Original article

**In vitro** assessment of anti-inflammatory and anti-arthritic effects of *Helicanthes elasticus* (Desv.) Danser accessions collected from six different hosts

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1. Introduction

Herbal medicine has been developed as a tradition in most of the developing countries and being the land of origin of Ayurveda, India encourages herbal therapeutic practices. Arthritic conditions can be treated using traditional medicines with considerable cure and relief without any side effects (Sheelarani et al., 2014). It is considered as an autoimmune disorder having symptoms like pain, swelling, and inflexibility of joints (Srikanth et al., 2012). Destruction of articular cartilage and synovial proliferation is the reason behind these symptoms. Inflammation is a bodily response to any injury caused by physical trauma, noxious chemicals or microbial agents, along with other diseases like rheumatism is now a major problem affecting the morbidity of working force all over the world (Aishwarya et al., 2017). According to Montecucco and Mach (2009) rheumatoid joint inflammation affects more or less 1% of the people around the world and its reason are still unknown. Rheumatoid arthritis suddenly progresses into an inflammation affecting multiple systems of the body with irreversible joint destruction leading to an increased risk of mortality (Wang et al., 2012). It involves the breakdown of cartilage at joints and as a result bones rub together causing pain, swelling and stiffness. Joints then packed with white blood cells which secretes substances like interleukins and tumor necrosis factor-alpha (TNF-alpha) that also results in pain, joint swelling and damage (Kaur et al., 2012). Lysosomal enzymes were produced during inflammation and extracellular activity of these enzymes makes the condition worse (Chayen and Bitensky, 1971). According to Rajendran and Lakshmi (2008), stabilization of the lysosomal membrane is one of the crucial factors in reducing the inflammatory responses. Being very much similar to the lysosomal membrane, stabilization of the human red blood cell membrane by hypotonicity induced membrane lysis can be considered as an in vitro measure of anti-inflammatory activity of drugs and plant extracts (Sreekumari et al., 2015).

In the present investigation, a hemiparasitic plant *Helicanthes elasticus* (Desv.) Danser procured from six different host plants were studied in vitro for anti-inflammatory and anti-arthritic responses along with the six host taxa. The work also aims to find out whether there exist any correlation between the host and the hemiparasite towards these pharmacological responses.

2. Materials and methods

2.1. Collection of plant materials

*Helicanthes elasticus* procured from six different hosts such as *Nerium oleander* (NO), *Hevea brasiliensis* (HB), *Citrus maxima* (CM), *Saraca asoca* (SA), *Anacardium occidentale* (AO) and *Murraya koenigii* (MK) and the following abbreviations HEN, HEH, HEC, HES, HEA and HEM were used respectively for the hemiparasite collected from these hosts.

2.2. Preparation of the plant extract

Powdered samples measuring 20 g each of hemiparasite and hosts were extracted separately in 200 ml methanol for 10 h in the soxhlet apparatus. After filtration, the extract was then concentrated using a rotary vacuum evaporator and the semi-dried extracts were kept in airtight bottles.
2.3. Anti-inflammatory studies by HRBC membrane stabilization method

HRBC membrane stabilization method (Gandhidasan et al., 1991) was used to assess the in vitro anti-inflammatory activity of both host and hemiparasite plant extracts. Fresh human blood (10 ml) was collected and transferred to the heparinized centrifuge tubes. It is then mixed with an equal volume of Alsever’s solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 ml) and centrifuged with 0.85% isosolane (Dissolved 8.5 g NaCl in water and autoclaved for 15 min at 121 °C and cooled to room temperature). To 1 ml of HRBC suspension, equal volumes of plant extracts in four different concentrations (10, 20, 50, 100 μg/mL) were added. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage of hemolysis was calculated using the formula given below:

\[
\text{Percent of hemolysis} = \frac{\text{OD of test solution}}{\text{OD of control}} \times 100
\]

Anti-inflammatory activity is percent of protection and is calculated using the formula:

\[
\text{Percent of protection} = 100 - \text{Hemolytic percentage}
\]

Anti-inflammatory activity = percent of protection and is calculated using the formula:

\[
\text{OD of test} = \text{Optical density of test sample’s absorbance.}
\]

\[
\text{OD of control} = \text{Optical density of the negative control}
\]

Negative control used = Alsever’s solution with blood

2.4. Anti-arthritic studies by protein denaturation method

In vitro anti-arthritic assay was conducted by protein denaturation method given by Mizushima and Kobayashi (1968). The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (PBS with pH 6.4), and 2 ml of varying concentrations (10, 20, 50, 100 μg/L) of plant extract. A similar volume of double distilled water is used as control. Then the mixture was incubated at 37 °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm by using the vehicle as blank.

