EVALUATION OF ANTHELMINTIC ACTIVITY OF CAESALPINIA CRISTA LINN. SEED EXTRACTS

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ABSTRACT

Caesalpinia crista Linn. Synonym Caesalpinia bonduc, Caesalpinia bonducella belonging to family Caesalpiniaceae/Fabaceae is a moderately size deciduous tree growing wildly throughout the deciduous forest of India. In Indian traditional plant medicine it is an important remedy for the treatment of several diseases. It is popular in indigenous system of medicine like Ayurveda, Siddha, Unani and Homoeopathy. The various parts of this plant like leaves, stem, roots and seeds are used as anthelmintic, antidiarrhoeal, antidiabetic, anti-inflammatory, antimalarial, antimicrobial and anticonvulsant in traditional system of medicine. The crude seed extracts of Caesalpinia crista were investigated for their anthelmintic property against Pheretima posthuma and Ascardia galli. Three concentration 5%, 10% and 15% w/v of each extract were studied in the experiment, which involved the determination of time of paralysis and time of death of the worms. Piprazine citrate was included as standard drug and normal saline as control. The result of the present experimental study indicated that all the extract significantly demonstrated paralysis and also caused death of worms at the higher concentration 15% w/v as compared to the standard drug.

KEYWORDS: Anthelmintic, Piperazine citrate, Caesalpinia critas, Pheretima posthuma.

INTRODUCTION

Caesalpinia crista Linn Syn. Caesalpinia bonducella (L.) Fleming, Syn. Caesalpinia bonduc (L.) Roxb belonging to family Caesalpiniaceae/Fabaceae is a prickly shrub widely distributed all over the world specially in India, Sri Lanka and Andaman and Nicobar islands. In India
specially found in tropical regions. All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in traditional system of medicine.\textsuperscript{[1]} Phytochemical analysis of seeds of \textit{Caesalpinia bonducella} has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids.\textsuperscript{[2]} In Hindi, it is known as karanjwa and seed kernels of this plant have been used as an antimalarial and anthelmintic. The plants of this family have been reported for various activities e.g. \textit{Caesalpinia crista} is used as tonic for the treatment of rheumatism and backache while \textit{Caesalpinia pulcherrima} is applied as abortifacient and emmenagogue.\textsuperscript{[3]} The different parts such as leaves, seeds, root and bark is also used in colic fever, intermittent fever, malaria, menstrual complaints, pneumonia, skin disease, swelling, tonic, pulmonary tuberculosis and as a uterine stimulant to cleans the uterus. It also alleviates the fever, edema and abdominal pain during the period.\textsuperscript{[4]} Pharmacologically various extracts of \textit{Caesalpinia crista} Linn has evaluated for anti diabetic activity (Gupta et al 2013),\textsuperscript{[5]} anthelmintic activity (A.Jabbar et al 2007),\textsuperscript{[6]} Hepatoprotective activity (R.Sarkar et al 2012),\textsuperscript{[7]} anti bacterial, anti diarrhoeal and cytotoxic activities (Billah et al 2011),\textsuperscript{[8]} anti cancer (Bodakhe et al 2011),\textsuperscript{[9]} antioxidants, anti inflammatory, and analgesic activity (Gill et al 2011)\textsuperscript{[3]} in experimental animals.

The literature survey revels that, the crude seed extracts of \textit{Caesalpinia crista} Linn for anthelmintic activity, has not been systematically investigated so far. Therefore the present study was an effort to investigate the anthelmintic properties of crude seed extracts of \textit{Caesalpina crista} against Indian earthworms \textit{Pheretima posthuma} and roundworms \textit{Ascardia galli}.

**MATERIALS AND METHODS**

**Plant material**

The seeds of \textit{Caesalpinia crista} Linn were procured from local market of Old Delhi and identified by Prof. A.K.Sharma, Head Department of Dravyaguna, Vaidya Yagya Dutt Sharma Ayurvedic Medical College Bulandshahar, U.P. The voucher specimen 0158/YDS have been deposited in department of Dravyaguna and Sir Madan Lal Institute of Pharmacy, Etawah, U.P.

**Preparation of Extracts**

The seed material was shade dried for 2-4 week and the material was subjected to pulverization, made course powder. Successive solvent extraction was performed with Pt-Ether (60-80), Ethyl Acetate and 95% Ethanol. The dried seed powder about 90 gm was
exhaustively extracted by hot continuous extraction using soxhlet apparatus with Pt-Ether (60-80), ethyl acetate, and 95% ethanol in increasing order of polarity up to 40-42 siphons separately. The extracts were filtered and concentrated by distillation process. The concentrated mass was dried under vacuum till constant weight for each of extract. For aqueous extract the dried seed powder about 200 gm was macerated with 1000 ml chloroform water (1:9) for seven days. The extractive was filtered and concentrated over a water bath at 40-45°C and further dried in vacuum oven till constant weight.

