EXPERIMENTAL EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF HELICTRESS ISORA LINN

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ABSTRACT

Hydroalcoholic and Aqueous extract of Helictress isora linn leaves was investigated anti-inflammatory effect against carrageenan and histamine induced paw edema in rats. In the present study hydroalcoholic showed highly significant (p≤0.001) anti-inflammatory effect when compared with control rats. Aqueous extract showed significant anti-inflammatory effect against carrageenan but less than hydroalcoholic extract of Helictress isora linn leaves. Hydroalcoholic and aqueous extract showed significant effect against carrageenan and histamine. In both models the observation were observed 0-6hrs. Hydroalcoholic extract of Helictress isora linn leaves showed anti-inflammatory effect near to standard group in both models. The present study indicates that hydroalcoholic extract of Helictress isora linn leaves may be used as an anti-inflammatory drug.

KEYWORDS: Anti-inflammatory, carrageenan, histamine, paw edema, Helictress isora linn.

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.\(^1\) The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped
response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.\[^2\] Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.\[^3-4\]

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus.\[^5\] It is characterized by five cardinal signs:\[^6\] Both chronic and extreme inflammation are associated with disruptions of anabolic signals initiating muscle growth. Chronic inflammation has been implicated as part of the cause of the muscle loss that occurs with aging.\[^7-8\] Increased protein levels of myostatin have been described in patients with diseases characterized by chronic low-grade inflammation.\[^9\] Increased levels of TNF-\(\alpha\) can suppress the AKT/mTOR pathway, a crucial pathway for regulating skeletal muscle hypertrophy,\[^10\] thereby increasing muscle catabolism.\[^11-13\] Cytokines may antagonize the anabolic effects of Insulin-like growth factor 1 (IGF-1).\[^14-15\] In the case of sepsis, an extreme whole body inflammatory state, the synthesis of both myofibrillar and sarcoplasmic proteins are inhibited, with the inhibition taking place preferentially in fast-twitch muscle fibers.\[^14,16\] Sepsis is also able to prevent leucine from stimulating muscle protein synthesis.\[^17\] In animal models, when inflammation is created, mTOR loses its ability to be stimulated by muscle growth.\[^18\] Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory condition. However the long term administration of NSAIDs may induce gastro-intestinal ulcers, bleeding and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes.\[^19\] Many herbal plants are used in various medical systems for the treatment and management of various disease. Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care.
Many herbal plants are used in various medical systems for the treatment and management of various diseases. The plant *Helictress isora linn* traditionally used as an anti-inflammatory drug. There are number of active constituents present in plant such as saponins, phenolic compound, and tannin, gums and mucilage and carbohydrates, and absence of flavonoids, alkaloids. All above active constituents like flavonoids, phenolic compound, alkaloids has been used for the treatment of inflammation.\[20-21\]

**MATERIALS AND METHODS**

**Plant material**: Plant material was collected from Betul (M.P) India in between the last October-November. It was made completely clean and dust free. And Plant material was identified and authentified by Prof. P.Patil (Prof&HOD) Department of botany, Government M.L.B Girls Autonomous College Bhopal (M.P) and specimen voucher no.308. The leaves were dried under shade for 6-7 days. It was pulverized to coarse powder with the help of hand grinder. The coarse powder was packed in to airtight container and stored in cool and dry place.

**PLANT EXTRACT**

**Hydro alcoholic extract**: The powdered leaves (150g) was extracted with hydro-alcohol(70:30) solvent by maceration process for about 7 days. Solvent was concentrated under reduced pressure using rotatory evaporator and dried below 40\(^0\)c. the extract was brown in color and the percentage yield of the extract was calculated. Thus hydro-alcoholic extract of *Helictress isora linn.* was obtained.

**Aqueous extract**: The powdered leaves (150g) was macerated with distilled water for seven days. 2ml of ethanol was added to prevent the growth of microorganism in the extract. Solvent was concentrated under reduced pressure using rotatory evaporator and dried below 40\(^0\)c. the extract was brown in colour and the percentage yield of the extract was calculated. Thus aqueous extract of *Helictress isora linn.* was obtained.

