**ASSESSMENT OF ANTI-ASTHMATIC ACTIVITY AND PHYTOCHEMICAL SCREENING IN URTICA DIOICA**

1. **SHAKTHI\*,R.DEVI,Dr.R.SRINIVASAN,N.JAYARAMAKANI,Dr.R.SARAVANAN**

**Faculty of Pharmacy,Bharath Institute of Higher Education and Research,Selaiyur,Chennai,Tamil Nadu,India**

**Corresponding Author email: [devivarshni@gmail.com](mailto:devivarshni@gmail.com)**

**ABSTRACT:**

The major purpose of this study is to evaluate anti-asthmatic activity and its phytochemical screening in the leaves of Urtica dioica in guinea pig ileum. This plant is herbaceous and perennial angiosperm plant belonging to the family. Urtricaceae and the genus urtica. This plant also called as stinging nettle and widely distributed to various parts of Europe, North America, Southwestern china and North Africa. Urtica dioica is mostly used to treat several disease such as diuretic, asthma, diabetes, skin injury, anemia, blood purifier, hay fever, arthritis, cleansing tonic, hair problems, headaches, chest pain, joint pain, gout, neuralgia, reduce excessive flow of menstrual, and nose bleeding. Phytochemical screening test was performed by wagner and bladt. It help to identify the phtyochemicals and its characteristic of the compound. The material that are used in this experiment are histamine dihydrochloride aerosol, Sherrington rotating drum, histamine closed chamber and guinea pig ileum. In this work Mepyramine is used as standard drug which is used to compare the extract of urtica diocia and the extract shows the result of having anti-asthmatic property.

**KEYWORDS:**

Stinging nettle, Urtricaceae, wagner and bladt, histamine dihydrochloride aerosol, Mepyramine and Sherrington rotating drum.

**PLANT - URTICA DIOICA**

Urtica dioica otherwise called as stinging nettle. Urtica is derived from the word urere in Latin language which means "to burn" for the reason of trichome present in it. The word diocia referred as "two houses" for the reason that plant consists of two flowers such as male and female [1]. This plant is a perennial angiosperms and dicotyledons plant it covered with the stinging hairs called trichomes that produce chemicals like histamine, serotonin and acetylcholine it gives hot sensation and itchy, rashes when it is touched. It grows upto the height of 2 metres, soil containing moisture content, nitrogen and having pH 5 - 8. This plant parts are used as vegetable in soup, tea and salad [2]. Roots and stems are used to treat asthma, jaundice, diuretic,cough, joint pain, cold and applied in inflammation, wounds, cuts and burns. Leaves are enriched in chlorophylls, protein, fats, carbohydrates, minerals, fibre, carotenoids and vitamins [3]. It is boiled in water and filtered then the filters are given to menstrual disorder, pregnant women after childbirth for gaining strength and also used in jaundice. In traditionalmedicine this herb is used orally to treat haemorrhage, hay fever, influenza, rheumatism, diabetics and gout as well as problems of the gastrointestinal tract, locomotor system, skin, and cardiovascular system [4]. Stinging nettle is used in the bast fibre and also in the cosmetic industry. This plant have greater pharmacological activities, such as antiarthritis, anticancer, antimicrobial, cardioprotective, antioxidant, antihelmintic, nephroprotective, antiviral, antidiabetic, antiendometriosis, hepatoprotective, antiaging, and anti inflammatory activity [5].



**BIOLOGICAL SOURCE:**

PARTS USED : Leaves, stem and root.

SPECIES : Urtica ardens.

FAMILY : Urtricaceae.

GENUS : Urtica [6].

**SYNONYMS:**

The urtica dioica is commonly known as stinging nettle, common nettle, nettle, burn nettle, American stinging nettle, stringer, nettle leaf, hoary nettle, stinger and European stinging nettle [7].

**GEOGRAPHICAL SOURCE:**

Urtica dioica originated from Finland, North America, Germany, France, Himalayas, New Zealand, Denmark, Belgium, Ireland, Iran, Netherlands, Nepal, Mexico, Poland, Switzerland, Sweden, North Africa, Italy and Southwestern china [8].

**DISEASE - ASTHMA:**

Asthma is a long-term inflammatory disorder with obstruction of hyper responsiveness in bronchial tubes occurring due to allergens that lead to wheezing, increasing mucous

secretion, cough, chest tightness, rapid respiration and shortness of breath [9]. When allergens like dust particles, pollens, pet hairs and mould are enter into the body, discharge the cytokines like interleukin IL-4, IL-5, IL-9 and IL-13 by increases the T-helper cell [10], then promote the production of immunoglobulin E, release of inflammatory mediators like histamine and cysteinyl leukotrienes, it leads to contractions of bronchial airways [11]. The drugs that are used to treat asthma are called anti-asthmic drugs, some example of asthma drugs are Mepyramine Salbutamol, Montelukast, Theophylline, predinosone, omalizamab, ketotifen, predinosolone [12].

