In-Vitro Antileishmanial Activity of Raphanus sativus L. var. caudatus

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Abstract: Leishmaniasis is a major public health problem causing significant morbidity and mortality around the world, specially Asia, Africa and Latin America. The present preliminary in-vitro study reports antileishmanial potential of Raphanus sativus L. var. caudatus. Various concentrations of ethanolic extract of plant were used. The tested extract showed noteworthy inhibition (concentration – dependant) of Leishmania promastigotes. The IC₅₀ of extract was found to be 39 μg/ml. The results of present study could be helpful in future for antileishmanial therapy.

Keywords: Leishmaniasis, Raphanus sativus L. var. caudatus, ethanolic extract.

INTRODUCTION

Leishmaniasis are a complex of diseases caused by at least 17 different species of protozoan parasites belonging to the genus Leishmania [1]. Leishmaniasis is considered as a major public health problem (WHO, 1989, 1990) causing significant morbidity and mortality around the world, specially Asia, Africa and Latin America.

Among the drugs, antimonial agents are still used as the first choice for leishmaniasis therapy. It is now well known that these drugs are not only toxic and costly but also require long-term use during therapy. In addition, emerging resistance to these pentavalent antimonial drugs pose significant hindrance in the eradication of Leshmaniasis [2, 3]. Thus it is necessary to explore new agents for the treatment of Leishmaniasis.

Raphanus sativus Var. caudatus are commonly known as rat tailed radish. These are actually aerial parts (Pods) of radish and belong to the family Brassicaceae. These are known as Mungraa or Sungraa in Pakistan & India [4].

Radish has been reported to possess antimicrobial [5], anti-fungal [6], anti-inflammatory [7] antiurolithiatic [8] and antioxidant properties [9]. Different parts of Raphanus have been reported for variety of therapeutic uses [10]. Among plants, Brassicaceae have been reported for remarkable antimicrobial potential [11, 12]. However radish has not been tested against leishmaniasis so far. The present in-vitro investigation is an attempt to evaluate antileishmanial activity of Raphanus sativus L. var. caudatus.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

Fresh pods of Raphanus sativus L. were purchased from Karachi, Pakistan, identified from herbarium, University of Karachi, Pakistan and washed with double distilled water. The aerial parts of plants were then dried in air under shade at room temperature (25°C) and stored in tightly well closed labeled containers.

Later on, dried pods were powdered with the help of mechanical grinder and subjected to extraction with ethanol (98% w/v) using soxhlet apparatus [13]. After filtration the extract was dried using Rotary evaporator at 45°C to obtain semi- solid extract. Percentage yield of extract was found as 12.56% and stored in the refrigerator at a temperature of 4°C.

In-Vitro Leishmanicidal Assay

Leishmania promastigotes (DESTO. Pakistan) were grown in bulk early in modified Novy-MacNeal-Nicolle medium (NNN) biphasic medium using normal physiological saline. Leishmania parasite promastigotes were cultured with Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, St. Louis, USA) supplemented with 10% heat inactivated fetal Calf serum (PAA Laboratories GmbH, Austria). Parasites at log phase were centrifuged at 2000 rpm for 10 minutes, and washed three times with saline at same speed and time. Parasites were diluted with fresh culture medium to a final density of 1x 10⁶ cells/ml.

In a 96-well micro titer plate, 180μl medium was poured in different wells; 20μl of ethanolic extract of
Raphanus caudatus L. was added in medium and serially diluted. 100μl of parasite culture was added in all wells. Two rows were left for "control" (Negative control) and reference drug (Positive control). "Control" consists of medium only whereas "reference" consists of varying concentrations of standard antileishmanial drug Pantamidine (ICN Biomedical Inc). The plate was incubated at a temperature range of 22-25 °C for 72 hrs [14, 15].

The culture was examined microscopically on an improved Neubauer counting chamber for cell viability by counting the number of motile cells and IC\textsubscript{50} (50% Inhibitory Concentration) values were calculated by Software Ezfit 5.03 Perrella Scientific. All assays were carried out in triplicate and their mean and standard deviation were calculated by using Microsoft excel sheet.

Percentage inhibition of Leishmania promastigotes were calculated by using following formula:

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\text{Percentage inhibition} = 100 - \left( \frac{\text{Total no. of cells calculated in test}}{\text{Total number of cells calculated in control}} \right) \times 100
\]

### Statistical Analysis

Data were expressed as Mean (IC\textsubscript{50}) ± standard deviation (SD). Cut off values were considered significant as \( p < 0.05 \).

### RESULTS

In the present investigation, in-vitro antileishmanial potential of Raphanus caudatus was evaluated. Different concentrations (25 – 200 μg/ml) were used for this purpose. Ethanolic extract of Raphanus caudatus L. showed a concentration-dependent inhibition of Leishmania promastigotes, as indicated in Figure 1. Both Raphanus caudatus extract as well as Pentamidine showed significant \((p<0.05)\) potential against Leishmania when compared with control. The IC\textsubscript{50} of extract was found to be 39 μg/ml whereas Pentamidine had an IC\textsubscript{50} of 3.13 μg/ml.

### DISCUSSION

Plants are extensively and successfully used for the prevention and treatment of different type of diseases. At present, there has been great enthusiasm and strides are made for development of therapeutic agents from plants [16].

Radishes have long been grown as a food crop and are of high medicinal value. Raphanus sativus root, seed, leaves and pods have been reported to possess strong antibacterial and antifungal potential [17-19]. Radish seed oil in the folk medicine has been used for its antimicrobial properties. This led us to determine its anti-leishmanial potential.

In the present study, Raphanus caudatus displayed significant potential against Leishmania promastigotes. Isothiocyanate components and raphanin have been reported in Raphanus caudatus [20] and anti-leishmanial activity in the present investigation could be linked to the occurrence of these compounds.

Moreover, among flavonoids, Quercetin is commonly occurring and widely distributed in Brassicacea plant including Raphanus [21].

In another study, different naturally occurring flavonoids were investigated for their antileishmanial activity. Radishes have been reported as rich source of different type of flavonoids like quercetin, kaempferol and luteolin [22]. It was found that luteolin and quercetin blocked the growth of Leishmania donovani promastigotes. In addition the researchers found that these two naturally occurring flavonoids targets the DNA through topoisomerase-II [23].

Thus antileishmanial activity of Raphanus caudatus in the present study could be attributed to the occurrence of flavonoids or related compounds.

### CONCLUSION

The results of present preliminary in-vitro investigation indicate that ethanolic extract of Raphanus caudatus may be used to exhibit an adjuvant treatment for the control of Leishmaniasis. Additionally, it is suggested further to isolate and investigate naturally occurring flavonoids and isothiocyanate components in Raphanus caudatus against Leishmaniasis.