In vitro anthelmintic activity of fresh juice and ethanolic extract of

Garcinia cambogia (Clusiaceae)

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ABSTRACT

The crude ethanolic extract and fresh juice from the fruits of Garcinia cambogia, were evaluated for their anthelmintic activity using adult earth worms. Five concentrations viz 5, 10, 15, 20, 25 mg/ml of ethanolic extract and 10, 20, 30, 40 and 50 μg/ml of fresh juice of G. cambogia were studied for anthelmintic activity, which involved the determination of colour change, time of paralysis and time of death of worms. Both the ethanolic extract and fresh juice exhibited a dose-dependent inhibition of spontaneous mobility (paralysis) and evoked response to pin-prick and death with higher doses (25mg/ml of alcoholic extract and 50μl of fresh juice of G.cambogia), Piperazine citrate in 15mg/ml concentration was included as standard reference and normal saline as control. The results shows that both the extract and fresh juice posses anthelmintic activity.

Key Words: Garcinia cambogia, Ethanolic extract, Fresh juice, Paralysis, Time of death, Anthelmintic activity.

INTRODUCTION

Garcinia cambogia is a small or medium sized tree with rounded crown and horizontal branches is well known as Malabar tamarinds and brindle berry. It is used as condiment in south East Asia cuisine and used to preserve fish [1-4].

Traditionally it has been used for the treatment of edema, delayed menstruation, constipation, ulcers, hemorrhoids, diarrhoea, dysentery, fever, open sores, intestinal parasites, anti-microbial agent, anti-fungal, and as an anti-cancerous [4-8]. There is no report on anthelmintic study of this plant till date.

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Based on this, an attempt has been made to evaluate the anthelmintic effect of fresh juice and crude ethanolic extract G. cambogia.

MATERIALS AND METHODS

Collection of Plant Materials
G. cambogia was collected from Pallor district, Mahe of Kerala, India in the month of March 2009. This plant material was identified and authenticated by Dept. of Botany, Pondicherry University, Puducherry, India. The voucher specimen of the plant was deposited in the Dept. of Pharmacognosy, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry, India.

Around 2kg of fruits was collected and washed under running tap water, dried and were cut into small pieces. These fruits were then sun dried for 30 days and homogenized to get a coarse powder. This powder was stored in an air tight container and used for further extraction process.

Preparation of Fresh Juice
The freshly collected fruits were cleaned with Distilled water and juice was extracted by Juicer. The fruits were filtered through muslin cloth and solutions were prepared as 10,20,30,40 and 50 µg/ml.

Preparation of Crude Ethanolic Extract
Crude ethanolic extract was done by using 70% alcohol as solvent in soxhlet apparatus. The reflux time was 40 cycles. The extracts were cooled at room temperature and filtered. The filtrate was dried under reduced pressure in rotary evaporator to get a semi-solid form and weighed [9]. The solutions were prepared as 5, 10, 15, 20, 25 mg/ml.

Drugs
Piperazine citrate and other chemicals used were of analytical grade and purchased from local suppliers.

Animals
Indian earth worms were collected from the water logged areas from the Ossudu Lake in Puducherry, India. Indian adult earth worms (Pheretima Posthuma) of 8 ± 1 cm in length and 0.1 - 0.2cm in width were taken for the experiment. The earth worm resembles both anatomically and physiologically to the intestinal round warm parasites of human beings, hence can be used to study the anthelmintic activity [10, 11].

Anthelmintic Activity of Garcinia Cambogia
Selected worms were divided into groups and treated as shown in the Table 1 & 2. Piperazine Citrate 15 mg/ml given as standard drug. Observations were made for the time taken to paralysis, and death of individual worms. Time for paralysis were noted, when no movement of any sort could be observed except when the worms were shaken vigorously; Death was included, when the worms lost their mobility followed with fading away of their body color [10, 12-14].

Statistical analysis:
The data obtained were expressed as mean ± SEM (n=6). Statistical analysis were performed by one way analysis of variance (ANOVA) followed by student ‘t’ test. At 95% confidence interval,
* P< 0.001, **P<0.02, ***P<0.5 values were considered significant. The results were tabulated in Table 1 & 2 [15].

