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# *IN-VIVO* ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF *ASPARAGUS RACEMOSUS* ROOTS

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**ABSTRACT:** In this research work we elucidated the anti-inflammatory and anti-arthritic activity of hydroalcoholic extract of *Asparagus racemosus* roots (ARHE) *in-vivo*. Carrageenan induced paw edema methodology is used to induce inflammation and Diclofenac sodium is used as standard drug whereas Freund's Complete adjuvant used to induce arthritis and Dexamethasone is used as standard drug. *Asparagus racemosus* roots were showed significant anti-inflammatory and anti-arthritic activity at oral dose of (200mg/kg and 400mg/kg).

**INTRODUCTION:** Inflammatory diseases including different types of rheumatic diseases are a major cause of morbidity of the working force throughout world. This has been called the 'King of Human Miseries'.

Although rheumatism is one of the oldest known diseases of the mankind, it affects a large percentage of population of the world [1].

Inflammation is a local response of living mammalian tissues to the injury [2]. It is a complex response in the vascularized connective tissue occurs due to exogenous and endogenous stimuli [3].

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Inflammation, clinically, causes, as shown by Cornelius Celsus of Rome 2000 years ago, rubor (redness), calor (heat), dolor (pain) of the affected region and is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells [4]. NSAIDs are among the most commonly used drugs worldwide [5]. These drugs are anti-inflammatory and used to ease pain in various conditions including: arthritis, muscle, and ligament pains [6]. These drugs cause various side effects like, renal and hepatic abnormalities, metabolic disturbances and concomitant disease such as arterial hypertension or type 2 diabetes mellitus [7].

According to the WHO report, about 70–80% of the world's population rely on nonconventional medicine mainly from herbal sources in their primary health care. Especially, its demand is increasing day by day in developing countries where the cost of consulting a physician and price of medicine are beyond the limit of most people [8].

Nature has offered a complete store-house of remedies for all ailments of mankind by providing drugs from herbs, whole plants and algae most of which are of moderate toxicity relative to western medicines [9]. This is where, nature provides us drugs in the form of herbs, plants and algae have to cure the incurable diseases without any toxic effect [10]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [11].

*Asparagus racemosus* (Liliaceae) is one of the herbal plant whose roots are used as anti-inflammatory and anti-arthritic which is proved by this research work.

## MATERIALS AND METHODS:

**Chemicals:** Carrageenan and Freund's Complete Adjuvant were purchased from UGO Basil, Sigma Aldrich (USA) and the entire reagents used in the study were analytical grade.

**Preparation of extract:** Roots of plant collected and shade dried. The dried roots were coarsely powdered and the powder plant material was extracted with hydroalcohol (30% Ethanol and Water 70%). The extract (ARHE) was filtered and evaporated to dryness to yield the dry extract. The dry extract was kept in a vacuum desiccator until use.

**Selection of Animal:** Wistar albino rats of either sex of weighing 150-200g were procured from animal house of Jaipur College of Pharmacy, Jaipur, Rajasthan. They were fed with the standard food pellets and water (*ad libitum)*. They were housed in polypropylene cages maintained under standard conditions.

**Pharmacological studies:**

1. **Carrageenan induced paw edema:** Healthy albino rats of either sex, weighing 100-160 gm were selected and provided a standard rat food and water *ad libitum*. Animals were divided into five groups of six animals each (one normal, one control, one standard and two test groups). Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay [12]. The acute hind paw edema in rats was produced by 0.05 ml of carrageenan (prepared as 1% w/v solution 0.9% w/v in Nacl) locally injected into sub plantar region of the left hind paw of rats [13].
2. The extracts were administered orally into the rats 1 hour prior to Carrageenan injection. Diclofenac sodium (4 mg/kg) was given to standard group.

**Treatment Groups:**

Normal: 1% aqueous solution of Tween80, p.o.

Control: Carrageenan + 2% Tween80 (10ml/kg).

