IJPSR (2021), Volume 12, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 August 2018; received in revised form, 28 December 2018; accepted, 17 January 2019; published 01 February 2019

ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF WHOLE PLANT OF *MERREMIA TRIDENTATA* ON MURINE MODELS

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Keywords:

Inflammation, Anti-inflammatory drugs, *Merremia tridentata*, Paw edema method

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ABSTRACT: The present study aims to reveal the anti-inflammatory activity of the ethanolic plant extracts of *Merremia tridentata* against murine models. The Wistar Albino rats of mixed sex were induced inflammation *via* injecting histamine and formalin. The anti-inflammatory effect of *Merremia tridetata* was compared with the presence of positive control, Indomethacin. The Paw volume of the control group (group I and group II) have revealed the following mean and standard deviation values of 7.91±0.22 ml and 5.66±0.27 ml, respectively. Whereas, the minimum and maximum dosages of plant extracts have shown the values 6.1±0.29 (200 mg/kg) and 5.49±0.18 ml. similarly, the histamine and Indomethacin control group I have revealed the following values 7.96±0.2 ml (group I) and 6.02±0.53 ml (group II). Likewise, the minimum and maximum dosage of plant extracts has revealed the maximum anti-inflammatory effect with the following values 6.30±0.60 ml (200 mg/kg) and 6.13±0.47 ml (400mg/kg). Hence, the present study have exerted the potential biological effect of *M. tridetata* might be useful for the development of an alternative medicine for inflammation.

INTRODUCTION: Inflammation and pain are the most common strategies of nonspecific mani-festations of many diseases and it is natural defense mechanism focus to remove the injurious stimuli and initiate the healing process wound. There are many endogenous mediators are responsible for inflammation such as, histamine, serotonin, bradykinin and prostaglandins. These are the most abundant substances/secretions in inflammatory cells and among them prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation [6].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(5).1000-06

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(5).1000-06

The prolonged state of inflammation can cause numerous diseases /disorders such as rheumatoid arthritis (RA), psoriasis and inflammatory bowel disease [8]. The balancing and functioning of the immune system is well maintained through the finely pro-inflammatory & anti-inflammatory process of inflammation. The major mediators / agents of the inflammation are cytokines, eicosanoids and free radicals are having direct or indirect effect on the path physiology of inflammatory diseases [9].

Acute inflammation due to with or without clinical background develops in to chronic inflammation. In this connection macrophages & lymphocytes are being activated and release certain inflammatory mediators. Neutrophils are the first blood leukocytes which arrive to the inflammatory site. Tran endothelial migration from the blood to the tissue is a tedious process which requires the

involvement of certain chemotactic factors and binding to adhesion molecules at the surface of the endothelium [10]. This process involves the transfer of blood neutrophils to the tissues where it involved in different functions. The blood neutrophils are having different biological role in immune system *viz;* phagocytosis, production of reactive oxygen & nitrogen species that damage DNA & cell membranes & production of chemoattractants such as IL-8 which recruit further competent cells [7]. The responses from neutrophils are usually terminated when they undergo apoptosis and are phagocytosed by macrophages. Prostaglandins are chemical substances secreted by inflammatory cells which inturns increases the production/expression of a key enzyme cycloxygenase thus Inflammation, which spreads to the normal cells which inturn activates oxidant generating enzymes like NADPH oxidase, xanthine oxidase, myeloperoxidase, *etc*. The active oxidant also synthesizes superoxide anion and other reactive nitrogen species like nitric oxide through activation of inducible nitric oxide synthase (iNOS) [4].

Free radicals (FR's) play a major role in process of inflammation. During the process of inflammation, inflammatory cells secrete chemically reactive oxidants, radicals and electrophilic compounds that bring about the elimination of the infectious agents. Such inflammatory mediators are highly toxic and effect the normal function of the tissue. Many researchers are focusing on inflammation, due to the non-availability of effective, side effect free and safe medication from natural origin. Presently available chemical/synthetic based commercial drugs may cure the inflammation associated with severe side effects.