RESULTS AND DISCUSSION

The results are summarized in Tables 1 and 2. The standard drug piperazine citrate acts by blocking the response of worm muscle to acetylcholine, probably by causing hyperpolarization (increasing chloride ion conductance on worm muscle) of nerve endings, resulting in flaccid paralysis of the worm. Flaccid paralysis leads to expulsion of worm from human by peristalsis [16, 17].

Fresh juice of G. cambogia (Table 1) is more potent than the standard drug (Piperazine Citrate, 15mg/ml). It showed a short time of paralysis and death at the concentration of 40µl/ml and 50µl/ml of fresh juice. It showed significant activity (P<0.001) at the concentration of 10µl/ml, 40µl/ml and 50 µl/ml.

**TABLE: 1 Anthelmintic activity of Fresh Juice of Garcinia cambogia (Clusiaceae)**

<table>
<thead>
<tr>
<th>Group/ Treatment</th>
<th>Concentration</th>
<th>Time Taken for Paralysis (Min)</th>
<th>Time Taken for Death (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal Saline)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>15 mg/ml</td>
<td>19 ± 1.304</td>
<td>2.5 ± 1.576</td>
</tr>
<tr>
<td></td>
<td>10 µg/ml</td>
<td>38.33 ± 0.5815 *</td>
<td>40.41 ± 0.2811 *</td>
</tr>
<tr>
<td></td>
<td>20 µg/ml</td>
<td>20.53 ± 1.4023 ***</td>
<td>28.2 ±0.3486</td>
</tr>
<tr>
<td></td>
<td>30 µg/ml</td>
<td>20 ± 1.301 ***</td>
<td>27.23 ± 0.2582 ***</td>
</tr>
<tr>
<td></td>
<td>40 µg/ml</td>
<td>10 ± 1.1204 *</td>
<td>23.52 ±0.1687 ***</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>9.27 ± 1.0385 *</td>
<td>20.39 ±0.1895 **</td>
</tr>
</tbody>
</table>

n=6 for all the Groups.; Values are recorded as Mean ± SEM.; * P< 0.001, **P<0.02, ***P<0.5 as compared to standard.

From the observation made, the ethanolic extract of G. cambogia (Table 2) showed anthelmintic activity in dose-dependent manner, giving short time of paralysis and death at the concentration of 25mg/ml. The anthelmintic activity of ethanolic extract of G. cambogia increased significantly (P<0.001), showing decrease in time of paralysis and death with increase in concentration of extract.

**TABLE: 2 Anthelmintic activity of Etanolic extract of Garcinia cambogia (Clusiaceae)**

<table>
<thead>
<tr>
<th>Group/ Treatment</th>
<th>Concentration</th>
<th>Time Taken for Paralysis (Min)</th>
<th>Time Taken for Death (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal Saline)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperazine Citrate</td>
<td>15 mg/ml</td>
<td>19 ± 1.304</td>
<td>2.5 ± 1.576</td>
</tr>
<tr>
<td></td>
<td>5 mg/ml</td>
<td>59.5 ± 0.648 *</td>
<td>60 ± 0.5815 *</td>
</tr>
<tr>
<td></td>
<td>10 mg/ml</td>
<td>52.4 ± 1.4106 *</td>
<td>58.2 ±0.7084 *</td>
</tr>
<tr>
<td></td>
<td>15 mg/ml</td>
<td>48.1 ± 1.0812 *</td>
<td>51.29 ± 0.946 *</td>
</tr>
<tr>
<td></td>
<td>20 mg/ml</td>
<td>46.23 ± 1.2117 *</td>
<td>49.02 ± 1.1077 *</td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>35 ± 1.049</td>
<td>44.43 1.098 *</td>
</tr>
</tbody>
</table>

n=6 for all the Groups.; Values are recorded as Mean ± SEM.; * P< 0.001, as compared to standard.
G. cambogia showed presence of Hydroxy citric acid (HCA), dimeric flavonoids, xanthones, Benozophenones, organic acid [18, 19, 20]. Some of these phyto constituents may be responsible to show a potent anthelmintic activity.

CONCLUSION

The traditional use of fruits of G. cambogia as an anthelmintic have been proved as the fresh juice and crude ethanolic extract showed potent anthelmintic activity against Indian earth worm. Further studies using in vivo models are required to carry out and establish the effectiveness and pharmacological rationale for the use of G. cambogia as an anthelmintic drug. The drug may further explore for its phytochemical profile to identify the active constituents responsible for anthelmintic activity.

REFERENCES