Standard: Carrageenan + Diclofenac sodium (4mg/kg)

Test group (Low dose): Carrageenan + ARHE (200mg/kg)

Test group (High dose): Carrageenan + ARHE (400mg/kg)

Mean paw volume was measured 1 h prior to carrageenan injection using plethysmometer and at 1, 2, 3, 4, 5, 6 and 24 hours after the carrageenan injection [14]. Reduction in the paw volume is compared with the vehicle treated controlled animals with that of the test groups and the anti-inflammatory activity was carried on the basis of the percentage (%) of inhibition of edema [15]. The percentage of inhibition of edema was calculated by using the formula;

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

Where, Vt = Paw volume in test group animals and Vc = Paw volume in control group.

3. **Freund's Complete Adjuvant (FCA) induced Arthritis in rats:** Freund's adjuvant induced Arthritis model was used to access the anti-arthritic activity in albino rats [16]. Animals were divided into five groups containing six animals in each group (one normal, one control, one standard and two test groups). Arthritis was induced by a single sub-planter injection of 0.1 ml of Complete Freund's adjuvant (CFA) (Sigma Chemicals) containing 1.0 mg dry heat-killed *Mycobacterium tuberculoi* per milliliter sterile paraffin oil into a foot pad of the left hind paw of male rats [17].

**Treatment groups:**

Normal: 1% aqueous solution of Tween80

Control: FCA + 2% Tween80 (10ml/kg).

Standard: FCA + Dexamethasone (5mg/kg)

Test group (Low dose): FCA + ARHE (200mg/kg)

Test group (High dose): FCA + ARHE (400mg/kg)

The dosing of all the groups was started from day $12^{th}$ once daily orally. Various parameters i.e. body weight, joint diameter, paw volume, arthritic score, motor incoordination, analgesic have been evaluated on day $0^{th}$, $4^{th}$, $7^{th}$, $10^{th}$, $12^{th}$, $14^{th}$, $17^{th}$, $19^{th}$, $21^{th}$, and $28^{th}$. On last day ($28^{th}$ day), blood was withdrawn by retro-orbital puncture for assessment of hematological parameters i.e. WBC, RBC, Hb, ESR.

**Behavioral assessment:**

- **Paw Volume:** Paw volumes of both hind limbs were recorded on the day of FCA injection, and again measured on day $0^{th}$ till day $28^{th}$ using mercury plethysmometer [18]. The change in paw volume was measured as the difference between the final and initial paw volumes [19].
- **Joint Diameter:** Paw thickness was measured by compressing the joint by rotating the screw of micrometer screw gauge till the pain elicited as indicated by squeaking or leg withdrawal. The distance moved by the screw gauge was recorded[20].
- **Arthritic Score:** The arthritic severity in each paw was graded from 0 to 4:

0= paws with no swelling and focal redness.

1= paws with swelling of finger joints.

2= paws with mild swelling of ankle or wrist joints.

3= paws with severe inflammation of the entire paw.

4= paws with deformity or ankylosis.

Each paw was graded and the four scores were totalled so that the possible maximum score per rat was 16 [21].

**Statistical analysis:**

**RESULTS:**

1. **Carrageenan induced inflammation:** Anti-inflammatory effect of ARHE of roots was evaluated after subplantar injection of carrageenan in animals. The standard Diclofenac (4 mg/kg) showed significant and dose-dependent decrease ($P < 0.05$, $P < 0.01$ and $P < 0.001$) in paw edema on $4^{th}$, $5^{th}$, $6^{th}$ and $24^{th}$ hours as compared to control group animals. Whereas treatment with ARHE (400 mg/kg) showed significantly ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively) decrease in paw edema as compared to control group animals on $3^{rd}$, $4^{th}$, $5^{th}$, $6^{th}$ and $24^{th}$ hours. ARHE (200 mg/kg) showed significantly decrease in paw edema as compared to control group animals ($P < 0.05$, $P < 0.01$) on $5^{th}$, $6^{th}$ and $24^{th}$ hours **(Table 1, Fig. 1)**