Hence, more studies are required for the development of novel, natural, side effect alternative medicine to treat inflammation from terrestrial plants. Plants are having a potent phytochemicals which are having various biological properties are the prime research focus. Presently available many commercial drugs used for inflammation are the products of terrestrial plant origin. Hence, the bio molecules of plant origin should be focused to assess their biological efficacy as they are having low cost process and high availability. *Merremia tridentata* (L.) Hallier belonging to the family Convolvulaceae, grows naturally as a perennial, spreading herb with thick root stock, and is distributed throughout India on hedges and open waste lands. Traditionally, the plant is used in piles, swellings, rheumatic affections, stiffness of the joints, hemiplegia, urinary infections and general debility apart from being a good laxative and astringent [1]. The previous studies conducted on *M. tridentata* have strong wound healing, anti-inflammatory and anti-arthritic activities. *M. tridentate* is also used as a supplementary feed to the grass Panicum maximum for young West African Dwarf Sheep. The aerial plant parts of the *M. tridentata* contains variety of phytochemical *viz*; flavonoids, diosmetin, luteolin and their 7-O-β-D-glucosides. The acetone extract of *M. tridentata* root has reveals the presence of high phenolic which enchances the antioxidant activity [5]. Hence, the anti inflammation property of *M. tridentata* is highly warrented. Thus, the present study focusses the anti-inflammatory potential of Ethanolic extracts of the *Merremia tridentata* using paw edema method induced by histamine and formalin on Wistar albino rats comparable with a standard drug Indomethacin.

MATERIALS AND METHODS:

Procurement of Medicinal Plants: Fresh leaf samples of *Merremia tridentata* were collected from Coimbatore district, Tamil Nadu, India, the voucher specimen sample was authenticated in Tamilnadu Agricultural University (TNAU Campus), Coimbatore, Tamilnadu.

Preparation of Ethanolic Leaf Extracts: The fresh plant leaves were washed thrice with running tap water and then with distilled water to remove associated biota and contaminants followed and air dried in shade condition. The dried leaf samples were grand using kitchen eclectic blender. Coarsely powdered plant sample was drenched with 100% ethanol and subjected to cold percolation method. Then the plant extract was concentrated through air drying method and stored at 4 °C until its use. The extract was concentrated under vacuum to the solvent free residues. The percentage yield of extract was 41.64% (w/w).

Experimental Animals: Wistar rats weighing 200-250g were purchased from SreeVenkateshwara Enterprises, Bangalore. The animals were housed in groups of four in standard cages at room

temperature (25±3 °C) in 12 h dark/12 h light control, with both food and water and *libitum*. Twelve hours before the experiments they were transferred to the laboratory and were maintained only with water *ad libitum*. Wistar albino rats weighed around 200–250 were used for this study. The work was carried out in according to the ethical guidelines of the 466/01a /CPCSEA

Histamine Induced Inflammation:

- **Group-I:** Served as negative control (only histamine 50 µg/paw S.C).
- **Group-II:** Served as standard which received Indomethacin (10mg/kg, p.o.).
- **Group-III:** Received extract with dose of 200mg/kg.
- **Group-IV:** Received extract with dose of 400mg/kg.

Group I served as negative control and received histamine 50 ug/paw subcutaneously. Group II received indomethacin (10 mg/kg,po) and Group III and IV animals received the extract at the dose of (200 & 400 mg/kg po) respectively. After 30 min, the rats were challenged with subcutaneous injection of histamine 50 µg/paw S.C into the sub plantar region of right paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The paw volume was measured at 0, 1, 2, 3, 4 and 5 h after histamine injection using Digital Plethysmometer. The difference between initial and subsequent reading gave the actual edema volume.

Formalin Induced Inflammation:

- **Group-I:** Served as negative control (0.1 ml of solution of 2.5% formalin/ paw S.C)
- **Group-II:** Served as standard which received Indomethacin (10mg/kg, p.o.)
- **Group-III:** Received extract with dose of 200mg/kg
- **Group-IV:** Received extract with dose of 400mg/kg.

Group I received 0.1 ml of solution of 2.5% formalin/ paw subcutaneously. Group II received indomethacin (10mg/kg,p.o) and groupIII and IV animals received the extract at the dose of (200 & 400 mg/kg p.o) respectively. After 30 min, the rats were challenged with subcutaneous injection of formalin 50 ug/paw S.C into the sub plantar region of left paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The paw volume was measured at 0, 1, 2, 3, 4 and 5 h after formalin injection using Digital Plethysmometer. The difference between initial and subsequent reading gave the actual edema volume.