The percentage of inhibition of protein denaturation was calculated by using the following formula:

\[
\text{Inhibition} = \frac{AC_{660} - AT_{660}}{AC_{660}} \times 100
\]

\[AC = \text{Absorbance of control solution, } AT = \text{Absorbance of the test sample.}\]

2.5. Statistical analysis

All the results were expressed in mean ± standard deviation through tables and graphs. The statistical significance in the results obtained from hemiparasite collected from the different hosts was analyzed by 2-way ANOVA followed by post hoc Tukey HSD. Pearson product-moment correlation was used to determine the relationship in the activities between hemiparasite and respective hosts.

3. Results

3.1. Anti-inflammatory studies- HRBC membrane stabilization method

HRBC membrane stabilization method to determine the anti-inflammatory activity showed that six samples of Helicantes elastica responded positively to the tests conducted. The sample hemiparasite collected from Muruya koenigii host (HEH) showed the least effect whereas all other samples provided positive results (Table 1). The highest percentage of protection of more than 60% was obtained in HEH, HEA and HEN at 100 μg/L (Fig. 1). When host plants were studied, SA has maximum protection at 100 μg/L followed by AO and MK (Table 2). The result obtained was very poor in Nerium oleander as compared to its parasite HEA and Hevea brasiliensis, the host of HEH also found to have less anti-inflammatory activity.

There is a statistically significant difference in the interaction effect of plant and concentration with that of percentage of protection in the anti-inflammatory assay conducted as given by 2-way ANOVA with p = 0.0001 (Table 3) except the samples HEH and HEA (p = 1), HEA and HEH (p = 0.929), HEA and HEH (p = 0.967). Correlation analysis proved that a very strong correlation existed between H. elastica and its respective hosts in anti-inflammatory responses except for HEH and its host HB, HEC and its host CM (Table 4).

3.2. Anti-arthritic study by protein denaturation method

Samples of Helicantes elastica collected from the selected hosts lack significant results in anti-arthritic assay using the protein denaturation method (Table 5). Even at a high concentration of 100 μg/L, only HEN crossed 50% of inhibition (51.31 ± 1.66). Increase in the absorbance of test samples with respect to control indicated the stabilization of protein. All the other samples exhibited less activity with the least value for HEA and HEM. Among the host samples tested, Saraca asoca manifested lower inhibition compared to others with a range of inhibition percentage between 50% – 60% (Table 6). Maximum inhibition was obtained in AO (57.45 ± 2.35) followed by HB (56.62 ± 1.77).

The samples of the hemiparasite showed somewhat similar pattern of inhibition and a close resemblance could be obtained between HES and HEA (Fig. 2). A statistically significant variance was observed in 2 way ANOVA between parasites collected from different hosts with F (48, 15) = 6.944, p = 0.0001 (Table 7) except between groups HEA and HEM (p = 0.648), HEA and HEC (p = 0.091), HEM and HEC (p = 0.841) and also in HES and HEN (p = 0.917). Inhibition mean value obtained in anti-arthritic studies of H. elastica and respective hosts showed statistically significant linear relationship as determined by Pearson product mean correlation except for HEA and AO (Table 8).

4. Discussion

Helicantes elastica of Loranthaceae effectively proved to have anti-inflammatory and anti-arthritic properties. Denaturation of proteins is one of the predominant reasons for inflammatory and arthritic diseases (Hossain et al., 2015). The decrease in the absorbance of a plant extract with an increase in concentration indicates the stabilization of HRBC membrane and albumin protein. Stabilization of the lysosomal membrane had a crucial role in reducing inflammatory responses and preventing the extracellular release of its contents and damage of tissues (Azeem et al., 2010). Therefore, it has to be generalized that the extract of Helicantes elastica collected from different hosts has the property to prevent the hypotonicity induced hemolysis of erythrocyte membrane that is similar to lysosomal membrane and thus prevents the efflux of intracellular components. These properties might have been imparted to the plant due to the various phytoconstituents produced within the plant. It was reported that phenolics and flavonoids have remarkable biological activities like anti-oxidant, anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory, cardiovascular protection as well as inhibition of angiogenesis and cell proliferation activities (Rice-Évans et al., 1996). According to Singh...
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Anti-inflammatory studies of six host plants under study.

