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Research Article

ANTIPLASMODIAL ACTIVITY OF *CAESALPINIA CRISTA* SEED EXTRACTS

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ABSTRACT

Objective: To evaluate antiplasmodial activity of *Caesalpinia crista* seed extracts**Methods:** Antiplasmodial activity of the seed extracts of *Caesalpinia crista* against rodent malaria infections in chloroquine sensitive *Plasmodium falciparum* strain was investigated, and oral acute toxicity of seed extracts of *Caesalpinia crista* was also evaluated.**Results:** The findings of this study revealed significant ($P < 0.05$) and dose dependent decrease in parasitaemia in the parasitized groups treated with varying doses of the extract (50-200 mg/kg p.o.) in both suppressive and curative tests. There was also significant decrease in parasitaemia density in the chloroquine treated group. The alcoholic extract was found no toxicity in wistar rats and the oral LD50 was determined to be greater than 5000 mg/kg.**Conclusion:** Seed extracts of *Caesalpinia crista* extract possesses potent antiplasmodial activity and may therefore, serve as potential sources of new antimalarial agents**Keywords:** Plasmodium falciparum, *Caesalpinia crista*, Plant extracts, Phytochemicals, Toxicity tests, malaria.**Article Info:** Received 26 July, 2018; Review Completed 22 Aug 2018; Accepted 25 Aug 2018; Available online 15 Sep 2018

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INTRODUCTION ^{1, 2, 4, 5}

Malaria still remains one of the killer diseases plaguing Africa and other developing countries. The development and spread of drug resistant strains of the causative agent *Plasmodium falciparum* has limited the effectiveness of the currently used malarial drugs. This creates the need for new antimalarial drugs. Plants have over the years proved to be a good source of chemotherapeutic agents.

Caesalpinia crista (*Caesalpinaceae*) is a large scandant prickly shrub found throughout the interior parts of India, Sri Lanka and West Indies. It is common in southern parts of India and is often grown as a hedge plant. *Caesalpinia* is a pantropical genus with 120-130 species, but has a complex taxonomic history. This

plant has profound medicinal use and is proved to have adaptogenic activity, anthelmintic activity, anti-inflammatory activity, antipyretic activity, analgesic activity, anti-amyloidogenic activity, antibacterial activity, antidiabetic activity, antifilarial activity, antioxidant activity, nootropic activity, immunomodulatory activity, hypoglycemic activity and hepatoprotective activity. The macro and microscopical features of the seed, leaf and flowers have been studied.

MATERIALS AND METHODS

Plant collection and sample preparation

Collection of plant part: The seeds of *Caesalpinia crista* were procured from local areas of Warangal, Telangana in the month of January, 2017 Seeds were authenticated and voucher specimen was deposited in

Department of Botany, Kakatiya University, Warangal for further reference.

Preparation of extract: Dried seed kernels of *Caesalpinia crista* Linn. were taken. Seed coat was broken and testa was separated. The kernel was powdered and passed through sieve No. 40 and stored in an airtight container for the extraction. It was extracted with ethanol for 6 h in soxhlet assembly. The ethanolic extract was then concentrated on rotary evaporator. The residue was yellowish brown sticky mass.

Phytochemical screening³

The phytochemical screening of ethanolic seed extract of *Caesalpinia crista* was carried out to check the presence of chemical constituents like flavonoids, alkaloids, tannins, triterpenoids, coumarin glycosides and proteins using standard procedures of analysis (Harborne, 1973)

Acute toxicity test^{6,7,8}

The LD50 of the seed extract was examined to determine the safety of the agent in rats, *in vivo* following OECD (2010) method. Dose levels used ranged from 10-5000 mg/kg. The rats were observed for signs of toxicity such as salivation, stretching of the body, weakness, paw licking, respiratory distress, coma and death for 72 h

Animals: Wistar albino rats (120-150 g) of either sex were divided into five groups of six rats each (24 rats). Animals were housed under standardised animal house conditions like 12 h light/dark cycle, temperature (24±1°C), relative humidity (55-65%) in all the experiments. They had free access to pelleted food and water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week prior to experimentation. All the animal experiments were carried out in accordance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and study was approved by Institutional Animal Ethical Committee with registration no. 1663/PO/RE/S/12/CPCSEA. The animals were assigned to different groups to be treated in experiments according to their weight range.

Parasite

Chloroquine-sensitive *Plasmodium falcipurum* NK65 obtained from National Institute for Malarial Research (NIMR) New Delhi was used for the study.

Infection of wistar rats with *Plasmodium falcipurum*

All the wistar rats used for the study were inoculated intraperitoneally on day 1 with 1×10^7 P. falcipurum parasitized erythrocytes obtained by suitable dilution with normal saline (0.9%) of a donor infected mouse by cardiac puncture.

