Phytochemical Investigation and Anti-Arthritic Activity of Hydroalcoholic Extracts of *Trichosanthes dioica*

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**ABSTRACT**

*Trichosanthes dioica* Roxb. is a Cucurbitaceous perennial herb widely distributed in tropical areas of Asia and Australia. This herb is cultivated across the globe firmly as a vegetable source. *Trichosanthes dioica* are used in traditional medicinal system as anti-inflammatory agent, liver tonic, cardiotonic, skin infection, antiulcer, anti-diabetic etc. Number of phytochemical and pharmacological work has been carried out on deferent parts of *Trichosanthes dioica*, prime objective of the study to find out Anti-Arthritic activity of hydroalcoholic extract of *Trichosanthes dioica*. Hydroalcoholic extracts are rich of flavonoids, Vitamins, Alkaloids, proteins and saponins. Although flavonoids are also responsible for inflammatory induced Antiarthritic activity, presence of secondary metabolite phytoconstituents encourages the current work. Results of the current study shows that *Trichosanthes dioica* Roxb. having significant anti-arthritic activity.

**Keywords:** Anti-arthritic; Hydroalcoholic extract; *Trichosanthes dioica*; Phytoconstituents

**INTRODUCTION**

Globally around 80,000 plant species are used for medicinal and aromatic purposes. Although there is wide use of herbal medicine, traditional knowledge of the use of medicinal plants is influenced by rapid urbanization, migration, climate change, and the increasing number of modern healthcare systems throughout the world [1-4]. About 90% herbal raw drugs used in the manufacture of vegetable drugs are obtained from the wild source which is limited. With the increasing esteem of herbal medicine and ayurveda, use of medicinal plants is expected to rise globally. Since the side effects and cost of synthetic drugs are higher it demands increased utilization of herbs [5]. Rheumatoid Arthritis (RA), classified as autoimmune affecting approximately 5% of the human population [6]. RA is a chronic inflammatory polyarthritis, affecting multiple diarthroidial joints in a Characteristic distribution, and leading to pain, joint deformities and a reduced quality of life. RA characterised by extensive synovitis resulting in erosions of articular cartilage and marginal bone that lead to joint destruction [7]. More than 100 of rheumatic diseases are characterized by inflammation and pain, are called as autoimmune diseases because they occur when the immune system shows significant activity with response to serious infection. Clinically RA manifests with a symmetric polyarthritis characterized by pain, swelling, loss of function and a morning stiffness lasting more than one hour [8]. A common onset synovitis involves the metacarpophalangeal, the proximal interphalangeal, the wrist and the metatarsophalangeal joints, although all the joints may be affected. Several constitutional symptoms can precede the onset of RA, such as fatigue, malaise, weight loss, fever and depression [9].

*Trichosanthes dioica* (T. dioica) Roxb. A herb of Cucurbitaceae family and commonly known as “Sespadula” in English “Parwal” or Patal in Hindi. In India fruits of the parval are used as vegetable in almost all seasons [10]. Their Anti-Inflammatory, antipyretic, diuretic, cardiotonic, and laxative and many more pharmacological activity are already established, Study of extensive ethnobotanical survey and its Anti-Inflammatory activity excites us to go for Anti-Arthritic activity of its hydro alcoholic extract because it contains more relevant phytochemicals as compared to other extracts [11].
MATERIALS AND METHODS

Identification of *Trichosanthes dioica*

*Trichosanthes dioica* Roxb. fruits are collected freshly from nearby villages of Varanasi. Vouchered herbarium specimen of *Trichosanthes dioica* were prepared and preserved (Cucurbitaceae 2014/9) in faculty of science, department of botany, Banaras Hindu University, Varanasi (U.P.), India. Plants are Shade dried, reduced to powder and preserved for further use.

Animals

- Male wistar rats were chosen for the study.
- The animals were used after an acclimatization period of 7 days to the laboratory environment.

**Preparation of extracts**

Accurately weight 1.232 kg of powdered drug was taken in soxhlet apparatus, maceration was carried out till the extraction completed with petroleum ether up to 80°C. It was filtered hot and solvents were removed by distillation under reduced pressure. Marc left after petroleum ether extraction was dried in hot again loaded in soxhlet with ethanol (70%) until extraction completed. It was filtered hot and solvents were removed by distillation under reduced pressure and the hydroalcoholic extracts were stored for further use (Table 1).