**Phytochemical screening**: The preliminary phytochemical screening of hydro alcoholic extract and aqueous extract of leaves of *Helictress isora linn* was performed by the standard methods.\[22\]

**Animals**: Male albino Wister rats weighing 150-250g. were used in experiment. The experimental animals were maintained under standard laboratory condition in an animal
house. The whole research work were carried out under the regulation of CPCSEA. All animals were kept in animal house in SIRT-Pharmacy. The research work were performed in Pharmacological laboratory in SIRT-Pharmacy.

**Acute toxicity study:** The acute oral toxicity study of *Hellicitress isora linn*. Extracts were carried out according to OECD 425 guideline (Organization for Economic Co-operation and Development) which is based on a stepwise procedure with the use of a minimum number of animals per step. Wister albino rats were administered 2000mg/kg of hydro-alcoholic fraction of *Hellicitress isora linn* for the sign and symptoms of acute toxicity as well as they were examined for the effect of extract on general behavior for 48 hours and further up to 14 days for delayed toxicity.\(^{[23]}\)

**Anti-inflammatory Activities**

**Carrageenan induced paw edema in rat:** Carrageenan induced paw edema is a suitable experimental animals model for evaluating an anti-edematous effect edema develop following injection of index of acute inflammatory changes can be determined from differences in the paw volume measured immediately after carrageenan injection. Acute inflammation was produced by sub-planter administration of 0.1ml of 1% w/v carrageenan in normal saline in the right hand paw of the rats.\(^{[24]}\)

**Normal Group:** Treatment with (5ml/kg.p.o) + normal saline + mineral oil  
**Control Group:** Treatment with (5ml/kg.p.o) + control saline + carrageenan  
**Standard Group:** 10mg/kg i.p Indomethacin + carrageenan consider as [standard]  
**Test Group-I** Hydro-alcoholic extract + carrageenan  
**Test Group-II** Aqueous extract + carrageenan

**Histamine induced rat paw edema:** Histamine is known to cause edema and excitation of small diameter primary afferent neurons. Histamine is a biogenic amine and through its specific membrane receptors, H1, H2, H3, and H4, plays an important role in physiological and pathological processes such as gastric acid secretion, smooth muscle contraction, neurotransmission, immunomodulation, angiogenesis, and allergic disorders. Histamine affects the local inflammatory responses by activation of vasodilatation, vascular permeability, edema formation, polymorphonuclear leukocyte infiltration, and cytokine release. For induction of paw edema, each rat was subcutaneously injected with 100 μl
histamine (0.1%) in the ventral surface of the right hind paw using a 29-gauge injection needle.\textsuperscript{[25]}

**Normal Group:** Treatment with (5ml/kg.p.o) + normal saline+ mineral oil
**Control Group:** Treatment with (5ml/kg.p.o) + control saline+ histamine
**Standard Group:** 10mg/kg.i.p Indomethacin + histamine consider as [ standard]
**Test Group-I** Hydro-alcoholic extract + histamine
**Test Group-II** Aqueous extract + histamine.

**STATISTICAL ANALYSIS**
All values were expressed as mean ± standard error of mean (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett multiple comparison test p≤0.01 was considered as statistically significant.

**RESULTS**
**Preliminary phytochemical tests:** Preliminary phytochemical screening showed the presence of steroids, triterpenoids, flavanoides, reducing sugar, tannins and saponins in extracts of leaves of *Helictrss isora linn*.

**Acute toxicity study:** Acute oral toxicity study has been conducted as per OECD guideline 425. At higher dose 2000mg/kg p.o. their was no sign and symptoms of toxicity observed in hydro alcoholic and aqueous extracts.

**Carrageenan induced paw edema in rats:** In the present study control group showed increase the inflammation 1h to 5h. standard group and test group-II showed significant decrease the inflammation on 2h to last h of the study When compared with control group. Test group-I decrease significant paw edema when compared with control group.