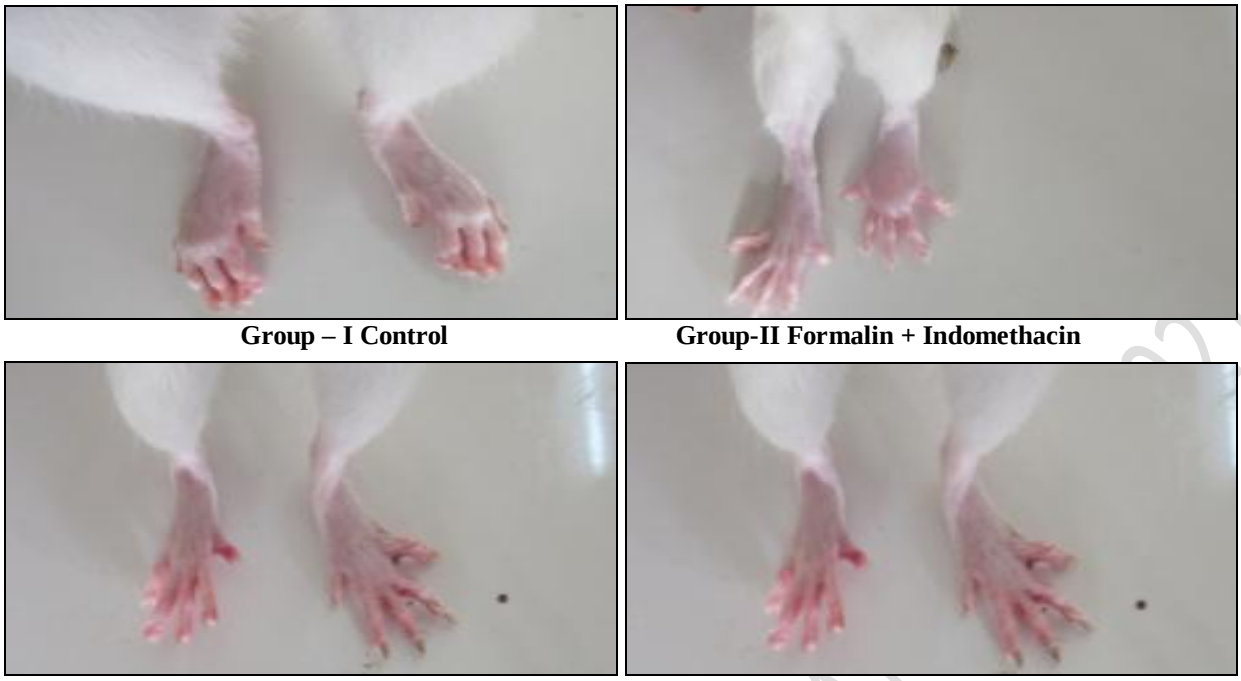
- **RESULTS:**

Effect of *Merremia tridentata* on Formalin Induced paws Edema in Rats: The paw volume of the Negative control group was found to be increased to 8.28±0.29 ml at the 5 h and 7.91±0.22 ml at the 6 h. The standard drug showed reduction in paw volume 7.9±0.20 ml at 3 h, 6.03±0.15 ml at 5 h, 5.66 ±0.27 ml at 6 h. Two doses (200 mg/kg and 400 mg/kg) of Ethanolic plant extracts were given to the animal groups (Group-III and Group-IV). The paw volume after the treatment of 200 mg/kg of ethanolic plant extract (Group-III) at the 0min was found to be 3.69±0.25 ml, 8.10±0.12 ml at the 3 h and 6.1±0.29 ml at 6h. Similarly, the paw volume of High doses (400 mg/kg) of the ethanolic plant extracts (Group-IV) was observed to be 4.28±0.09 ml at 0 h, 8.16±0.10 ml at 3 h and 5.49±0.18 ml at 6 h. The high doses of plant extract showed the significant reduction in paw volume than the standard drug at 6 h **Fig. 1**. **Table 1** represents the mean paw volume at different durations of formalin induced paw edema in rats.

TABLE 1: ANTI-INFLAMMATORY EFFICACY OF *MERREMIA TRIDENTATA* AGAINST FORMALIN - INDUCED PAW EDEMA IN RATS

Test group	Mean paw volume (ml)							
	0 min	30 min	1h	2h	3h	4h	5h	6h
I	4.10±0.16	7.42±0.15	7.48±0.14	8.34±0.20	8.3±0.10	7.8±0.08	8.28±0.29	7.91±0.22
II	4.08±0.16	7.03±0.10	7.81±0.19	7.7±0.24	7.95±0.20	7.78±0.12	6.03±0.15	5.66±0.27
III	3.69±0.2	6.97±0.19	7.64±0.15	8.15±0.20	8.10±0.12	7.44±0.25	6.63±0.38	6.1±0.29
IV	4.28±0.09	7.06±0.10	7.48±0.22	7.8±0.15	8.16±0.10	7.75±0.037	6.23±0.15	5.49±0.18

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns-Not significant **P< 0.05 calculated by comparing treated group with control group.