<table>
<thead>
<tr>
<th>Types of Extract</th>
<th>Percentage Yield</th>
<th>Visual Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum extract</td>
<td>Hydroalcoholic Extract</td>
</tr>
<tr>
<td><em>Trichosanthes dioica</em> Roxb.</td>
<td>3.2</td>
<td>25.1</td>
</tr>
</tbody>
</table>

**Table 1**: Extractive values and appearance of various extracts.

**Table 2**: Presence of various phytoconstituents.

<table>
<thead>
<tr>
<th>Name of phytoconstituents</th>
<th>Pet. Ether Extract</th>
<th>Ether Hydro alcoholic Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of Alkaloid</td>
<td>Hager’s test</td>
<td>Mayer’s test</td>
<td>-</td>
</tr>
<tr>
<td>Presence of Glycosides</td>
<td>Borntrager’s test</td>
<td>Keller killiani test</td>
<td>-</td>
</tr>
<tr>
<td>Presence of Carbohydrates</td>
<td>Benedict’s test</td>
<td>Fehling’s test</td>
<td>-</td>
</tr>
<tr>
<td>Presence of Phenols and Tannin</td>
<td>Bromine water Test</td>
<td>Lead acetate Test</td>
<td>-</td>
</tr>
<tr>
<td>Presence of Flavonoid</td>
<td>Molish test</td>
<td>Shinoda Test</td>
<td>-</td>
</tr>
<tr>
<td>Presence of Protein</td>
<td>Biuret Test</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Presence of phytoconstituents like flavonoids, tannins, phenols, alkaloids and glycosides, fats and carbohydrates were investigated by using preliminary phytochemical screening of *Trichosanthes dioica* and the maximum contents were observed in hydroalcoholic extracts of *Trichosanthes dioica* [12] (Table 2).
Estimation of phenolic content of *Trichosanthes dioica*

The hydroalcoholic extract of *Trichosanthes dioica* Roxb. was evaluated total phenolic content concentrations. Folin-Ciocalteu method was used for estimation of total phenolic content in extracts of *Trichosanthes dioica* data expressed as gallic acid was equivalents. For calculating absorption data gallic acid was dissolved in water and absorbance were recorded. Total phenolic content of hydroalcoholic extract of *Trichosanthes dioica* were found to be 94.65 ± 1.05 mg GAE/gm [13-15] (Table 3).

Table 3: Total Phenol content of hydroalcoholic extract of *Trichosanthes dioica*.

<table>
<thead>
<tr>
<th>Type of Extract</th>
<th>Total Phenol content (mg GAE/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic Extract</td>
<td>94.65 ± 1.05</td>
</tr>
</tbody>
</table>

Determination of flavonol content of *Trichosanthes dioica*

Spectrophotometric methods by using aluminum chloride were used for estimation of flavonoid content and the contents were expressed in terms of quercetin equivalents. Standard curve of quercetin was plotted by dissolving it in distilled water. Content of flavonoids identified in the hydroalcoholic extract of *Trichosanthes dioica* are shown in Table 4. The concentrations of flavonoids in hydroalcoholic extract of *Trichosanthes dioica* Roxb. 74.11 mg QE/gm [16-19].

Table 4: Flavonol content of hydroalcoholic extract of *Trichosanthes dioica*.

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Amount of flavonol content (mg QE/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic Extract</td>
<td>74.11 ± 0.64</td>
</tr>
</tbody>
</table>

Estimation of anti-arthritis activity of *Trichosanthes dioica*

Adjuvant induced chronic arthritis model: There are many experimental models available but autoimmune arthritis are mainly accelerated by mycobacterium infection via T-Cell intermediate pathway. For our study arthritis was induced by using injection of dead mycobacterium in combination with liquid paraffin. After treatment with FCA there is significant increase in paw volume of rat was observed when it is compared with hydroalcoholic and standard drug treated rat. Dose of 25 mg/kg of hydroalcoholic extract shows significant decrease in paw edema of rat. After a time interval of 28 days it was found that hydroalcoholic extract of *Trichosanthes dioica* reduces paw volume significantly and which is totally dose dependent. Reductions in paw volume after treatment with Hydroalcoholic extract of *Trichosanthes dioica* were found to be 0.39 ± 0.51 mL. Indomethacin which is used as standard drug reduces paw volume was found to be 0.23 ± 0.91 mL [20-29].

Before the start of experiment rats were divided into 5 groups each group contains 6 rats. On starting day 0.1 mL of FCA (Freund’s complete adjuvant) were injected sub plantrly in left paw of rats. Standard drug Indomethacin and hydroalcoholic extract were injected after the next day of FCA injection and it will continue till 28th day. Left paw was marked with marker and paw volumes were recorded with the help of plethysmometer after injection routinely on 7th and 14th, 21st and 28th day of experiment (Table 5).

Control group marked as Group I: Arthritic infected rats treated with distilled water.

Group II: Arthritic infected rats treated with Indomethacin i.e., Standard drug with the dose of 10 mg/kg.

Group III: Arthritic infected rats treated with Hydroalcoholic extract of *Trichosanthes dioica* with the dose of 25 mg/kg.

