In vitro evaluation of anti-asthmatic activity of Stinging nettle leafs

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ABSTRACT

Stinging nettle (Urtica dioica) has a long history use as a medicinal herb found throughout the world that has used for medicinal purposes for centuries. It has been used to treat asthma but synthetic drugs for the treatment of asthma and allergy in India found more side effects. Synthetic drugs used for the treatment of asthma and allergy in India but more side effects are reported. Over the centuries, they are using medicinal herbs in daily life and approximately 6000 plants species are known to have medicinal properties in India. As per the literature survey will be expressed medicinal plants and traditional systems of medicines, Ayurveda, Yunani, Siddha and Homeopathy for the treatment of asthma and allergy but no scientific validation. Several literatures are indicated that the herbal drugs have lesser adverse effects when compared to synthetic drugs. The Urtica dioica is not scientifically validated and which was traditionally using herb. The work provides scientific validation for use of leaves against asthma by revealing the chemical compounds may be present in the plant. The present study is attempts to develop a novel plant-based antihistamine work through anti asthmatic drug which will be evaluated by in vitro and in vivo.

KEYWORDS- Mephramine, anti-asthmatic activity; Stinging nettle leafs,

INTRODUCTION

Stinging Nettle (Urtica dioica L.) is one such species that is found widely in temperate and tropical Asia, Europe, northern America and northern Africa and consumed by traditional societies. Urtica dioica is a perennial herb that grows commonly in waste lands, gardens, farmers field (as weed), as hedges in terraced fields. It is distributed between 1200 to 3000 m in Himalaya from Jammu & Kashmir to Arunachal Pradesh (Wealth of India 1998). Nettle has been used for centuries in various folk medicine systems in China, Persia, Turkey, Russia, India and various other countries to cure humans and animals. For treatments it is used as extract (juice), in dried form, as tincture, ointment and/or as a supplement. It is used to treat allergies, kidney stones, burns, anemia, rashes, internal bleeding, diabetes, etc. Commonly called as Nettle, Common nettle or Stinging nettle, all over the world Urtica dioica is known with the different names. Allergy is one of the common diseases that affect mankind with diverse manifestations. Asthma is a respiratory disease characterised by recurrent episode of chest tightness, cough, wheezing and difficulty breathing brought about by bronchial constriction, inflammation and excessive mucus secretion due to bronchial hyperresponsiveness. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Allergic diseases are responsible for significant morbidity and have severe economic impact. Various epidemiological studies have identified the causes for an increase in the prevalence of upper and lower respiratory tract allergic diseases. Some of the postulated
reasons are increasing environmental pollution and increased predisposition of individuals producing excessive IgE through a major change in the gene pool, changing lifestyles, and an increasing awareness of the disorders. The application of nettle to cure diseases with good healing properties can be attributed to the presence of certain phytochemicals, such as flavonoids, lignans, fatty acids, sterols, polysaccharides, glycoproteins, carotenoids, plastocyanins, tannins and lectins (Sajfrtová et al., 2005, Ghaima et al., 2013). Efforts are being made to identify and isolate such phytochemicals from different parts of the plant that has direct effect (Krystofova et al., 2010). It is reported that nettle comprised polysaccharides, vitamin C and carotene, betasitosterol, and the flavonoids quercetin, rutin, kaempferol, and beta-sitosterol (Newall et al., 1996; Schottner et al., 1997; Konrad et al., 2000). The leaves comprised diterpene lactone and Phlogantholide A. Polar extracts of the nettle roots contain the lignans that have binding affinity to SHBG in the in vitro assay (Schottner et al., 1997). Other than the lignans nettle is reported to have lectins, sterols, phenylpropanes, ceramides, hydroxyl fatty acids, triterpenes, phenols, coumarins, fatty acids and carotinoids, flavonoids, amines, chlorophylls and carotinoids (Seliya and Kothiyal, 2014). The main components of essential oil in nettle are carvacrol (38.2%), carvone (9.0%), naphthalene (8.9%), (E)-anethol (4.7%), hexahydrofarnesyl acetone (3.0%), (E)-geranyl acetone (2.9%), (E)-ionone (2.8%) and phytol (2.7%) (Gul et al., 2012).

MATERIALS AND METHODS

The leaves of *urtica dioica* were collected.

