DETERMINATION OF ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF OCIMUM SANTUM LEAVES

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Abstract:
Ocimum sanctum leaves were used to check anthelmintic activity of ethanolic extract of ocimun sanctum. The paralysis time and death time was checked by this study. The anthelmintic activity of leaves was checked by preparing its ethanolic extract in a concentration of 20mg/ml, 40mg/ml, and 60mg/ml. The standard drug Albendazole was used to check the anthelmintic activity of ethanolic extract. The observations and results suggest that ethanolic extract of ocimun sanctum leaves found to possess concentration dependent anthelmintic activity on worms (shows better activity with increasing concentration of extract). The ethanolic extract 20 mg/ml shows significant activity as compare to the standard drug. The study involves the determination of paralysis time and death time of the worms in different doses of the methanolic extracts.

Helminthiasis also known as helminth infection or intestinal worm infection in which humans beings or other animal were infected with parasitic worms known as helminths. The parasitic worms were classified as tapeworms, flukes, and roundworms.

Tulsi is an important symbol of the Hindu religious tradition. Although the word ‘Tulsi’ gives the connotation of the incomparable one, its other name, Vishnupriya means the one that pleases Lord Vishnu. Found in most of the Indian homes and worshipped, its legend has permeated Indian ethos down the ages. Known in English as Holy Basil and botanically called Ocimum sanctum, Tulsi belongs to plant family Lamiaceae.

Keywords: ocimun sanctum leaves, albendazole, time for paralysis, time for death, anthelmintic activity.

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Please cite this article in press Dasrao A Patil et al., Determination of Anthelmintic Activity of Ethanol Extract of Ocimum Santum Leaves., Indo Am. J. P. Sci, 2018; 05(09).
INTRODUCTION:
Helminthiasis also known as helminth infection or intestinal worm infection in which humans beings or other animal were infected with parasitic worms known as helminths. The parasitic worms were classified as tapeworms, flukes, and roundworms. Typically, the worms reside in the gastrointestinal tract of their host but may also burrow into the liver and other organs. Infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation. [1] Some other people may be infected by ingesting eggs or larvae in contaminated food or through penetration of the skin by infective larvae in the soil (hookworms). Helminthiasis spread worldwide and is one of the common diseases of all ages. The parasitic diseases cause severe morbidity by affecting population in endemic areas with major economic and social consequences. [2] Helminth infections are among the most common infections in man, affecting a large proportion of the world’s population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilias, and pneumonia. The most severe cause of parasitic diseases is morbidity including filariasis, onchocerciasis (river blindness), and schistosomiasis. [3] Although the majority of the infections due to worms were generally limited to tropical regions, they can occur to travellers who have visited those areas. [4] As per WHO only synthetic drugs are frequently used for treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity by use of herbal drug

Tulsi is an important symbol of the Hindu religious tradition. Although the word “Tulsi” gives the connotation of the incomparable one, its other name, Vishnupriya means the one that pleases Lord Vishnu. Found in most of the Indian homes and worshipped, its legend has permeated Indian ethos down the ages. Known in English as Holy Basil and botanically called Ocimum sanctum, Tulsi belongs to plant family Lamiaceae. It has made important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal properties. Tulsi has been described as of two types—vanya (wild) and gramya (grown in homes). Although having identical usage, the former has darker leaves. Tulsi is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, catarhal fever, otalgia, lumbago, hiccough, ophthalmic, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders [5-6]. It has also aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, alexiteric, vermifuge and febrifuge properties. In view of these facts, an attempt has been made to review on the various pharmacological activities of OS based on the experimental and clinical studies reported in different literatures. Tulsi grows wild in the tropics and warm regions. The plant is distributed and cultivated throughout India. It is an erect, much branched, fragrant and erected plant attaining a height of about 30-60 cm when mature. Its aromatic leaves are simple, opposite, elliptic, oblong, obtuse or acute with entire or sub serrate or dentate margins, growing up to 5 cm long. The Tulsi flowers are small, purplish in elongate racemes in close whorls. The fruits are small and the seeds are reddish-yellow in color. The plant is bitter and acrid. [6-7]

MATERIALS AND METHODS:
Materials
The drug Albendazole was gift sample from Micro Labs Pvt. Ltd. (Goa, India). All other chemicals petroleum ether, methanol, distilled water etc. were used of analytical grade.