Table 5: Effect of hydroalcoholic extract of *Trichosanthes dioica* in paw volume.

<table>
<thead>
<tr>
<th>Name of the Group</th>
<th>Observation of Paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On Day</td>
</tr>
<tr>
<td></td>
<td>0th</td>
</tr>
<tr>
<td>Control Group</td>
<td>0.21 ± 0.73</td>
</tr>
<tr>
<td>Indomethacin treated Group</td>
<td>0.27 ± 0.53</td>
</tr>
<tr>
<td>Hydroalcoholic extract</td>
<td>0.22 ± 0.63</td>
</tr>
</tbody>
</table>

Study of hematological parameters

Table 6: Hematological study of hydroalcoholic extract of *Trichosanthes dioica*.

<table>
<thead>
<tr>
<th>Name of the Group</th>
<th>Reading of RBC level</th>
<th>Reading of WBC level</th>
<th>Reading of Hb level</th>
<th>Reading of ESR level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>3.25 ± 23.17 * 8.31</td>
<td>1.53 ± 35.26 * 1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin treated Group</td>
<td>6.12 ± 7.63 * 11.74</td>
<td>0.77* ± 2.31 * 0.27*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroalcoholic treated Group</td>
<td>4.32 ± 0.72 * 0.95</td>
<td>0.45* ± 3.24 * 1.25*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After the administration of hydroalcoholic extract of *Trichosanthes dioica* on Freund’s adjuvant treated rats it was observed that the hemoglobin (Hb) and Red Blood Cell Count (RBC) levels are increased significantly when it is compared to distilled water treated rats. Whereas the White Blood Cell (WBC) and Erythrocyte Sedimentation Rate (ESR) count were
significantly decreased after the administration of *Trichosanthes dioica* Hydroalcoholic extract [30-32] (Table 6).

**DISCUSSION**

After preliminary phytochemical screening it was observed that hydroalcoholic extract contains maximum amount of phytoconstituents which are responsible for inflammation and arthritic activity therefore we have chosen hydroalcoholic extract. Anti-inflammatory activities are already established therefore we have not gone through Anti-inflammatory activity. Based on earlier established data we have carried out our work for rheumatoid arthritis. Rheumatoid arthritis is significantly characterized as inflammatory process therefore on folk medicinal information and previous study we have conducted Rheumatoid Arthritis activity.

RA is an autoimmune disease, therefore immunologically mediated FCA induced arthritic model is considered for ongoing study. Treatments of animals with FCA results and induction of systemic inflammation.

FCA insertion developed a chronic swelling of joints, erosion of joint cartilage, remodeling and bone destruction which results complete destruction of joint stability and mobility in the arthritic rats. Swelling of Ankle joints was observed which is termed as edema [33].

In our study, experimental arthritis were developed with repeated sub plantar injection of 0.1 mL of FCA up to 28 days which was characterized by development of tissue edema and reported highest on 7th day.

The progression of arthritis was confirmed in our study by scoring total arthritis lesions. Polyphenol content and presence of tannins also reveals anti-inflammatory action of *Trichosanthes dioica*. Therefore inhibition of lipid peroxidation and enzyme activity like cyclooxygenase, lipoxygenase may be due to tannins and polyphenols components [34].

In our study, arthritic control rats showed a decreased level of RBC and Hb where as ESR ratio is increased. Above symptoms are indications of anemia. Groups which are treated with *Trichosanthes dioica* Hydroalcoholic extract shows a significant recovery from anemic condition. Increase in leukocyte count in rats may be due to the stimulation of immune system against FCA therefore hydroalcoholic extract treated groups shows immunomodulatory effect. Resulting immunomodulation effect indicates the anti-arthritic activity of Hydroalcoholic extract of *Trichosanthes dioica*. Hydroalcoholic extract of *Trichosanthes dioica* are responsible for its anti-arthritic activity which are also supported by presence of various phytoconstituents.

**CONCLUSION**

*Trichosanthes dioica* Roxb. are rich in secondary metabolite such as alkaloid, glycoside, flavonoids, polyphenol etc. Polyphenol and flavones are responsible for various pharmacological activities. Therefore in our study an attempt was made to determine phytoconstituents and relate their anti-arthritic activity. Although anti-inflammatory activity are previously established therefore only for confirmation we have conducted phenol content and flavonol content study, presence of flavones on our hydroalcoholic extract supports our study. Hydroalcoholic extract of *Trichosanthes dioica* shows moderate anti-arthritis activity. This is also supported by presence of various plant metabolites like alkaloids, tannins, phenols and flavones. For estimation of possible mechanism responsible for anti-arthritis activity further study in cellular level and isolation of hydroalcoholic fractions are required.

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**REFERENCES**