| Concentration (µg/mL) | HEN | HHE | HEC | HES | HEA | HEM |
|-----------------------|-----|-----|-----|-----|-----|-----|
| 10                    | 44.05 ± 1.02 | 38.46 ± 1.08 | 19.23 ± 2.1 | 14.68 ± 0.75 | 37.76 ± 1.42 | 37.76 ± 1.42 |
| 20                    | 51.04 ± 0.59 | 49.47 ± 1.86 | 31.29 ± 1.65 | 27.09 ± 0.94 | 51.74 ± 1.48 | 51.74 ± 1.48 |
| 50                    | 57.34 ± 1.74 | 61.71 ± 1.06 | 45.45 ± 0.93 | 36.53 ± 1.58 | 62.76 ± 0.91 | 62.76 ± 0.91 |
| 100                   | 63.46 ± 1.23 | 66.6 ± 1.83 | 50.17 ± 0.62 | 47.37 ± 1.47 | 65.73 ± 2.01 | 65.73 ± 2.01 |

HEN: H. elastica (Desr.) Danser obtained from Nerium oleander L. (NO). HEC: H. elastica (Desr.) Danser obtained from Hevea brasiliensis (Willd.ex A.Juss., Mull.Arg. (HB)). HES: H. elastica (Desr.) Danser obtained from Citrus maxima (Burm., Merr. (CM)). HEA: H. elastica (Desr.) Danser obtained from Saraca asoca (Roxb.) Willd. (SA). HEA: H. elastica (Desr.) Danser obtained from Anacardium occidentale L. (AO). HEM: H. elastica (Desr.) Danser obtained from Murraya koenigii (L. Spring. (MK)).

and Sharma (2016), anti-arthritic activity could be attributed to constituents like phenols, tannins, and flavonoids. It was also reported that many flavonoids and related polyphenols are responsible to the antioxidant and anti-inflammatory activities of many plants (Govindappa et al., 2011). Hence, the presence of such bioactive principles in the extracts of Helianthus elastica could be expected and these compounds have membrane-stabilizing properties. Such compounds are well known in their ability to prevent the release of phospholipases that enhances the production of inflammatory mediators (Aitadafoun et al., 1996).

Helianthus elastica obtained from six different hosts had phenolics and flavonoids in its methanolic extract and hence it was mandatory for these samples to show a positive response towards anti-inflammatory and anti-arthritic assays. Reports of Hevea brasiliensis regarding this activity were obscure but according to Kittigowittana et al. (2013), the seed oil of rubber had anti-oxidant and non-cytotoxic property and the hemiparasitic plant Loranthus micranthus from H. brasiliensis was reported to have rich polyphenol content and anti-inflammatory properties (Agbo et al., 2014). Various biological activities such as anti-analgesic, anti-inflammatory, anti-diabetic, and anti-arthritic were attributed to Citrus maxima (Vijaylakshmi and Radha, 2015, Shivananda et al., 2013) as well as Nerium oleander (Nagourney et al., 2001). In vivo anti-inflammatory responses were shown by leaf and bark extracts of Saraca asoca (Satish et al., 2014) and Anacardium occidentale.

Table 2

| Concentration (µg/mL) | NO | HB | CM | SA | AO | MK |
|-----------------------|----|----|----|----|----|----|
| 10                    | 10.31 ± 1.56 | 12.93 ± 1.76 | 25.87 ± 1.34 | 45.97 ± 2.03 | 45.8 ± 1.78 | 23.07 ± 1.86 |
| 20                    | 13.81 ± 1.09 | 16.95 ± 1.35 | 52.62 ± 0.86 | 54.02 ± 1.78 | 58.39 ± 2.07 | 25.02 ± 1.86 |
| 50                    | 18.53 ± 1.47 | 21.15 ± 0.81 | 59.44 ± 0.74 | 63.81 ± 0.96 | 64.51 ± 1.83 | 36.18 ± 0.56 |
| 100                   | 24.82 ± 0.84 | 31.29 ± 1.06 | 63.98 ± 1.56 | 71.32 ± 0.45 | 66.95 ± 1.63 | 66.43 ± 0.98 |