Preparation of plant extract
Cold maceration technique was used for the extraction of plant material and a total of 200 g of *Urtica dioica* leaves the coarse powder was used. During the process 100 g of the coarse powder was soaked in an Erlenmeyer flask with 1 L of 50% of Ethyl Acetate and then placed on a shaker (Bibby Scientific Limited Stone Staffo Reshire, UK) tuned to 120 rpm with occasional shaking for 72 h at room temperature. The extract was filtered first using a muslin cloth and then Whatman grade No-1 filter paper and the marc was re-macerated for a second and third time by adding another fresh solvent. The filtrates were left overnight in a deep freezer and then lyophilized using freeze dryer. The dried plant extract was reconstituted with distilled water for oral administration.

**Phytochemical Test**

**Maeyer’s reagent** - 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

**Test for alkaloids** - About 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer’s and Dragendorff’s).

**Test for steroids** - About 0.5 g of the methanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

**Dragendorff’s reagent** - Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

**Test for terpenoids** - An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of CHCl3 in a test tube. 3 ml of concentrated H2SO4 was carefully added to the mixture to form a layer.

**Test for flavonoids** - To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.
Test for tannins: The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

Test for Phytosterol
1. Foam Test: 5 ml of the test solution taken in a test tube was shaken well for five minutes.
2. Olive oil test: - Added a few drops of olive oil to 2 ml of the test solution and shaken well.

Test for glycosides
1. Keller-Killiani test: Added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully.
2. Hydroxyanthraquinone Test To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution.

Test for Phytosterol
1. Foam Test: 5 ml of the test solution taken in a test tube was shaken well for five minutes.
2. Olive oil test: - Added a few drops of olive oil to 2 ml of the test solution and shaken well.

Experimental animals
The Adult female Swiss mice weighing between (20-30 g) were used to calculate LD50 and female and male guinea pigs with an average weight of 220-250 g were used antihistamine study. They were housed in clean polypropylene cages and maintained under standard conditions of light (12 hours with alternative day/night cycles), relative humidity (60-70%) and temperature (26 ± 1 °C). The animals were fed daily with rodent pellet diet and tap water ad-libitum under strict hygienic conditions.

Histamine induced convulsion by using histamine chamber
Animals with nearly same pre convulsion time were selected and randomly divided into three groups of six animals each-
- GROUP I – Asthmatic control-0.5% Histamine HCL aerosol.
- GROUP II – Standard treatment 0.5% Histamine HCL aerosol with Mepyramine (8mg/kg.p.o).
- GROUP III - High dose (200mg/kg) of aqueous ethanolic extract of urtica dioica.

The experimental animals were kept in a closed chamber and exposed to an aerosol of 0.5% of histamine dihydrochloride and preconvulsion time was measured two hours after the above drug treatment, animals were exposed to histamine aerosol and pre convulsion time was noted. As soon as dyspnea occurs, it leads to the appearance of convulsion. animals were removed from the chamber and placed in fresh air to recover.
Percentage protection = $\{1-T1/T2\} \times 100$ where, T1 = time in second for PCD before treatment; T2 – time in second for PCD after treatment.
RESULTS AND DISCUSSION

*Urtica dioica* were a light semisolid brownish color extract and the percentage yield was found to be 16.35%.

**Phytochemical Analysis**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemicals</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Phytosterol</td>
<td>+</td>
</tr>
</tbody>
</table>

+, Presence of the compound
-, Absent

**Histamine induced convulsion by using histamine chamber**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment Groups</th>
<th>% increase in PCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Disease control</td>
<td>4.16 ±0.83</td>
</tr>
<tr>
<td>2.</td>
<td>Mephramine (8mg/kg)</td>
<td>58.5 ±0.22 *</td>
</tr>
<tr>
<td>3.</td>
<td><em>Urtica Dioica</em> leaves extract 200mg/kg</td>
<td>47.83 ±0.47 *</td>
</tr>
</tbody>
</table>
CONCLUSION

This work will be useful to find new anti-asthmatic drug with help of in vitro and in vivo models. Ethanolic extract will be possess highly substantial anti-asthmatic activity by significantly inhibited the histamine induced bronchoconstriction of guinea pig representing its H1 receptor antagonistic activity and support the plants by its anti-asthmatic properties.

REFERENCES