Group – I Control **Group-II Formalin + Indomethacin**

Group –III formalin + 200mg/kg Extract **Group –IV Formalin + 400mg/kg Extract**

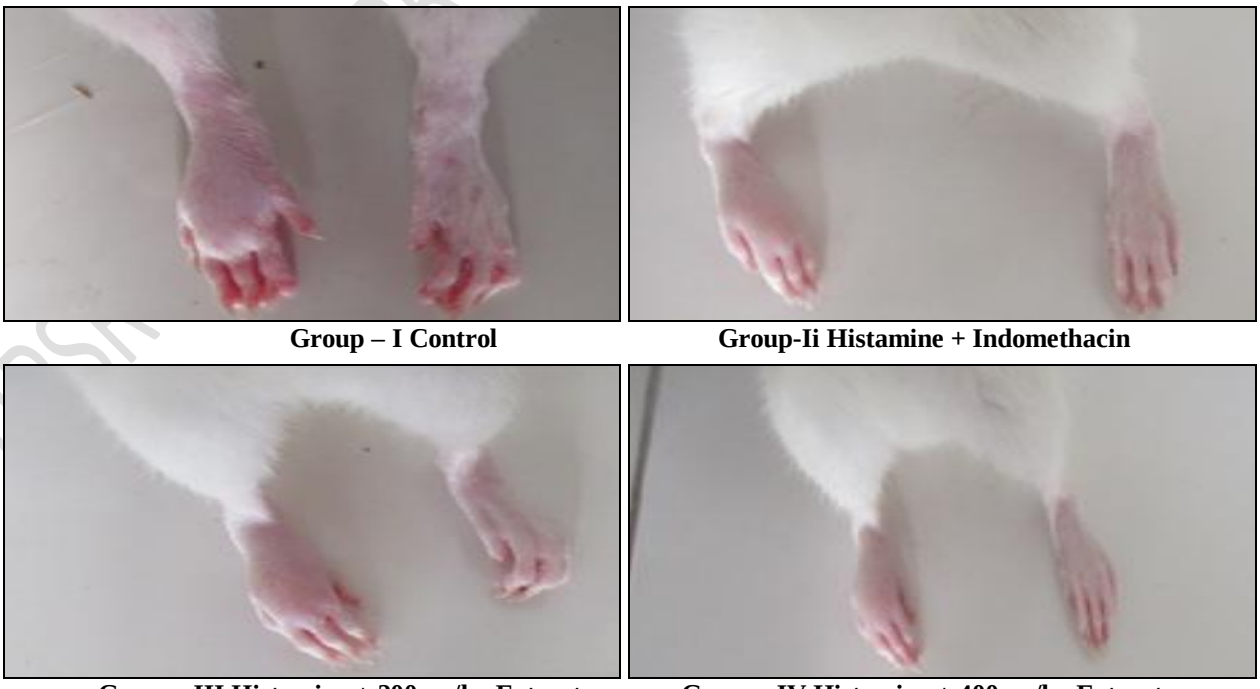
FIG. 1: EFFECT OF *MERREMIA TRIDENTATA* ON FORMALIN INDUCED PAW EDEMA IN RATS

Effect of *Merremia tridentata* on Histamine Induced Paw Edema in Rats: From the analysis of Histamine induced paw odema, the paw volume of the control group was found to be 4.5±0.42 ml at the 0 min, 8.0±0.22 ml at the 5 h and 7.9±0.29 ml at the 6th h **Table 2**.

The standard drug showed reduction in paw volume of 3.75±0.27 ml at 0 min, 7.6±0.15 ml at 3h, 6.0±0.53 ml at 6 h.

The paw volume after the treatment of low doses of ethanolic plant extract at the 0 min was found to be 4.2±0.6 ml, 8.0±0.07 ml at the 3 h and 6.3±0.60 ml at 6 h.