In vivo antiplasmodial study^{9,10,11,12}

Suppressive study: This was the four-day suppressive study adopting the methods of Akuodor *et al* and David *et al* with slight modification. Thirty healthy albino wistar rats (male or female) were inoculated intraperitoneally with infected blood suspension (0.2

mL) containing 1×10^7 NK65 *Plasmodium falcipurum*. After inoculation, the animals were grouped into 5 of 6 wistar rats per cage. The first 3 groups were treated with the extract under study (50, 100 and 200 mg/kg, p.o.), while the last 2 groups were treated with the standard drug, chloroquine and normal saline (10 and 20 mL/kg, p.o.). The treatments were continued for 2nd, 3rd and 4th day of the study. On the 5th day, thin films were made from the tail blood of each mouse. The films were allowed to dry, fixed in methanol, stained with Giemsa, and parasitaemia density was examined microscopically (CX 21, Olympus) by counting the parasitized red blood cells in 10 different field.

Curative study:

The curative potential of *Caesalpinia crista* seed extracts was evaluated following the methods of Akuodor *et al* and Peter *et al* with slight modification. Thirty wistar rats employed for this study were intraperitoneally inoculated with blood suspension (0.2 mL) containing 1×10^7 *Plasmodium falcipurum* on day one. Seventy-two hours post-inoculation, the experimental animals were grouped and divided into 5 groups of 6 wistar rats per cage. Groups 1-3 were treated with the seed extract (50, 100 and 200 mg/kg, p. o. respectively). Groups 4 and 5 (positive and negative control) were treated with chloroquine [10 mg/kg and normal saline (20 mL/kg)], orally. Treatments were continued for days 4, 5 and 6. On the 7th day, thin films were prepared from the tail blood of each mouse and dried. Thereafter, the air-dried films were fixed in methanol and stained with Giemsa. The parasitaemia density were later examined microscopically (CX 21, Olympus Corporation) by counting the parasitized red blood cells in 10 different fields. The mean survival time (in days) of each group was determined by finding the average time of wistar rats in each group over a period of 30 days.

Statistical analysis

Data obtained were expressed as mean \pm standard error of mean. Results were analyzed using one-way analysis of variance, differences between means were considered significant at $P < 0.05$.

RESULTS

Phytochemical test

Phytochemical screening of the seed extracts of *Caesalpinia crista* showed the presence of flavonoids, tannins, saponins, terpenoids, steroids.

Acute toxicity test

There was no mortality recorded after oral administration of the alcoholic extract at 5000 mg/kg. Hence the doses used (50, 100 and 200 mg/kg, p.o.) were experimentally with in safe margin.

Suppressive activity

The alcoholic extract highly showed chemo-suppressive activity in a dose dependent manner. Doses used (50, 100 and 200 mg/kg) exhibited 85%, 88% and 94% Chemo-suppression of parasitaemia, respectively. The activity of the seed extract was significant ($P < 0.05$)

when compared with the control. The standard drug chloroquine (10 mg/kg) caused 96% inhibition (Table

1).

Table 1: suppressive effect

Drug	Dose (mg/kg)	Mean Parasitaemia (D5)	Suppression (%)
Control	20	32.5±0.5	-
<i>Caesalpinia crista</i>	50	6.98±0.2	78.5
	100	3.93±0.3	87.9
	200	2.61±0.9	91.9
Chloroquine	10	1.8±0.2	94.4

Note: results are expressed as mean ± standard error of mean. Significantly different from the control at $P < 0.05$ ($n = 6$).