**TABLE: 1. EFFECT OF (ARHE) ON PAW EDEMA INDUCED BY CARRAGEENAN**

| S. No. | Time (Hour) | Control | Normal | Standard | ARHE (200mg/kg) | ARHE (400mg/kg) |
|---|---|---|---|---|---|---|
| **1.** | 1 | 0.200 ± 0.000 | 0.00 ± 0.000 | 0.200 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| **2.** | 2 | 0.333 ± 0.067 | 0.00 ± 0.000 | 0.333 ± 0.067 | 0.200 ± 0.000 | 0.133 ± 0.067 |
| **3.** | 3 | 0.400 ± 0.000 | 0.00 ± 0.000 | 0.333 ± 0.067 | 0.267 ± 0.067 | 0.200 ± 0.000* |
| **4.** | 4 | 0.400± 0.000 | 0.00 ± 0.000 | 0.267± 0.067* | 0.333 ± 0.067 | 0.200 ± 0.067* |
| **5.** | 5 | 0.400 ± 0.000 | 0.00 ± 0.000 | 0.067± 0.067** | 0.400 ± 0.000* | 0.133± 0.067** |
| **6.** | 6 | 0.333± 0.000 | 0.00 ± 0.000 | 0.000 ± 0.000*** | 0.200 ± 0.067** | 0.067± 0.067*** |
| **7.** | 24 | 0.333 ± 0.000 | 0.00 ± 0.000 | 0.000 ± 0.000*** | 0.067 ± 0.067** | 0.000 ± 0.000*** |

Values were expressed Mean ± SEM. *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group animals.

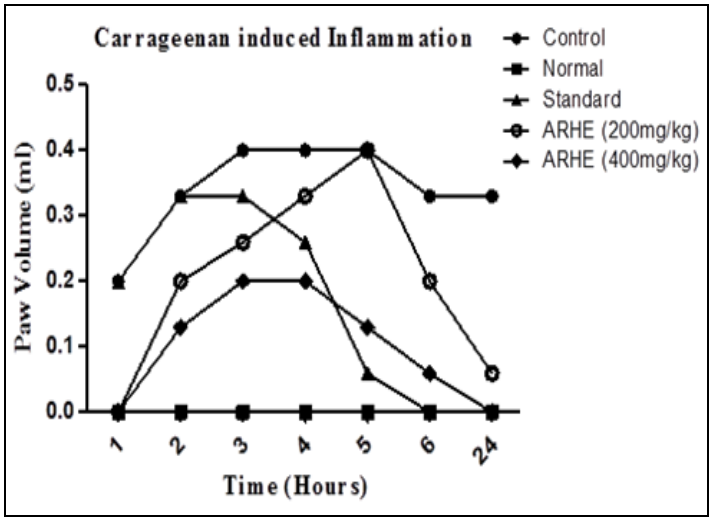


**FIG: 1: EFFECT OF ARHE ON CARRAGEENAN INDUCED INFLAMMATION**

## 1. FCA induced arthritis:

### Behavioral assessment

**1.1 Effect of ARHE on body weight:** Standard group animals showed significant increase in body weight ($P < 0.05$, $P < 0.01$ and $P < 0.001$) from 14th day to 28th day as compared to the control group animals. Treatment with ARHE (400 mg/kg) showed significant increase in body weight ($P < 0.05$, $P < 0.01$ and $P < 0.001$) as compared to control group animals from 14th day to 28th day. Treatment with ARHE (200 mg/kg) showed significant increase in body weight ($P < 0.05$ and $P < 0.01$) as compared to control group animals from 17th day to 28th day. **(Table.2, Fig. 2**.)