HEN: H. elastica (Desr.) Danser obtained from Nerium oleander L. (NO). HEC: H. elastica (Desr.) Danser obtained from Hevea brasiliensis (Willd.ex A.Juss., Mull.Arg. (HB)). HES: H. elastica (Desr.) Danser obtained from Citrus maxima (Burm., Merr. (CM)). HEA: H. elastica (Desr.)Danser obtained from Saraca asoca (Roxb.) Willd. (SA). HEA: H. elastica (Desr.) Danser obtained from Anacardium occidentale L. (AO). HEM: H. elastica (Desr.) Danser obtained from Murraya koenigii (L. Spring. (MK)).

Table 3

Two-way ANOVA for Anti-inflammatory studies.

| Source                  | Type III Sum of Squares | df | Mean Square | F     | Sig |
|------------------------|-------------------------|----|-------------|-------|-----|
| Corrected Model        | 209553.506*             | 23 | 911.022     | 503.382 | 0.0001 |
| Intercept              | 124096.638              | 1  | 124096.638  | 6.857  | 0.0001 |
| Plant                  | 13588.753              | 5  | 2717.751    | 1.502  | 0.0001 |
| Concentration          | 6794.259               | 3  | 2264.753    | 1.151  | 0.0001 |
| Plant × Concentration  | 570.494                | 15 | 38.033      | 21.015 | 0.0001 |
| Error                  | 86.871                 | 48 | 1.81        |       |     |
| Total                  | 145137.015             | 72 |             |       |     |
| Corrected Total        | 21040.377              | 71 |             |       |     |

Dependent Variable: Percentage of inhibition. Superscript a: R Squared = 0.996 (Adjusted R Squared = 0.994) HEN: H. elastica (Desr.) Danser obtained from Nerium oleander L. (NO). HEC: H. elastica (Desr.) Danser obtained from Hevea brasiliensis (Willd.ex A.Juss., Mull.Arg. (HB)). HES: H. elastica (Desr.) Danser obtained from Citrus maxima (Burm., Merr. (CM)). HEA: H. elastica (Desr.) Danser obtained from Saraca asoca (Roxb.) Willd. (SA). HEA: H. elastica (Desr.) Danser obtained from Anacardium occidentale L. (AO). HEM: H. elastica (Desr.) Danser obtained from Murraya koenigii (L. Spring. (MK)).
Danser obtained from *Helicanthes elasticus* (Desr.) Danser obtained from *Cuscuta australis* (L.) Spring. 

**Table 4** Pearson correlation effect between *H. elasticus* and respective hosts in Anti-inflammatory studies.

| Parasite | HEN | HEH | HEC | HES | HEA | HEM |
|----------|-----|-----|-----|-----|-----|-----|
| Pearson correlation(r) | 0.987* | 0.917 | 0.944 | 0.996* | 0.994* | 0.985* |
| Sig.(2 tiled)(P) | 0.013 | 0.083 | 0.056 | 0.004 | 0.006 | 0.015 |
| N | 4 | 4 | 4 | 4 | 4 | 4 |
| Host | NO | HB | CM | SA | AO | MK |

**Table 5** Anti-arthritic studies of *H. elasticus* collected from six different hosts.

| Concentration (µg/mL) | HEN | HEH | HEC | HES | HEA | HEM |
|-----------------------|-----|-----|-----|-----|-----|-----|
| 10 | 17.39 ± 0.86 | 11.98 ± 0.75 | 5.28 ± 0.73 | 18.04 ± 1.44 | 5.63 ± 0.56 | 5.32 ± 1.64 |
| 20 | 29.19 ± 1.52 | 20.7 ± 0.56 | 9.42 ± 0.98 | 28.81 ± 1.27 | 7.91 ± 0.69 | 10.8 ± 0.57 |
| 50 | 37.58 ± 2.05 | 24.82 ± 1.55 | 18.73 ± 0.84 | 38.83 ± 0.53 | 12.46 ± 0.82 | 16.91 ± 1.84 |
| 100 | 51.31 ± 1.66 | 31.36 ± 1.68 | 30.12 ± 1.06 | 45.88 ± 0.87 | 21.56 ± 1.7 | 20.53 ± 2.22 |