**Histamine induced paw edema in rats:** In the present study control group increase the inflammation 1h to 5h compared with initial value. Standard and test group-II showed significant reduction in paw edema 1h to 5h when compared with control group. Test group-I showed significant reduction in paw edema from 2h to last h of the study.
Table-1: Effects of *Helictress isora linn.* leaves extracts on carrageenan induced paw edema in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Paw volume (ml)</th>
<th>% inhibition In paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour</td>
<td>2 hour</td>
</tr>
<tr>
<td>Control Group</td>
<td></td>
<td>0.21±0.020</td>
<td>0.41±0.018</td>
</tr>
<tr>
<td>Standard Group</td>
<td></td>
<td>0.17±0.012</td>
<td>0.19±0.014*</td>
</tr>
<tr>
<td>Test group I</td>
<td></td>
<td>0.17±0.008</td>
<td>0.22±0.010*</td>
</tr>
<tr>
<td>Test group II</td>
<td></td>
<td>0.19±0.017</td>
<td>0.31±0.009</td>
</tr>
</tbody>
</table>

No. of each animals in each group=6, Data expressed as mean ± One way ANOVA Followed by Dunuett multiple comparison test drug treated group compared with control group. P Value less than b. *P<0.05, **P<0.01, ***P<0.001. considered as significant.

Table-2: Effects of *Helictress isora linn.* leaves extracts on Histamine induced paw edema in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Paw volume (ml)</th>
<th>% inhibition In paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hour</td>
<td>2 hour</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td>0.35±0.020</td>
<td>0.31±0.014</td>
</tr>
<tr>
<td>Standard Group</td>
<td></td>
<td>0.10±0.015*</td>
<td>0.13±0.006*</td>
</tr>
<tr>
<td>Test group I</td>
<td></td>
<td>0.13±0.018*</td>
<td>0.18±0.014*</td>
</tr>
<tr>
<td>Test group II</td>
<td></td>
<td>0.21±0.012</td>
<td>0.24±0.012</td>
</tr>
</tbody>
</table>

No. of each animals in each group=6, Data expressed as mean ± One way ANOVA Followed by Dunuett multiple comparison test, drug treated group compared with control group. P Value less than b. *P<0.05, **P<0.01, ***P<0.001. considered as significant.

DISCUSSION

The purpose of the study was to evaluate anti-inflammatory properties of *Helictress isora linn* leaves extracts. The result of present study indicate that both extracts showed significant anti-inflammatory activity. Hydro alcoholic extract 200mg/kg showed highly potent anti-inflammatory activity. Currently available therapies include corticosteroids, NSAID’s and immunosuppressive drugs in case of severe patient. these agent are not fully effective and have significant risk of toxicity. Biochemical parameters indicate that there is no sign and symptoms of toxicity. *Helictress isora linn* is a proposed promising candidate for the treatment of chronic inflammatory disease of joints. Hydro alcoholic and aqueous extracts of *Helictress isora linn* has proved to possess with potential anti-inflammatory. The delayed
toxicity study needs to be performed. The inflammation is a complex process, which is frequently associated with pain and several events such as the increase of muscular permeability, increase of granulocytes and mono nuclear cells migration as well as the granulomatus tissue. Proliferation. Obtained result showed that *Helictress isora linn* has anti-inflammatory activity on acute inflammatory process like carrageenan induced paw edema in rat paws. It is well known that leukocytes migration to the injured tissue is an important aspects of the inflammatory process. Histamine is played important role in arthritis. Hydro alcoholic and aqueous extracts showed anti-inflammatory effect against histamine induced paw edema in rats.

CONCLUSION

Both extracts of *Helictress isora linn* leaves showed anti-inflammatory effects. However Hydro alcoholic extract 200mg/kg p.o. showed significant anti-inflammatory effects. The extract of *Helictress isora linn* showed significant effect in arthritic rats. Hydro alcoholic extract showed potent anti-inflammatory activity against carrageenan induced paw edema in rats. positive results of study indicate potent anti-inflammatory effect of hydro alcoholic extract of H. isora linn leaves . also its ability to overcome its complications by improvement of clinical signs in joint space evidenced by histopathology.

REFERENCES

6. Chandrasoma Parakrama, Taylor Clive R. "Part A. General Pathology, Section II. The Host Response to Injury, Chapter 3. The Acute Inflammatory Response, sub-


