ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF THE AQUEOUS LEAF EXTRACT OF ARTEMESIA VULGARIS IN EXPERIMENTAL ANIMAL MODELS

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INTRODUCTION

Inflammation and pain is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow (Ialenti et al., 1995).

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects.

Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorpho nuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase
A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are major components that induce pain and inflammation (Higgs et al., 1984; Vane, 1971).

The present study was used to evaluate acute toxicity, anti-inflammatory, analgesic activity of Artemisia Vulgaris in swiss wistar rats.

**MATERIALS AND METHOD**

**Collection of Plant Material:**

The leaves were collected from the talakona forest, Andhra pradesh, India during month of January 2016 and its identity as Artemisia Vulgaris was confirmed by Assistant professor in the department of botany, Sri Venkateswara University, Tirupati.

**Preparation of extracts and Phytochemical screening**

Extraction was done by using soxhlet apparatus with 70% ethanol (hydro alcoholic) as solvent. The extracts were concentrated under reduced pressure dried and stored at 4°C temp in air tight containers for further studies.

**Phytochemical screening:**

The ethanolic extract of A. Vulgaris was analyzed for preliminary phytochemical screening by using spray reagents: Liebermann–Burchard spray reagent (Briekskorn and Capuano, 1953) was used to detect the presence of steroids and pentacyclic triterpenes in the form of violet spots. Neutral ferric chloride was used to detect phenolic compounds that appear in the form of blue spots. Shinoda test (Shindo, 1928) and Fiegel’s test (Feigl, 1954) were used to detect flavonoids and glycoside, respectively. The extract was found to be devoid of alkaloids as detected by the DragonDroff’s reagent (Bollinger et al., 1965). Further isolation and characterization of pure compounds from the extract is in progress.

**PHARMACOLOGICAL SCREENING**

**Animals used**

Wistar rats of either sex, weighing 150 - 200 gm, were procured from the animal house of Venkateswara Enterprises, Banglore, Karnataka, India. Animals were kept in polypropylene cages and fed on standard laboratory diet (Lipton India Ltd) with water ad libitum, maintained at an ambient temperature of 26 ±2°C. The ethical clearance was obtained by the Institutional Animal Ethics committee.

**Chemicals:**

Diclofenac sodium was obtained from Sigma-Aldrich, Bangalore. Formalin, Acetic acid were obtained from SD fine chemicals Ltd Mumbai. and all other reagents used were of analytical grade.

**Instruments:**

Plethysmometer, and electronic balance (Shimadzu, Model no: DS-852 J).

**Acute toxicity study**

Wistar rats of either sex, weighing 150 - 200 g and of 90 days age were used to evaluate acute toxicity of the aqueous extract. Animals were then administered by oral route with aqueous extract (50 - 4000 mg / kg), suspended in 2% w/ v gum acacia solution (vehicle).

Control group received only vehicle. The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The aqueous extract was found to be nontoxic up to dose of 4000 mg/kg body weight and hence 1/10 of this dose was taken as the screening dose.

**Treatment schedule**

As extract (100, 200, 400 mg/kg body weight) and standard anti-inflammatory and analgesic drug diclofenac sodium (10 mg/kg body weight) (sigma-aldrich chemicals, bangalore) was prepared in 1% sodium carboxymethylcellulose (cmc) vehicle as suspension and administered orally. Animals were randomized into three groups, each carrying 6 rats.

1. Experimental group, treated with different doses of extract.
2. Experimental group treated with diclofenac sodium.
3. Control group of animals that were treated with vehicle similar to experimental group.

**Anti-inflammatory study:**

In this experiment, formalin-induced rat hind paw oedema was used as the animal model of acute inflammation according to Winter et al., 1962 and described previously (Saha et al. 2007). Briefly, acute inflammation was produced by sub-plantar injection of 0.1ml of 1% suspension of Formalin with 2% gum acacia in normal saline, in the right hind paw of the rats 1h after the oral administration of test materials. The paw volume was measured by plethysmometer (Ugo Basile, Italy) at 1, 2, 3, and 4 h after the Formalin injection. The extract was administered at 100,200 and 400 mg/kg body weight. Diclofenac 10 mg/kg body weight was used as standard anti-inflammatory agent.
### Table 1: Phytochemical screening.