Similarly, the paw volume of High doses of the ethanolic plant extracts was observed to be 3.7±0.08 ml at 0 min, 7.8±0.2 ml at 3 h and 6.13±0.47 ml at 6 h **Fig. 2.**



Group – I Control **Group-Ii Histamine + Indomethacin**

Group –III Histamine + 200mg/kg Extract **Group –IV Histamine + 400mg/kg Extract**

FIG. 2: EFFECT OF *MERREMIA TRIDENTATA* ON HISTAMINE - INDUCED PAW EDEMA IN RATS

TABLE 2: ANTI-INFLAMMATORY ACTIVTY OF *MERREMIA TRIDENTATA* ON HISTAMINE - INDUCED PAW EDEMA IN RATS

Test group	Mean paw volume (ml)							
	0 min	30 min	1h	2h	3h	4h	5h	6h
I	4.58±0.42	7.75±0.54	7.76±0.28	8.31±0.23	8.34 ±0.13	8.33±0.45	8.02±0.22	7.96±0.298
II	3.75±0.27	7.32±0.26	7.65±0.15	7.76 ±0.25	7.69 ±0.15	6.42±0.19**	7.01 ±0.18*	6.02±0.53*
III	4.21±0.6	7.89±0.34	8.12±0.463	7.87±0.23	8.07±0.076	7.45±0.45	6.76±0.39*	6.30±0.60
IV	3.78±0.083	7.73±0.411	8.02±0.57	7.73±0.19	7.83±0.267	6.14±0.33**	6.238±0.40**	6.13±0.47

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns-not significant **P< 0.05 calculated by comparing treated group with control group.

DISCUSSION: Medicinal plants of terrestrial background are considered to have potent active substance than the substances of any other natural origin, hence its use in many pharmaceutical and medical industries are warrented. Plants are effective supplementary sources of drugs as they have rich dietary resource. They have been utilized for various medications for thousands of years (Samuelson, 2004). The phytochemicals of each plant extracts have variety of active chemicals with different chemical structures. The history of the medicinal plants is so important which explain the continuity and can be essential to treat many diseases and importance of medicated plants from generation to generation where and how it was discovered and who observed the medicinal importance in different era as the plants provide biologically active and important molecules that can be used for treatment of different disease.

The present research focused on the development of new anti-inflammatory compounds from a medicinal plant *Merremia tridentata*. The experimental modes of infla-mmation are induced by different agents by releasing different types of inflammatory mediators. Each is known to elicit distinct mechanisms of action for producing inflammation by increased in vascular perme-ability, the infiltrations of leukocytes from the blood into the tissue or granuloma formation and tissue repair. Among them methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniquesis based upon the ability of such agents to inhibit hind paw edema of the rat after the injection of a phlogistic agent. Many phlogistic agents (irritants) have beenused, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, Aerosil®, sulfated polysaccharides like carrageenan or naphthoylheparamine. The Herbals extracts were found to reduce the inflammation in the formalin induced paw edema models. The control group (Group-I) showed paw volume of 7.91±0.22 ml and the Group-II showed 5.6±0.27 ml. Lower doses (Group-III) and higher doses (Group-IV) of plant extracts showed 6.1±0.29 ml and 5.49±0.18 ml. The high doses of plant extract showed the significant reduction in paw volume than the standard drug at 6 h. Similarly on histamine induced paw edema. The control group (Group-I) showed paw volume of 7.9±0.29 ml and the Group-II (Indomethacin) showed 6.02±0.53 ml. Lower doses (200mg/kg) and higher doses (400mg/kg) of plant extracts showed 6.30±0.60 ml and 6.13±0.47 ml. Thus, the plant extracts showed higher reduction inpaw volume of the histamine induced rats than the standard drugs. From the above analysis, it is confirmed that the *Merremia tridentata* plant extracts showed paw volume reduction similar to the drug. Thus, the *Merremia tridentata* plant extracts with active phytochemical compounds could be used as an anti-inflammatory agent.

CONCLUSION: It may be concluded that the results of the present study support the traditional use of *Merremia tridentata* plant in inflammation and painful conditions which confirm the presence of active chemical compounds related to these activities. The plant extract showed significant anti-inflammatory extract when compared with the standard drug (Indomethacin). Thus, the *Merremia tridentata* can be utilized for the treatment of various inflammation associated diseases and can be used as an alternative against commercial drugs.

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How to cite this article:
Manimegalai M, Prasad SS, Subha TS and Suguna L: Anti-inflammatory activity of ethanolic extract of whole plant of *Merremia tridentata* on murine models. Int J Pharm Sci & Res 2021; 12(5): 1000-06. doi: 10.13040/IJPSR.0975-8232.12(5).1000-06.

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