Evaluation of Anti-arthritic activity of Vitex Negundo Linn

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ABSTRACT

The objective of the present research work was to evaluate the effect of antiarthritic activity of ethanolic extract of Vitex Negundo in laboratory animals. For arthritic study, the animals were divided randomly into 5 groups with 3 rats per group as following: Group I- vehicle control, Group II Arthritic control, Group III VNE (100mg/kg.p.o), Group IV VNE (200 mg/kg.p.o), Group V standard i.e. Indomethacin (10 mg/kg, p.o.). On day 1 and day 3, 0.1 ml of 2 % v/v formaldehyde in normal saline was injected into the subplantar region of the left hind paw. Dosing with standard drug (Indomethacin) and extracts were started on same day and continued for 10 days. The serum separated from the blood was collected for further biochemical estimations. Arthritis was assessed by following parameters as body weight, arthritic score, non injected, injected paw volume, biochemical estimations and haematological studies like Hemoglobin content, red blood cell, white blood cell counts and erythrocyte sedimentation rate. A significant increase in body weight, reduction in paw volume of both hind legs and reduction in total arthritic score were observed in formaldehyde induced arthritis in rats. There was a significant improvement in the levels of Hb and RBC in Vitex Negundo treated rats. The increased levels of WBC, ESR were significantly suppressed in the extract administered arthritic group. In the present study, we have evaluated Anti-Arthritic effects induced by formaldehyde, ethanolic extract of Vitex Negundo. Linn. This significantly suppressed the swelling of the paws of rats as compared to standard Indomethacin on dose dependent manner.

Key words: Antiarthritic activity, biochemical estimations, Vitex Negundo, and RA test
INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and progressive inflammatory disorder, characterized by synovitis and severe joint destruction. *Vitex Negundo* is used in the treatment of inflammation, bronchitis. *Vitex Negundo* L.(Verbenaceae) is a hardy plant, flourishing mainly in the Indian subcontinent. All parts of the plant, from root to fruit, possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant1-2. It is interesting to note that a single plant species finds for treatment of a wide spectrum of health disorders in traditional and folk medicine, some of which has been experimentally validated3-4. The plant is a component of a number of commercially available herbal formulations and has also shown potential as an effective bio-control agent5. Employment of techniques such as cell and tissue culture would provide means of rapid propagation and conservation of the plant species and, from the point of view of phytochemistry, give scope for enhancement of the quality and quantity of the bioactive secondary metabolites occurring in the plant6-7. It is frequently used in folk medicine to treat anti-convulsant, anti-inflammatory, antiarthritis8, antioxidant9, pesticidal and insecticidal activity, bronchial smooth muscle relaxant activity, hepatoprotective, antifertility, antimalarial, antibacterial, antifilarial, laxative activity10 and analgesic activity11-12.

MATERIALS AND METHODS

**Plant Material**
The plant *Vitex Negundo* was collected at the month of september at Tirupathi, Chittoor dist, A.P. The plant was authenticated by Dr.USHA RANI, Professor of Botany, B.T. College, Madanapalle, Andhra Pradesh, India.

**Drugs and reagents**
Indomethacin [Marketed Product], n-Hexane, Ethanol, Ethyl acetate, Chloroform, Glacial acetic acid, were used in this study.

**Preparation of Plant Extracts**
The plant *Vitex Negundo Linn* was shade air dried, coarsely powdered (1kg), and successive extraction [Hexane, Ethyl acetate, Chloroform & Ethanol] was done by hot continuous extraction for 72 hr. The extract was concentrated and kept in dessicator for further studies. The extract was studied for preliminary phytochemical analysis. The antiarthritic activity13 was studied with ethanolic extract of *Virtex Negundo Linn*, by using albino rats of either sex.

**Animals**
Adult wistar albino rats (180-230 gm) of either sex used for pharmacological activities. For both experiment the animals were kept in poly propylene cages (3 per cage) at 25±2°C with relative humidity 45-55% under 12 hr light and dark cycles. All the animals were acclimatized to laboratory condition for a week before use. They were fed with standard animal feed and water *ad libitum*.

**Anti arthritic activity:**

**Formaldehyde induced arthritis**
Antiarthritic activity was evaluated by using formaldehyde. The rats were divided into five groups, each group consists of three animals.