**Table 6** Anti-arthritic studies of six host plants under study.

| Concentration (µg/mL) | NO | HB | CM | SA | AO | MK |
|-----------------------|----|----|----|----|----|----|
| 10 | 28.69 ± 0.59 | 18.11 ± 0.89 | 18.32 ± 0.83 | 8.67 ± 1.78 | 27.81 ± 1.81 | 17.7 ± 1.75 |
| 20 | 41.36 ± 1.46 | 34.88 ± 1.89 | 34.88 ± 0.41 | 20.7 ± 0.69 | 44.04 ± 2.67 | 29.91 ± 2.07 |
| 50 | 49.15 ± 1.78 | 48.96 ± 1.24 | 43.39 ± 2.36 | 28.94 ± 0.83 | 52.58 ± 2.05 | 38.31 ± 0.98 |
| 100 | 54.12 ± 0.83 | 56.62 ± 1.77 | 53.16 ± 1.86 | 38.47 ± 1.08 | 57.45 ± 2.33 | 50.09 ± 1.11 |

(Pawar et al., 2000; Thomas et al., 2015) against induced paw edema in rats. Dried leaves and fruits of *Murraya koenigii* were found to have strong anti-arthritic and anti-inflammatory responses as reported by Pitchaiah et al. (2016) and Mathur et al. (2011).

Among the six samples of the hemiparasite, strong anti-inflammatory activity was shown by HEN, HEH, and HEA whereas the parasite and in both cases chemical signaling has an important role. It could be assumed that the production of such chemical sig-

ings might occur from both partners and transmit in both directions and this production and transmission were determined by the nature of hosts. Sometimes the production of such metabolites gets altered in partners as reported by Li et al. (2015) in the case of *Cuscuta australis* infection on *Bidens pilosa* in which total terpenoids in younger hosts found to be decreased due to infection but the same found significantly increased in older plants. Here, both HEM and HEA were found to have very low anti-arthritic activity whereas HEM alone was found to be least active in anti-inflammatory tests and this signifies the host’s effect on certain biological activity of the hemiparasite growing on it. The host plant often trying to inhibit the parasite by its anatomical or...
biochemical pathways as a means of defense and the parasite might produce more phenolics and other such compounds that get transported into the hosts. Thus the quantity of such secondary metabolites was less in the parasitic extract and thereby limiting its activity. The other reason might be that, the host plants might have an inhibitory role in the production of such metabolites within the parasite to check or limit the vast invasion strategy of the parasite over many hosts. The same reason could be attributed to Anacardium occidentale, the host of HEA. In other samples, the host and hemiparasite might have established a coexistence strategy and thus transmission of such therapeutically significant metabolites occurred in both directions or hosts being less efficient to eradicate the parasite and hence their suppressive strategy found less effective on hemiparasites to check its secondary metabolites. The influence of host’s chemistry on the phytochemical constituents of the hemi-parasites and holo-parasites growing on them might justify the importance of both host and parasite for their therapeutic and hence, the use of Loranthaceae members for such purposes often depended on a particular or specific host (Snyder et al., 1996; Preston et al., 2010). Such host specific procuring of hemiparastic plant Dendrophthoe falcata for treating various diseases is prevalent in India (Kulkarni and Kumbhojkar, 2002). Similarly, Cladocolea micrantha obtained from the host tree Anacardium occidentale is extensively used for the treatment of tumors and inflammatory diseases (Olapade, 2002; Guimarães et al., 2007).

5. Conclusion

Anti-inflammatory responses were found more in HEN, HEH and HEA but HEN was the only plant which showed more anti-arthritis efficacy. All the six hosts under study were effective against these malfunctions of the body. The same hemiparastic plant Helicanthes elaticus collected from different hosts showed difference in their potentiality against these diseases due to the influence of hosts. Phytochemical molecules like phenolics and flavonoids had crucial role in the anti-inflammatory and anti-arthritic properties of both hemiparasite and its hosts. It could be said that host relationship is a crucial factor in determining the metabolic activities of parasitic plants and there by the production of secondary metabolites which in turn established their pharmacological potential.

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