**AIM:**

This study mainly focus on to evaluated the anti-asthmic through the plant Urtica dioica by using mice also study the secondary metabolites for this plant using phytochemical screening method.

**MATERIAL REQUIRED:**

Shaker, muslin cloth, Whatman filter paper, frontal – writing lever , freeze dryer, Sherrington rotating drum, histamine chamber, histamine dihydrochloride aerosol, Mepyramine (standard drug) [13].

**PREPARATIONS OF EXTRACT:**

100g of urtica dioica was grinded into coarse powder and then soaked in the conical flask containing 1 liter of 50% ethyl acetate. The solution in the flask was placed on a shaker for shaking the content at 120 rpm from time to time for 72 hours in room temperature [14]. These extract are filtrate three times by using muslin cloth, then with Whatman filter paper and by adding fresh solvent. Leave the filtrate in freezer for overnight and the moisture is removed from filterate by using freeze dryer. The ethanolic extract of urtica diocia are also used directly with distilled water for treating asthma through oral administration [15].

**PHYTOCHEMICAL SCREENING METHOD:**

For identification and conformation of raw and final herbal product or herbal medicine by using the phytochemical screening methods and also determine the particular species of specific compounds and traits [16]. It has advantage for reveal the constituents of the plant as well as helpful to identify the bioactive agents that can be used in the manufacture of synthetic drugs. They are performed by as defined as Wagner and Bladt [17].

|  |  |  |  |
| --- | --- | --- | --- |
| **SI.NO** | **NAME OF THE TEST** | **EXPERIMENT** | **OBSERVATION** |
|  | Mayer's agent | Plant extract + few drops of maeyer's reagent (potassium iodide, mercuric chloride) are mixed and volume up the solution with 100 ml distilled water. | Formation of creamy white precipitate |
|  | Dragendorff's reagent | Plant extract + small amount of dragendroff's reagent (potassium bismuth and potassium iodide) | Formation of orangish red colour |
|  | Liebermann-burchard's test | Plant extract + acetic anhydride are boiled and cooled, then add the concentrate sulphuric acid  by the side of test tube | Appearance of green colour |
|  | Shinoda test | Plant extract + ethanol + concentrate hydrochloride acid + magnesium. | Appearance of pink colour |
|  | Keller Killiani test | Plant extract + glacial acetic acid + ferric chloride solution + concentrate sulphuric acid was add at the side of test tube. | Appears in blue colour |
|  | Foam test | Plant extract + 10 - 20 ml of water shaken for few minutes | Formation of foam about 2 cm high |
|  | Olive test | Plant extract + few drops of olive oil shaken for five minutes | Formation of foam about 2 cm high |
|  | Goldbeater's skin test | A small tissue of goldbeater's skin (tissue from ox intestine ) + immersed in hydrochloride acid and washed with distilled water then soaked in tannin solution. Again wash with distilled water and then transfer in ferrous sulphate solution. | Appears of brown or black colour |

[18,19,20,21,22]

**PREPARATION OF ISOLATED GUINEA PIG ILEUM:**

A piece of ileum were cut from the from guinea pig and then suspended in the 50ml tissue bath containing kerb solution at 37°c with the gas of oxygen (95%) and carbon dioxide (5% ) for 45 minutes [23]. After that observe the dose response curve of histamine and also with treatment of urtica diocia extract were incubated for 2 minutes, then observe the response of guinea pig. Aerator is connected with tissues bath which are used to maintain the aeration for its tissue survival [24]. Record the movement of ileum using frontal – writing lever are connected to the sherrington rotating drum.

**HISTAMINE INDUCED CONVULSION:**

Guinea pig were selected and separated into three groups. Each group contains six animals.

* GROUP 1 - For control - histamine dihydrochloride aerosol.
* GROUP 2 - Histamine dihydrochloride aerosol + Mepyramine.
* GROUP 3 - For test - 250 mg/ kg of urtica diocia extract [25].

Before and after the two hours of drug treatment the animals were placed in a histamine chamber and load the histamine dihydrochloride aerosol via nebulizer then the aerosol were exposed to an animal and finally noted the preconvulsion time of the animal. Convulsion appears as soon as shortness of breath (dyspnoea) occurs [26]. If the animal affect by convulsion then the animal were separated from the histamine chamber and kept in outside for recover.