- **Group I** - Control, received Carboxy Methyl Cellulose (1%, w/v).
- **Group II** - Arthritic control
- **Group III** - Served as test, received VNE at a dose of 100 mg/kg.
- **Group IV** - Served as test, received VNE at a dose of 200 mg/kg.
- **Group V** - Standard, received Indomethacin 10mg/kg.

On the 0th day, the basal paw volume of left hind paw of each animal was measured by using Plethysmometer. On day 1 and 3, 0.1 ml of 2 % v/v formaldehyde in normal saline was injected into the subplantar region of the left hind paw.14 Dosing with standard drug (Indomethacin) and extracts were started on same day and continued for 10 days15.
Statistical Analysis
The results were presented as Mean±S.E.M. One way analysis of variance (ANOVA) followed by Dunnett’s t-test for multiple comparison statistical evaluation. P-values less than 0.05 were considered as significant[16].

RA TEST (Latex slide test)
Rheumatoid arthritis is an auto immune disorder, where auto antibodies are produced against self-antigen (IgG). These auto antibodies are termed as rheumatoid factor, which are immunoglobulins of predominantly IgM class, which combines with FC portion of immunoglobulin IgG molecules. RF is present in sera of 80% cases of rheumatoid arthritis. The idea of using inert particles coated with gamma globulin is to detect RF factor which was developed from the sheep cell agglutination test like rose-waaler test[17].

Rheumatoid arthritis test involved that antigen consists of polystyrene latex particle coated with specifically modified preparation of human gamma globulin (IgG) in order to avoid non-specific agglutination was observed in figure 2 & 3. The suspension of coated latex particles agglutinate visible when mixed with serum containing rheumatoid factor the sensitivity of the reagent has been adjusted to detect=10 IU/ml of RF calibrated against an International standard[18].

RESULTS AND DISCUSSION
Rheumatoid arthritis is a chronic inflammatory auto immune disease which is characterised by series of pathological process of joints. There are drugs that improves the signs and symptoms, alter the nature of history of diseases and improve the quality of life, but there is still no cure. The most commonly prescribed medication for Rheumatoid arthritis is steroidal, non steroidal anti-inflammatory, disease modifying antirheumatic and immunosupressant drugs. The goals of these drugs are to relieve pain and to decrease joint inflammation. In this study we used standard Indomethacin and ethanolic extract of *Vitex Negundo*. The ethanolic extract of *Vitex Negundo* showed (figure 1) significant anti-arthritic activity as compared to standard drug. The anti-arthritic effect of ethanolic extract of *Vitex Negundo* Linn (100&200mg/kg) was tested in formaldehyde induced rats for different time intervals from 0-10 days each value represents mean ±, n=3 and showed in table No.1. The statistical analysis was carried out one way ANOVA method where, p<0.05.

RADIOGRAPHIC ANALYSIS
On the 10th day animals were anesthetized with diethyl ether and placed in digital x-ray machine for the radiographic analysis of the knee joints. X-rays was taken at the knee joints for the confirmation and evaluation of the severity of arthritis in formaldehyde induced rats.

Rheumatoid arthritis test [Latex slide test]
Arthritis is a chronic inflammatory disorder and the inflammation involves the release of mediators like cytokines (IL-1β and TNF-α), GM-CSF, Interferons and PGDF. These are responsible for the pain, destruction of bone and cartilage that can lead to severe disability.

On examination of RA test, diseased conditions produce auto antibodies (Rheumatoid Factors) which combine with test reagents and shows specific agglutination. From the above figure it clearly indicates the formation of rheumatoid factors. The above figures 2&3 shows that radio graphic analysis of the tibio tarsal joint in arthritic and drug treated animals further supports and confirms the potent anti-arthritic effect of *Vitex Negundo* Linn in dose dependent manner.

CONCLUSION
In the present work the rats were selected to induce arthritis because rats develop chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease. The determination of rat paw swelling was apparently simple, sensitive and one of quick procedure for evaluating the degree of inflammation and therapeutic effects of drugs. In this study, Anti-Arthritic activity was evaluated by induced with formaldehyde and ethanolic extract of *Vitex Negundo* Linn., which significantly suppressed the swelling of the paws of rats as compared to standard Indomethacin on dose dependent manner.

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