**TABLE: 2 EFFECT OF (ARHE) ON AVERAGE BODY WEIGHT**

| S. No. | Days | Control | Normal | Standard | ARHE (200 mg/kg) | ARHE (400 mg/kg) |
|---|---|---|---|---|---|---|
| **1.** | 0 | 130.83 ± 4.728 | 80.00 ± 5.000 | 150 ± 0.000 | 183.33 ± 8.333 | 175 ± 0.000 |
| **2.** | 4 | 125.00 ± 5.000 | 85.00 ± 7.637 | 126.66 ± 1.667 | 171.66 ± 9.280 | 165 ± 2.887 |
| **3.** | 7 | 118.33 ± 4.013 | 85.00 ± 7.637 | 123.33 ± 1.667 | 166.66 ± 9.280 | 158.33 ± 1.667 |
| **4.** | 10 | 98.33 ± 3.073 | 101.66 ± 13.017 | 111.66 ± 7.265 | 158.33 ± 6.009 | 151.66 ± 1.667 |
| **5.** | 12 | 103.33 ± 6.666 | 101.66 ± 13.017 | 120.00 ± 5.000 | 153.33 ± 3.333 | 150.00 ± 0.000 |
| **6.** | 14 | 119.16 ± 13.065 | 108.33 ± 8.333 | 166.6 ± 8.333* | 155.66± 4.410 | 157.33± 4.333* |
| **7.** | 17 | 119.16 ± 10.36 | 108.33 ± 8.333 | 166.6 ± 8.333* | 156.66± 4.410* | 157.33± 4.333* |
| **8.** | 19 | 135.00 ± 11.180 | 108.33 ± 8.333 | 191.66 ± 8.333** | 183.33 ± 4.410* | 188.33 ± 1.667** |
| **9.** | 21 | 129.16 ± 10.034 | 108.33 ± 8.333 | 206.66 ± 15.899*** | 185.00 ± 2.887** | 193.33 ± 4.410** |
| **10.** | 28 | 97.50 ± 4.787 | 116.66 ± 8.333 | 218.33 ± 9.280*** | 138.83 ± 9.523** | 210.00 ± 7.638*** |

Values were expressed Mean ± SEM. *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group

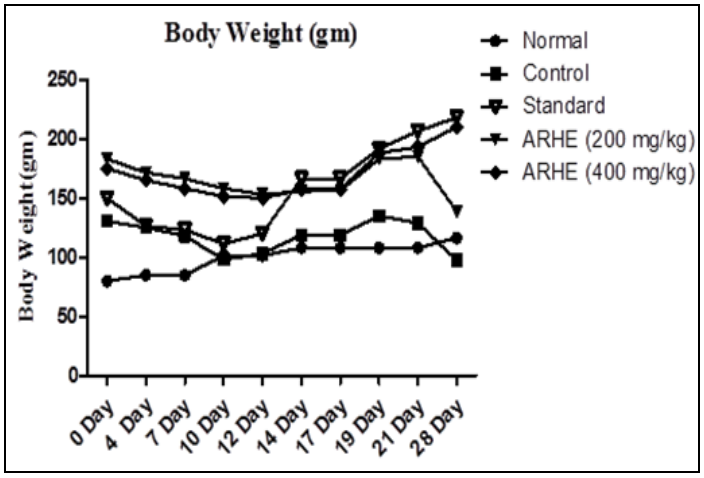


**FIG. 2: EFFECT OF ARHE ON AVERAGE BODY WEIGHT**

**1.2 Effect of ARHE on arthritic score:** All the groups of animals administered with FCA started showing signs of clinical inflammation in one or more hind paws, which was a biphasic response. The arthritic score was significantly increased from day 7th to 12th in control group animals which remained significantly increased till the end of the study i.e. up to 28th day. Animals treated with Standard drug showed significant and dose dependant decreased in arthritic score ($P < 0.01$ and $P < 0.001$) from day 14th onward till the end of the study as compared to control group animals.

Treatment with ARHE (400 mg/kg) showed significant decreased in arthritic score ($P < 0.05$, $P < 0.01$ $P < 0.001$) as compare to control group animals from 14th day to 28th day. Treatment with ARHE (200 mg/kg) showed significant decreased in arthritic score ($P < 0.05$ and $P < 0.01$) as compare to control group animals from 19th day to 28th day **(Table 3, Fig. 3.)**