Animals
Indian adult earthworms *Pheretima posthuma* collected from moist soil and washed with normal saline to remove all the faecal matters were used for the anthelmintic study. The earthworms of 3-5 c.m.in length and 0.1-0.2 c.m.in width were used for all the experimental protocol. *Ascardia galli* worms are easily available from freshly slaughtered fowls. The use of *Ascardia galli* as a suitable model for screening of anthelmintic drugs was advocated earlier.\textsuperscript{[10]} The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.\textsuperscript{[11]}

Experimental Method for Anthelmintic Study
The crude seed extracts of *Caesalpinia crista* were investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. The earthworms were divided in to fourteen groups containing six worms in each group in two sets respectively. All the extracts were dissolved in the normal saline at different concentrations and than volume was adjusted for each extract to 20ml with normal saline. The standard drug solution was prepared with distilled water and volume was adjusted 20ml with distilled water. All the extracts and standard drug solution were freshly prepared before stating the experiment. Different concentration 5%, 10% and 15% w/v of all the extracts and 5% w/v of standard drug solution at the volume 20 ml were poured in different petridishes and all the earthworms before released in petridishes were washed in normal saline.

The anthelmintic experiment was carried out as per Bhardwaj et al.\textsuperscript{[12]} with or without minor modifications. In the first set of the experiment each group of six earthworms (*Pheretima posthuma*) was released in to 20 ml of prepared formulations as per following manner respectively.
1. 1st Group. – Normal Saline as control.
In the second set of experiment the same experiment was carried out for *Ascardia galli* worms. Observations were made for the time taken to paralyze and death of individual earthworm. Time for paralysis was noted until there was no movement could be observed in earthworms. Paralysis was said to occur when the worms do not revive even in normal saline and death was concluded when the worms lost their mortality followed with fading away of their body colour.\[^{13}\]

**RESULT AND DISCUSSION**

The predominant effect of Piperazine citrate on the worms is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ions conductance of worms muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.\[^{14}\] From the observations [Table – 1] the seed extracts of *Caesalpinia crista* were showed not only paralysis also caused death of earthworms. All the extracts were found to show the anthelmintic activity when compared to standard drug. The ETE and AQE were more efficacious than EAE and PTE. The result showed ETE at higher concentration 15% w/v demonstrated paralysis at 16 min and death at 28 min of worms in shortest time as compared to piperazine citrate at 5% w/v, cause paralysis at 26.0 min and death at 59.0 min in case of *Pheretima posthuma*. While in case of *Ascardia galli* ETE showed paralysis at 24.0 min and death at 67.0 min respectively. AQE caused paralysis of *Pheretima posthuma* and *Ascardia galli* at 20.0 min and 27.0 min and death at 34.0 min and 71.0 min respectively. Whereas EAE and PTE also exhibited anthalmintic activity, EAE showed paralysis of *Pheretima posthuma* and *Ascardia galli* at 25.0 min and 33.0 min and caused death at 50.0 min and 78.0 min at higher concentration 15% w/v. PTE at higher concentration 15% w/v caused paralysis of *Pheretima posthuma* and *Ascardia galli* in 31.0 min and 38.0 min and death at 54.0 min and 86.0 min respectively.
Table 1: Anthelmintic activity of seed extracts of *Caesalpinia crista* Linn against earthworms - *Pheretima posthuma* and roundworms - *Ascardia galli*.

<table>
<thead>
<tr>
<th>Extract treatment of <em>C. crista</em> Linn</th>
<th>Concentration (% W/V)</th>
<th><em>Pheretima posthuma</em></th>
<th><em>Ascardia galli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P (Min)</td>
<td>D (Min)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>5</td>
<td>26±0.4</td>
<td>59±0.3</td>
</tr>
<tr>
<td>Pt. Ether Extract (PTE)</td>
<td>5</td>
<td>53±0.4</td>
<td>151±0.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>42±0.2</td>
<td>79±0.3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>31±0.1</td>
<td>54±0.2</td>
</tr>
<tr>
<td>Ethyl acetate Extract (EAE)</td>
<td>5</td>
<td>49±0.4</td>
<td>120±0.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>37±0.6</td>
<td>73±0.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>25±0.7</td>
<td>50±0.3</td>
</tr>
<tr>
<td>Ethanol Extract (ETE)</td>
<td>5</td>
<td>41±0.5</td>
<td>112±0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>27±0.2</td>
<td>49±0.3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>16±0.1</td>
<td>28±0.5</td>
</tr>
<tr>
<td>Aqueous Extract (AQE)</td>
<td>5</td>
<td>50±0.4</td>
<td>129±0.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>34±0.6</td>
<td>69±0.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>20±0.3</td>
<td>34±0.3</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ±SEM (n = 6). Values of P and D are in minutes.

Figure – 01

Fig-01; Comparative data of paralysis and death time for *Pheretima posthuma*. 
CONCLUSION

From above experimental results it is concluded that all the seed extracts of *Caesalpinia crista* exhibited anthelmintic activity in dose dependant manner. The ETE and AQE at 15% w/v concentration showed shortest time of paralysis and death for both types of worms as compared to standard drug. In the present study the anthelmintic experiment was performed on the earthworms *Pheretima posthuma* and roundworms *Ascardia galli* due to its anatomical and physiological resemblance with the intestinal roundworms parasite of human being.\(^{[11]}\)

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