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 IC_{50} 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 Lieshmaniasis [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 filteration 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 10^6 cells/ml.

In a 96-well micro titer plate, 180µl medium was poured in different wells; 20µl of ethanolic extract of

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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_{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:

$$\text{Percentage inhibition} = 100 - \frac{\text{Total no. of cells calculated in test}}{\text{Total number of cells calculated in control}} \times 100$$

Statistical Analysis

Data were expressed as Mean (IC_{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_{50} of extract was found to be 39 µg/ml whereas Pentamidine had an IC_{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.

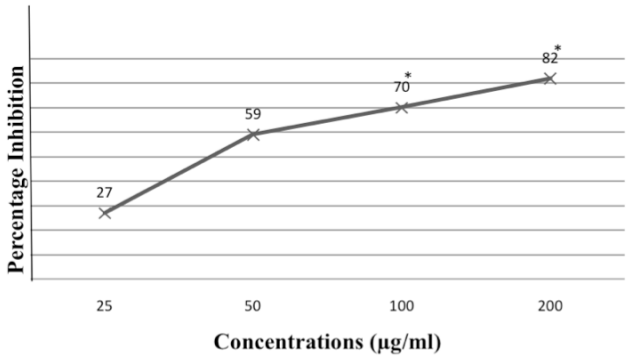

Figure 1: Antileishmanial activity of ethanolic extract of *Raphanus sativus* L. *var. caudatus* on Leishmania promastigotes. * $p < 0.05$ significant as compared to control.

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 isothiocyante components in *Raphanus caudatus* against Leishmaniasis.

REFERENCES

[1] Gazanion E, Vergnes B, Seveno M, Garcia D, Oury B, Ait-Oudhia K, *et al*. *In vitro* activity of nicotinamide/ antileishmanial drug combinations. Parasitol Int 2011; 60(1): 19-24. http://dx.doi.org/10.1016/j.parint.2010.09.005

[2] Berman J. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin Infect Dis 1997; 24(4): 684-703. http://dx.doi.org/10.1093/clind/24.4.684

[3] Olliaro P, Bryceson A. Practical progress and new drugs for changing patterns of leishmaniasis. Parasitology today 1993; 9(9): 323-8. http://dx.doi.org/10.1016/0169-4758(93)90231-4

[4] Khare C. Indian Medicinal Plants: an illustrated dictionary Library of congress Control Number: 2007922446 ISBN: 978-0-387-70637-5 Springer-Verlag Berlin. Heidelberg, Springer science+ Business Media, LLC; 2007.

[5] Abdou I, Abou-Zeid A, El-Sherbeeny M, Abou-El-Gheat Z. Antimicrobial activities of Allium sativum, Allium cepa, Raphanus sativus, Capsicum frutescens, Eruca sativa, Allium kurrat on bacteria. Qualitas Plantarum et Materiae Vegetabiles 1972; 22(1): 29-35. http://dx.doi.org/10.1007/BF01099735

[6] Terras FR, Goderis IJ, Van Leuven F, Vanderleyden J, Cammue BP, Broekaert WF. *In vitro* antifungal activity of a radish (Raphanus sativus L.) seed protein homologous to nonspecific lipid transfer proteins. Plant physiology 1992; 100(2): 1055-8. http://dx.doi.org/10.1104/pp.100.2.1055

[7] Kim KH, Moon E, Kim SY, Choi SU, Lee JH, Lee KR. 4-Methylthio-butanyl derivatives from the seeds of Raphanus sativus and their biological evaluation on anti-inflammatory and antitumor activities. J Ethnopharmacol 2014; 151(1): 503-8. http://dx.doi.org/10.1016/j.jep.2013.11.003

[8] Vargas S R, Perez G R, Perez G S, Zavala S M, Perez G C. Antiurolithiatic activity of Raphanus sativus aqueous extract on rats. J Ethnopharmacol 1999; 68(1-3): 335-8. http://dx.doi.org/10.1016/S0378-8741(99)00105-1

[9] Takaya Y, Kondo Y, Furukawa T, Niwa M. Antioxidant constituents of radish sprout (kaiware-daikon), Raphanus sativus L. J Agric Food Chem 2003; 51(27): 8061-6. http://dx.doi.org/10.1021/jf0346206

[10] Gutiérrez RMP, Perez RL. Raphanus sativus (Radish): their chemistry and biology. The Scientific World Journal 2004; 4: 811-37. http://dx.doi.org/10.1100/tsw.2004.131

[11] Duke J, Ayensu E. Medicinal Plants of China. Medicinal Plants of the World. Vol. 1. Algonac, MI. reference publications, Inc; 1985.

[12] Bown D. The Royal Horticultural Society encyclopedia of herbs & their uses: Dorling Kindersley Limited; 1995.

[13] Davey MR, Anthony P. Plant cell culture: essential methods: John Wiley & Sons; 2010. http://dx.doi.org/10.1002/9780470686522

[14] Habtemariam S. *In vitro* antileishmanial effects of antibacterial diterpenes from two Ethiopian Premna species: P. schimperi and P. oligotricha. BMC Pharmacol 2003; 3(1): 6. http://dx.doi.org/10.1186/1471-2210-3-6

[15] Choudhary MI, Yousaf S, Ahmed S, Yasmeen K. Antileishmanial physalins from Physalis minima. Chemistry & biodiversity 2005; 2(9): 1164-73. http://dx.doi.org/10.1002/cbdv.200590086

[16] Rates SMK. Plants as source of drugs. Toxicon 2001; 39(5): 603-13. http://dx.doi.org/10.1016/S0041-0101(00)00154-9

[17] Shukla S, Chatterji S, Yadav DK, Watal G. Antimicrobial efficacy of Raphanus sativus root juice. Int J Pharm Pharm Sci 2011; 3: 89-92.

[18] Rani I, Akhund S, Abro H. Antimicrobial potential of seed extract of Raphanus sativus. Pakistan Journal of Botany 2008; 40(4): 1793-8.

[19] Terras F, Schoofs H, De Bolle M, Van Leuven F, Rees SB, Vanderleyden J, *et al*. Analysis of two novel classes of plant antifungal proteins from radish (Raphanus sativus L.) seeds. J Biol Chem 1992; 267(22): 15301-9.

[20] Beevi SS, Mangamoori LN, Dhand V, Ramakrishna DS. Isothiocyanate profile and selective antibacterial activity of root, stem, and leaf extracts derived from Raphanus sativus L. Foodborne Pathog Dis 2009; 6(1): 129-36. http://dx.doi.org/10.1089/fpd.2008.0166

[21] Umamaheswari M, Ajith MP, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P, *et al*. *In vitro* angiotensin converting enzyme inhibitory and antioxidant activities of seed extract of Raphanus sativus Linn. Central European Journal of Experimental Biology 2012; 1: 11-7.

[22] Lugast A, Hovari J. Flavonoid aglycons in foods of plant origin I. Vegetables. Acta Alimentaria 2000; 29(4): 345-52. http://dx.doi.org/10.1556/AAlim.29.2000.4.4

[23] Mittra B, Saha A, Chowdhury AR, Pal C, Mandal S, Mukhopadhyay S, *et al*. Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. Mol Med 2000; 6(6): 527.

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