**TABLE: 3. EFFECT OF (ARHE) ON AVERAGE ARTHRITIC SCORE**

| S. No. | Days | Control | Normal | Standard | ARHE (200 mg/kg) | ARHE (400 mg/kg) |
|---|---|---|---|---|---|---|
| **1.** | 0 | 0.00 ± 0.000 | 0.0 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 |
| **2.** | 4 | 1.33 ± 0.333 | 0.0 ± 0.000 | 1.66 ± 0.333 | 1.33 ± 0.333 | 1.66 ± 0.333 |
| **3.** | 7 | 1.66 ± 0.333 | 0.0 ± 0.000 | 1.66 ± 0.333 | 1.66 ± 0.333 | 2.33 ± 0.333 |
| **4.** | 10 | 2.33 ± 0.333 | 0.0 ± 0.000 | 2.33 ± 0.333 | 2.33 ± 0.333 | 2.66 ± 0.333 |
| **5.** | 12 | 2.66 ± 0.333 | 0.0 ± 0.000 | 2.00 ± 0.000 | 2.66 ± 0.333 | 2.33 ± 0.333 |
| **6.** | 14 | 2.33 ± 0.333 | 0.0 ± 0.000 | 1.00 ± 0.000* | 1.66 ± 0.333 | 1.00 ± 0.000* |
| **7.** | 17 | 2.33 ± 0.333 | 0.0 ± 0.000 | 0.66 ± 0.333** | 1.66 ± 0.333 | 1.00 ± 0.000* |
| **8.** | 19 | 1.33 ± 0.333 | 0.0 ± 0.000 | 0.66 ± 0.333** | 1.00 ± 0.000* | 0.66 ± 0.333** |
| **9.** | 21 | 1.00 ± 0.000 | 0.0 ± 0.000 | 0.00 ± 0.000*** | 1.00 ± 0.000** | 1.00 ± 0.000** |
| **10.** | 28 | 0.33 ± 0.33 | 0.0 ± 0.000 | 0.00 ± 0.000*** | 1.00 ± 0.000** | 0.00 ± 0.000*** |

Values were expressed Mean ± SEM. *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

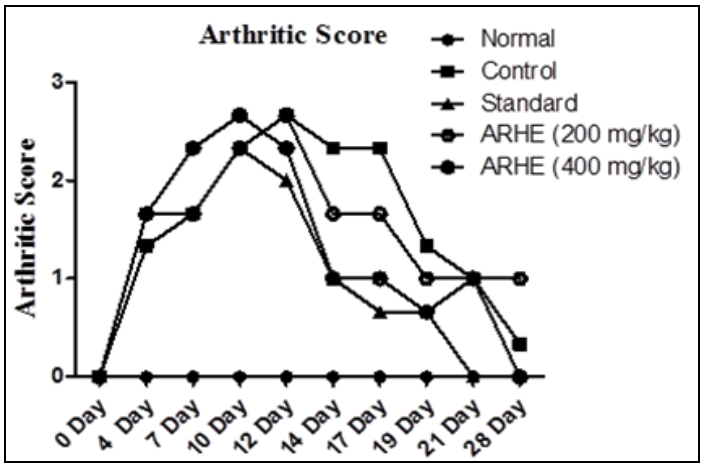


**FIG. 3: EFFECT OF ARHE ON ARTHRITIC SCORE**

**1.3 Effect of AREE on paw volume:** Standard group animals showed significant decrease in paw volume (P < 0.05, P < 0.01 and P < 0.001) 14$^{th}$ day to 28$^{th}$ day as compared to the control group animals. Treatment with ARHE (400 mg/kg) showed significant decreased in paw volume (P < 0.05, P < 0.01 and P < 0.001) as compare to control group animals from 17$^{th}$ day to 28$^{th}$ day. Treatment with ARHE (200 mg/kg) showed significant decreased in paw volume (P < 0.05, P < 0.01, P < 0.001) as compare to control group animals from 19$^{th}$ day to 28$^{th}$ day **(Table 4, Fig. 4).**