**FORMULA:**

Percentage of preconvulsion dyspnoea increased = (1- t1/t2) ×100

t1 = time for preconvulsion dyspnoea before treatment (second).

t2 = time for preconvulsion dyspnoea after treatment (second) [27].

**RESULT:**

**PHYTOCHEMICAL SCREENING METHOD:** [28, 29]

|  |  |  |  |
| --- | --- | --- | --- |
| **SI.NO** | **NAME OF THE TEST** | **PHTOCHEMICALS** | **INFERENCE** |
|  | Mayer's agent | Alkaloids | Presence |
|  | Dragendorff's reagent | Alkaloids | Presence |
|  | Liebermann-burchard's test | Steroids | Presence |
|  | Shinoda test | Flavonoids | Presence |
|  | Keller Killiani test | Glycosides | Presence |
|  | Foam test | Saponin | Presence |
|  | Olive test | Saponin | Presence |
|  | Goldbeater's skin test | Tannin | Presence |

**PREPARATION OF ISOLATED GUINEA PIG ILEUM:**

guinea pig ileum preparation is used for the identified the property of anti-asthmatic drugs. H1 receptor present in the ileum help to stimulates the contraction of ileum. In this study the extract of urtica dioica contain H1 receptor antagonist activity, so they bind to the H1 receptor containing in the guinea pig ileum and deactivate the contraction of ileum and help to relax the ileum [30].

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **No. of animal** | **Dose** | **Histamine conc.** | **Log conc.** | **Histamine CRC** | **Standard response (%)** | **Extract response (%)** |
|  | 0.2 | 20 | 0.301 | 47.66 | 23.976 | 36.65 |
|  | 0.5 | 50 | 0.856 | 69.59 | 37.887 | 44.87 |
|  | 0.8 | 80 | 1.202 | 76.12 | 38.097 | 48.98 |
|  | 1.5 | 150 | 1.985 | 81.54 | 34.657 | 51.76 |
|  | 1.9 | 190 | 2.138 | 91.63 | 38.982 | 54.87 |
|  | 3.2 | 320 | 4.060 | 100.45 | 35.761 | 50.09 |

**HISTAMINE INDUCED CONVULSION:**

The group of animals is treated with 0.5% histamine dihydrochloride aerosol result in constriction of bronchial tubes in the way of preconvulsion dyspnoea. Standard drug and ethanolic extract of urtica diocia are given before the animal expose to 0.5% histamine dihydrochloride aerosol and it slowly increased the preconvulsion dyspnoea time [31].

|  |  |  |
| --- | --- | --- |
| **Group** | **Drug treated** | **Preconvulsion dyspnoea increased (%)** |
|  | Control | 4.089 |
|  | Mepyramine | 59.674 |
|  | urtica diocia extract | 48.453 |

**CONCLUSION:**

This work shows that the anti-asthmatic activity by using in-vivo and in-vitro method. Ethanolic extract of urtica diocia have the excellent property of anti-asthmatic by inhibiting the H1 receptor using H1 receptor antagonist and also increase the preconvulsion time. This plant has contained secondary metabolites such as flavonoids, alkaloids, saponin, tannins, steroids, glycosides etc and also have excellent pharmacological property like anticancer, cardioprotective, antihelmintic, antiviral, antioxidant, antidiabetic, antimicrobial, antiendometriosis, antiaging, and anti inflammatory activity.