Antiplasmodial suppressive activity of *Caesalpinia crista*

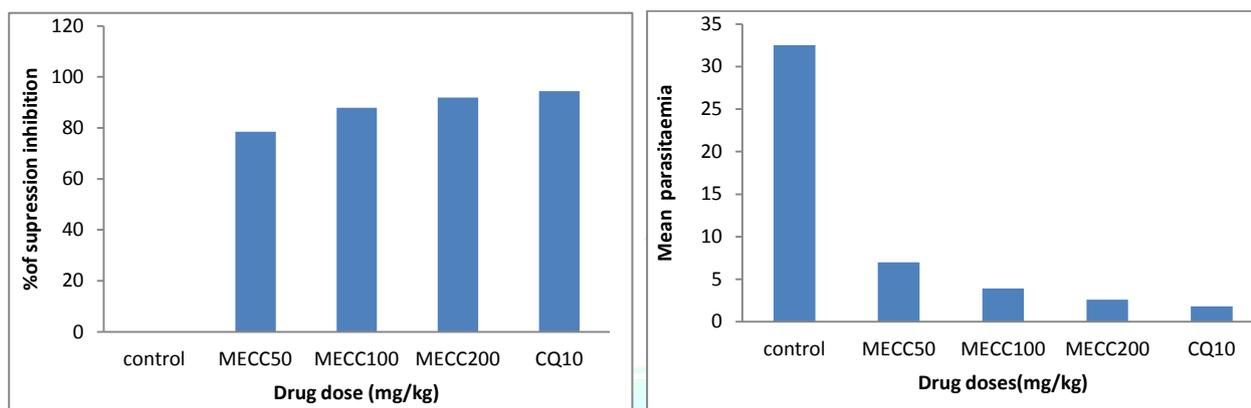


Figure 1: Antiplasmodial suppressive activity of *Caesalpinia crista*

Curative activity

The seed extracts significantly ($P < 0.05$) exhibited a dose dependent decrease in parasitaemia in both the extract treated and chloroquine treated groups. There was consistent increase in parasite density of the untreated group (negative control). Mortality was

recorded in the untreated group on day 7 and by day 10, all wistar rats in the group died. However, wistar rats in the groups treated with the extract survived beyond day 21, and some wistar rats in 200 mg/kg survived the 30-day observation period, while there was no mortality in the chloroquine treated group (Table 2).

Table 2: Curative effect

Drug	Dose (mg/kg)	Parasite density (D3)	Parasite density (D7)
Control	20	32.4±1.2	39.7±2.6
<i>Caesalpinia crista</i>	50	30.2±2.4	8.2±0.6
	100	29.4±2.6	6.4±0.2
	200	31.6±2.2	3.6±0.4
Chloroquine	10	30.5±1.4	1.6±0.6

Note: Results are expressed as mean ± standard error of mean. Significantly different from the control at $P < 0.05$ ($n = 6$)

Table 3: Mean survival time of rodents receiving various doses of ethanolic extract of *Caesalpinia crista*

Drug	Dose (mg/kg)	Mean survival time (day)
Control	20	10.4±1.2
<i>Caesalpinia crista</i>	50	24.4±0.4
	100	26.6±0.2
	200	28.4±0.8
Chloroquine	10	30.0±0.0

Note: results are expressed as mean ± standard error of mean. Significantly different from the control at $aP < 0.05$ ($n = 6$).

DISCUSSION

The seed extracts of *Caesalpinia crista* exhibited a high antiplasmodial activity in *Plasmodium falciparum* infected wistar rats as shown by the percentage inhibition of parasite development. The alcoholic extract showed a high suppression of malaria parasites comparable to chloroquine, the standard drug. The seed extract showed a significant chemosuppressive effect, as the dose increased antiplasmodial activity also increased. Moreover, the observed antiplasmodial activity suggests that the seed extract of *Caesalpinia crista* can suppress parasite growth to undetectable levels in erythrocytes in a long term treatment as it is in traditional herbal

medicine. It is imperative that herbal medicine preparations for claimed antimalarial agents are examined up to the point of knowing the level of suppression of parasite growth in red blood cells. The alcoholic seed extract of *Caesalpinia crista* also exhibited significant curative activity in the established infection. The observed antiplasmodial effect of the seed extract is consistent with the local use of the plant in traditional medicine against malaria, and indicative of its potential as a chemotherapeutic antimalarial drug. The antiplasmodial effect of the seed extract might be due to the presence of the secondary metabolites which have been variously implicated in antiplasmodial activity of numerous plants. High antiplasmodial activities on parasitaemia in this study are similar to the ones reported by Akuodor *et al* and Iwuanyanwu *et al*, whereby *Caesalpinia crista* leaf and stem bark extract at the dose of 400 and 600 mg/kg produced significant reduction of parasitaemia. The leaf extract of *Caesalpinia crista* also showed potent analgesic, anti-inflammatory and antipyretic properties in wistar rats and rats. Natural

products possessing the observed activities have been reported to provide relief for patients suffering from malaria attack. These findings support the ethnomedicinal use of this plant in the treatment of malarial and its associated symptoms. Furthermore, the active compounds observed in this study need to be identified. Some medicinal plants are known to exhibit antiplasmodial effect by either causing red blood cell oxidation or inhibiting protein synthesis depending on their phytoconstituents. However, the compounds observed in the seed extract may be acting singly or in synergy with one another to exhibit antiplasmodial activity. In conclusion, the findings of the present study have proved that the alcoholic seed extract of *Caesalpinia crista* possesses a strong antiplasmodial effect as observed in its ability to decrease parasitaemia density in the two models investigated. Further studies are ongoing in our laboratory to characterize and develop the actual compound(s) responsible for the observed antiplasmodial potential of this medicinally important plant.

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