**TABLE 4: EFFECT OF (ARHE) ON CHANGE OF PAW VOLUME**

| S. No. | Days | Control | Normal | Standard | ARHE (200 mg/kg) | ARHE (400 mg/kg) |
|---|---|---|---|---|---|---|
| **1.** | 0 | 0.36 ± 0.333 | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 |
| **2.** | 4 | 0.36 ± 0.333 | 0.00 ± 0.000 | 0.20 ± 0.000 | 0.26 ± 0.066 | 0.20 ± 0.000 |
| **3.** | 7 | 0.40 ± 0.05 | 0.00 ± 0.000 | 0.40 ± 0.000 | 0.40 ± 0.000 | 0.40 ± 0.000 |
| **4.** | 10 | 0.46 ± 0.04 | 0.00 ± 0.000 | 0.46 ± 0.066 | 0.53 ± 0.066 | 0.46 ± 0.066 |
| **5.** | 12 | 0.50 ± 0.0 | 0.00 ± 0.000 | 0.40 ± 0.115 | 0.60 ± 0.000 | 0.53 ± 0.066 |
| **6.** | 14 | 0.50 ± 0.04 | 0.00 ± 0.000 | 0.33 ± 0.066* | 0.53 ± 0.066 | 0.40 ± 0.115 |
| **7.** | 17 | 0.50 ± 0.04 | 0.00 ± 0.000 | 0.26 ± 0.066* | 0.26 ± 0.066 | 0.26 ± 0.066* |
| **8.** | 19 | 0.46 ± 0.04 | 0.00 ± 0.000 | 0.26 ± 0.066** | 0.26 ± 0.066* | 0.20 ± 0.000** |
| **9.** | 21 | 0.46 ± 0.06 | 0.00 ± 0.000 | 0.06 ± 0.066*** | 0.20 ± 0.066** | 0.20 ± 0.000** |
| **10.** | 28 | 0.36 ± 0.333 | 0.00 ± 0.000 | 0.06 ± 0.066*** | 0.13 ± 0.066*** | 0.06 ± 0.066*** |

Values were expressed Mean ± SEM
*P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group

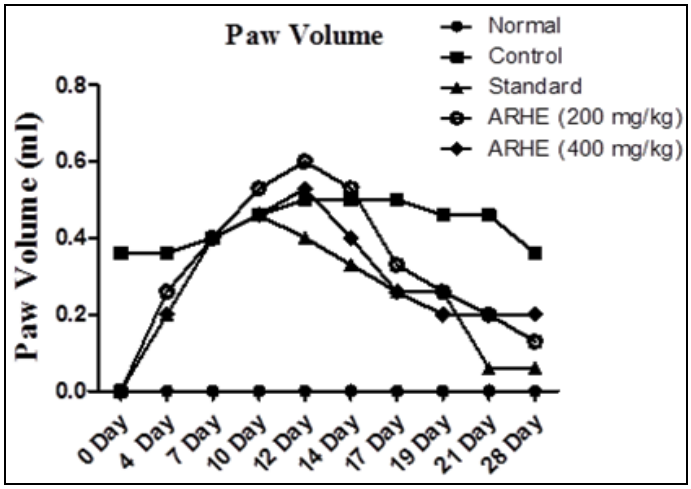


**FIG. 4: EFFECT OF ARHE ON PAW VOLUME**

**1.4 Effect of ARHE on joint diameter:** Standard group animals showed significant decreased in joint diameter ($P < 0.05$, $P < 0.01$ and $P < 0.001$) 14th day to 28th day as compared to the control group animals. There was significant decreased in joint diameter ($P < 0.01$ and $P < 0.001$) of ARHE (400 mg/kg) treated animals from 14th day to 28th day as compared to the control group animals. Treatment with ARHE (200 mg/kg) showed significant decreased in joint diameter ($P < 0.05$, $P < 0.01$) as compare to control group animals from 19th day to 28th day. **(Table. 5**, **Fig. 5)**