**REFERENCE:**

1. Wagner H, Willer F, Kreher B. Biologically active compounds from the aqueous extract of Urtica dioica. Planta Med. 1989; 55(5): 452-4
2. Riehemann K, Behnke B, Schulze-Osthoff K. Plant extracts from stinging nettle (Urtica dioica), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB. FEBS Lett. 1999 Jan; 442(1): 89-94.
3. Blumenthal M, Goldberg A, Brinckmann J. Herbal medicine: expanded Commission E monographs. Newton MA: Intergrative Medicine Communications, 2000, pp –367-75.
4. Randall C, Meethan K, Randall H, Dobbs F. Nettle sting of Urtica dioica for joint pain-an exploratory study of this complementary therapy. Comp. Ther. Med. 1999;7:126–131.
5. Adel M., Caipang C.M.A., Dawood M.A. Immunological responses and disease resistance of rainbow trout (Oncorhynchus mykiss) juveniles following dietary administration of stinging nettle (Urtica dioica) Fish Shellfish Immunol. 2017;71:230–238. [PubMed].
6. A.A.H., Otmani I.S.E., Derfoufi S., Benmoussa A. Highlights on nutritional and therapeutic value of stinging nettle (Urtica dioica L.) Int. J. Pharm. Pharmaceut. Sci. 2015;7(10):8–14.
7. Bnouham M., Merhfour F.Z., Ziyyat A., Mekhfi H., Aziz M., Legssyer A. Antihyperglycemic activity of the aqueous extract of Urtica dioica. Fitoterapia. 2003;74(7-8):677–681. [PubMed]
8. Le Moal MA, Truffa-Bachi P. Urtica dioica agglutinin, a new mitogen for murine T lymphocytes: unaltered interleukin-1 production but late interleukin 2-mediated proliferation. Cell Immunol. 1988; 115(1): 24-35.
9. Pourmorad F., Hosseinimehr S.J., Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 2006;5(11):1142–1145.
10. Tuberville T.D., Dudley P.G., Pollard A.J. Responses of invertebrate herbivores to stinging trichomes of Urtica dioica and Laportea canadensis. Oikos. 1996:83–88.
11. Tesfaye ZT, Gebreselase NT, Horsa BA. Appropriateness of chronic asthma management and medication adherence in patients visiting ambulatory clinic of Gondar University Hospital: a cross-sectional study. World Allergy Organ J. 2018;11(1):18.
12. Scirica CV, Celedón JC. Genetics of asthma: potential implications for reducing asthma disparities. Chest. 2007 Nov;132(5 Suppl):770S-781S. [PubMed].
13. Southworth T, Kaur M, Hodgson L, Facchinetti F, Villetti G, Civelli M, Singh D. Anti-inflammatory effects of the phosphodiesterase type 4 inhibitor CHF6001 on bronchoalveolar lavage lymphocytes from asthma patients. Cytokine. 2019 Jan;113:68-73. [PubMed].
14. Tahri A, Yamani S, Legssyer A, Aziz M, Mekhfi H, Bnouham M, Ziyyat A. Acute diuretic, natriuretic and hypotensive effects of a continuous perfusion of aqueous extract of Urtica dioica in the rat. J. Ethnopharmacol. 2000;73:95–100.
15. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 2015;64:555–559.
16. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem. 2015;43:1813–1819.
17. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 1998;46:4113–4117.
18. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother. 1968; 32:295–310.
19. Hajhashemi V, Ghannadi A, Sharif B. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill. J Ethnopharmacol. 2003;89: 67–71.
20. Hajhashemi V, Sajjadi SE, Zomorodkia M. Antinociceptive and anti-inflammatory activities of Bunium persicum essential oil, hydroalcoholic and polyphenolic extracts in animal models. Pharm Biol. 2011;49:146–151.
21. Amresh G, Reddy GD, Rao C, Singh PN. Evaluation of anti-inflammatory activity of Cissampelos pareira root in rats. J Ethnopharmacol. 2007;110:526–531.
22. Arulmozhi S, Papiya MM, Purnima A, Sathiya N. In-vitro antioxidant and free radical scavenging activity of Alstonia scholaris Linn. R. Br. Iranian J. Pharmacol. Ther. 2008;6:191–196.
23. Sreena KP, Poongothai A, Soundariya SV, Srirekha G, Santhi R, Annapoorani S. Evaluation of in-vitro free radical scavenging efficacy of different organic extracts of Morinda tinctoria leaves. Int. J. Pharm. Pharm. Sci. 2011;3:207–209.
24. Bors W, Saran M. Radical scavenging by flavonoid antioxidants. Free Radic Res Commun. 1987;2:289–294.
25. Dar SA, Ganai FA, Yousuf AR, Balkhi MU, Bhat TM, Sharma P. Pharmacological and toxicological evaluation of Urtica dioica. Pharm Biol. 2012.
26. Elisabetsky E, Amador TA. J Ethnopharmacol. Albuquerque RR, Nunes DS, Carvalho AC. 1995. Analgesic activity of Psychotria colorata (Willd. ex R. & S.) Muell. Arg. alkaloids; 48:77–83.
27. Hajhashemi V, Ghannadi A, Sharif B. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill. J Ethnopharmacol. 2003;89: 67–71.
28. Ghannadi A, Hajhashemi V, Jafarabadi H. An investigation of the analgesic and anti-inflammatory effects of Nigella sativa seed polyphenols. J Med Food. 2005;8:488–493.
29. Lachance PA, Nakat Z, Jeong WS. Antioxidants: an integrative approach. Nutrition. 2001;17:835–838.
30. Sonboli A, Mojarrad M, Ebrahimi SN, Enayat S. Free radical scavenging activity and total phenolic content of methanolic extracts from male inflorescence of Salix aegyptiaca grown in Iran. Iranian J. Pharm. Res. 2010;9:293–296.
31. Riehemann K, Behnke B, Osthoff KS. Plant extracts from stinging nettle (Urtica dioica), an antirheumatic remedy, inhibit the pro inflammatory transcription factor NF-KB. FEBS Let. 1999;442:89–94.