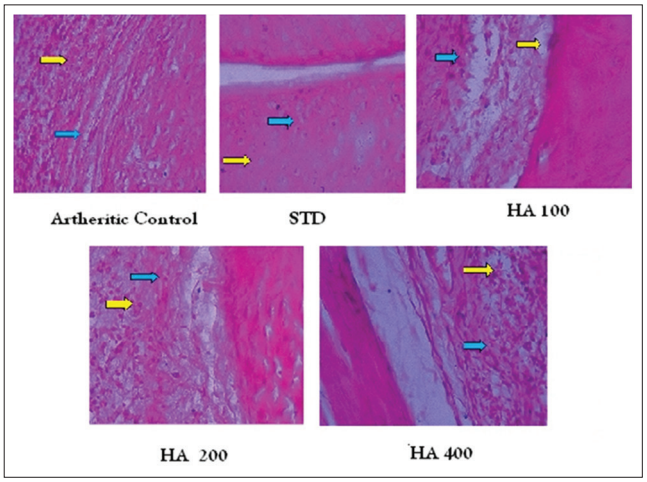


Fig. 2: Histopathological analysis of section of joint cavity photograph showing joint capsule with connective tissue proliferation (yellow arrow), and joint capsule with inflammatory changes (blue arrow)

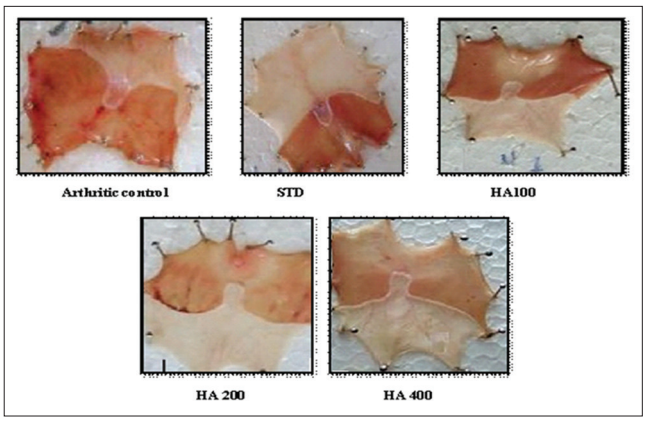


Fig. 3: Effect of *Helianthus annuus* on ulcerogenic index

restoration of tissue anti-oxidant enzymes, hematological parameters, lesser histopathological changes, and destruction of the knee joints induced by CFA.

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