**TABLE: 5. EFFECT OF (ARHE) ON JOINT DIAMETER**

| S.No. | Days | Control | Normal | Standard | ARHE (200 mg/kg) | ARHE (400 mg/kg) |
|---|---|---|---|---|---|---|
| **1.** | 0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.00 ± 0.000 | 0.00 ± 0.000 |
| **2.** | 4 | 2.415 ± 0.390 | 0.090 ± 0.078 | 1.19 ± 0.151 | 1.86 ± 0.651 | 0.590 ± 0.250 |
| **3.** | 7 | 2.33 ± 0.363 | 0.056 ± 0.073 | 2.40 ± 0.232 | 2.80 ± 0.654 | 1.86 ± 0.809 |
| **4.** | 10 | 4.375 ± 0.650 | 0.033 ± 0.056 | 3.28 ± 0.120 | 3.06 ± 0.516 | 2.66 ± 0.541 |
| **5.** | 12 | 3.122 ± 0.502 | 0.030 ± 0.020 | 2.57 ± 0.257 | 3.22 ± 0.478 | 1.88 ± 0.428 |
| **6.** | 14 | 2.837 ± 0.324 | 0.023 ± 0.010 | 1.09 ± 0.545* | 1.74 ± 0.510 | 1.16 ± 0.219* |
| **7.** | 17 | 2.353 ± 0.106 | 0.020 ± 0.025 | 1.460 ± 0.180* | 1.72 ± 0.382 | 1.16 ± 0.471* |
| **8.** | 19 | 2.257 ± 0.389 | 0.013 ± 0.023 | 0.25 ± 0.070** | 0.74 ± 0.255* | 1.42 ± 0.251** |
| **9.** | 21 | 2.522 ± 0.106 | 0.013 ± 0.013 | 0.28 ± 0.250*** | 1.64 ± 0.301** | 0.16 ± 0.092*** |
| **10.** | 28 | 1.908 ± 0.364 | -0.010 ± 0.015 | 0.28 ± 0.250*** | 1.64 ± 0.301** | 0.16 ± 0.092*** |

Values were expressed Mean ± SEM. *P < 0.05; **P < 0.01 and ***P < 0.001 significant as compared to control group.

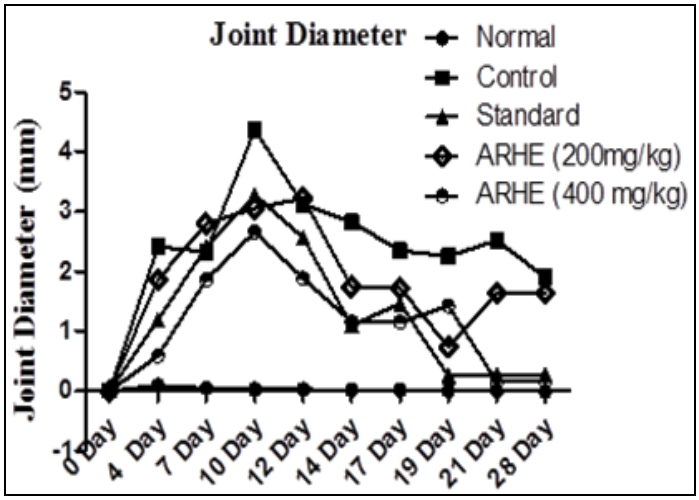


**FIG. 5: EFFECT OF ARHE ON JOINT DIAMETER**

## DISCUSSION:

1. **Carrageenan induced inflammation:** The primary phase of edema has been attributed to the release of histamine and serotonin; the edema maintaining during the plateau phase, attribute to kinin like substances and the secondary accelerating phase of swelling is attributed to the release of prostaglandin [22].

Mediators like leukotriene, prostaglandins, PAF and cytokines are reported to be responsible for the immediate hypersensitivity reaction, but it was observed that enhanced vascular permeability and leukocyte infiltration at the sites of allergen challenge [23].

**FCA induced arthritis:** The development of adjuvant-induced arthritis in the rat can be divided into three phases, just like human rheumatoid arthritis, starting with the induction phase without evidence of synovitis, followed by early synovitis, and finally late synovitis with progressive joint destruction [24].

Inhibition of COX-2 activity also modulated local and systemic cytokine production in arthritic rats. The development of arthritis was associated with increased levels of TNF-α and IL-6 mRNAs in affected paws and systemic IL-6 production. Both cytokines have been shown to be produced spontaneously by rheumatoid arthritis synovial cells [25].

**CONCLUSION:** Treatment with hydroalcoholic extract of *Asparagus racemosus* (200 and 400 mg/kg, p.o.) showed maximum reduction in paw volume as compared to vehicle treated animals in carrageenan induced rat paw edema. A significant increase in body weight, reduction in paw volume of both hind legs and reduction in total arthritic score were observed in FCA induced arthritis in rats. All these results thus predict that the drug provide pharmacological rationale for the traditional use of the drug against inflammatory disorders such as rheumatoid arthritis.

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