Collection of Earthworms
The earthworms of lengths (6-12cm) were obtained from the damp, cool, and covered area of the sangola Solapur (Maharashtra, India). The worms were transferred into a glass bottle with some quantity of the soil from which they were taken. The worms were identified and authenticated by approved zoologist.

Collection of Plant material
Ocimum sanctum plant red leaves was collected from near college Campus area sangola, Maharashtra in the month of April and authenticated by botanist sangola Science College (sangola, Maharashtra). After authentication the fresh leaves were collected cleaned and shade dried.

Preparation of Extract
Ocimum sanctum plant leaves were pulverized by mechanical grinder and passed through a 20# mesh sieve. The powdered leaves (500gm) were extracted successively with and ethanol extract by using a soxhlet apparatus and water extracted by a cold maceration. The extracts were filtered through a cotton plug, followed by whatmann filter paper (no.1). The extracts were evaporated under reduced pressure using a rotovac evaporator at a low temperature at 40oC - 50oC until all the solvent get removed to give extract sample. Then weights of each residue were noted.[8]
Anthelmintic Activity

Test samples of extracts were prepared at the concentrations, 20, 40 and 60 mg/ml in 25 ml of distilled water containing 2% Tween 80. Six earthworms of approximately same size were placed in petri-dish [diameter 9cm each] containing above solution of extracts. Albendazole [20 mg/ml] was used as standard drug and distilled water containing 2% Tween 80 was used as control. Anthelmintic activity of Albendazole mediates through hyperpolarization that leads to muscle relaxation and flaccid paralysis [9]. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was noted when the earthworms neither moved when shaken vigorously or when dipped in warm water [50oC]. [10,11]

RESULTS AND DISCUSSION:

The ultimate aim of present research work is to evaluate anthelmintic activity of ethanolic extract ocimum leaves against Indian earthworm Phertima posthma. In this study the paralysis time and death time of the earthworms in different doses of the extracts (20mg/ml, 40mg/ml, and 60 mg/ml) were determined. Albendazole drug at concentration (20 mg/ml) were used as standard/reference drug to compare anthelmintic activity of the ethanolic extract ocimum sanctum leaves against Indian earthworm Phertima posthma. The result suggests that ethanolic extract of ocimum sanctum leaves possess concentration dependent anthelmintic activity.

Table.1. Anthelmintic Activity of Ocimum Sanctum Ethanolic Extract Leaves

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Conc.mg/ml</th>
<th>Time for paralysis min</th>
<th>Time for death min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (normal saline)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Albendazole 20mg/ml</td>
<td>16</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract 20 mg/ml</td>
<td>40</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic extract 40mg/ml</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolic extract 60mg/ml</td>
<td>30</td>
<td>38</td>
</tr>
</tbody>
</table>

Fig 1: Ocimum sanctum leaves
Graph no. 1: Anthelmintic Activity of *Ocimum Sanctum* Ethanolic Extract Leaves

- **Fig 2**: Standard: Albendazole
- **Fig 3**: EEOS - 20 mg/ml
- **Fig 4**: EEOS - 40 mg/ml
- **Fig 5**: EEOS - 60 mg/ml
CONCLUSION:
The Anthelmintic activity of *ocimum sanctum* leaves (extract) shows better activity on helminth causing worms with increasing concentration of the extract. From this research work, it found that ethanolic extract of *ocimum sanctum* leaves were show concentration dependent anthelmintic activity. Further studies need to isolate and reveal the active compound in the crude extracts of *ocimum sanctum* and establish the mechanism of action are required.

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