| S.no | Phytochemicals | Ethanolic extract of *Artemesia Vulgaris* |
|------|----------------|-------------------------------------------|
| 1    | Alkaloids      | - ve                                      |
| 2    | Catechols      | - ve                                      |
| 3    | Flavanoids     | + ve                                      |
| 4    | Phenolic compounds | + ve                                           |
| 5    | Saponins       | - ve                                      |
| 6    | Steroids       | + ve                                      |
| 7    | Tannins        | - ve                                      |
| 8    | Triterpinoids  | + ve                                      |
| 9    | Glycosides     | + ve                                      |

### Table 2: Effect of *Artemesia Vulgaris* and diclofenac sodium on percent inhibition of Formalin induced paw edema.

| S.N O | Groups | Increase in paw diameter(cm) Mean±SEM | %Inhibition |
|-------|--------|--------------------------------------|-------------|
|       |        | 0hr | 1hr | 2hr | 3hr | 4hr |                |             |
| 1     | Control | 2.00±0.00 | 2.00±0.00 | 2.00±0.00 | 2.00±0.00 | 2.00±0.00 |                |             |
| 2     | Diclofenac sodium (10mg/kg) | 2.15±0.02 | 4.05±0.064 | 4.45±0.028 | 4.225±0.047 | 3.70±0.040 |                |             |
| 3     | A. Vulgaris (100mg/kg) | 2.2±0.040 | 2.675±0.047** | 2.4±0.040*** | 2.25±0.028*** | 2.12±0.025** | **p<0.0001** | 72.89%     |
| 4     | A. Vulgaris (200mg/kg) | 2.275±0.025 | 3.2±0.070*** | 3.225±0.075** | 3.17±0.062*** | 3.0±0.040*** | **p<0.0001** | 45.78%     |
| 5     | A. Vulgaris (400mg/kg) | 2.25±0.02 | 2.9±0.040*** | 2.675±0.085** | 2.375±0.047** | 2.2±0.040*** | **p<0.0001** | 69.87%     |

All values are shown as mean ±SEM and n=6. **p<0.0001 when compared with control (II) group.

### Table 2: Effect of *Artemesia Vulgaris* and diclofenac sodium on percent inhibition of Formalin induced writhings.

| S.NO | Groups | Writhings mean±S.E | %Inhibition |
|------|--------|---------------------|-------------|
| 1.   | Control | 41.50±0.6455 | 41.50±0.6455 | 72.89%     |
| 2.   | Diclofenac sodium (10mg/kg) | 12.50±0.6455*** | 72.89%     |
| 3.   | A. Vulgaris (100mg/kg) | 26.25±0.6292*** | 34.33%     |
| 4.   | A. Vulgaris (200mg/kg) | 22.25±0.4787*** | 45.78%     |
| 5.   | A. Vulgaris (400mg/kg) | 13.50±0.6455*** | 69.87%     |

All values are shown as mean ±SEM and n=6. **p<0.0001 when compared with control (II) group.
Acetic acid induced writhing test:
The peripheral analgesic activity of leaf extract of Artemesia Vulgaris was measured by the acetic acid induced writhing test as described earlier (Saha et al., 2007). Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Diclofenac at oral dose of 10 mg/kg was used as standard analgesic agent. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 min, 10 min after the application of acetic acid.

STATISTICAL ANALYSIS:
Data were analyzed by one-way ANOVA followed by Dunnet’s test and P values <0.0001 were considered statistically significant.

RESULTS & DISCUSSION
Acute toxicity:
Acute toxicity studies show that drug is safe up to the dose of 4000 mg/kg body weight. No mortality was observed at 14th day of the acute toxicity study.

Phytochemical Screening:
All extracts obtaining during successive extraction of Artemesia Vulgaris Leaves was examined for the presence of various phytoconstituents by performing qualitative phytochemical tests and the results are recorded in table 1.

Anti-inflammatory study: The results were tabulated in table 2

Acetic acid induced writhing test: The results were tabulated in table 3

CONCLUSION
This study has provided some justification for the folkloric use of the plant in several communities for conditions such as stomachache, pain and inflammations.

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