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In Vitro Anthelmintic Activity of Cassia fistula Against Pathogenic Liver Fluke Fasciola gigantica

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Abstract: *In vitro*, the anthelmintic activity of *Cassia fistula* was studied on the pathogenic liver fluke *Fasciola gigantica*. Live liver flukes were collected from the infected part of the liver and bile duct of the freshly slaughtered domestic goat at the local meat market of Udaipur, India. The *in vitro* anthelmintic efficacy of aqueous and alcoholic extracts of *Cassia fistula* leaves at different concentrations of 10, 20, 30, 40, and 50 mg/ml for 2 to 10 h of exposure time were evaluated against the pathogenic liver fluke *F. gigantica*. The study revealed that alcoholic and aqueous extracts of *C. fistula* caused more mortality compared to albendazole. The alcoholic extract was more potent than the aqueous extract of *C. fistula*. The present research work is of significance because it would help to prepare less costly, eco-friendly, anthelmintic, and veterinary herbal drugs to replace the synthetic drugs that are currently in use.

Keywords: Anthelmintic, *Cassia fistula*, *Fasciola gigantica*, *In vitro*, Liver fluke, Fascioliasis, Goat

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Introduction

Fascioliasis is one of the most important helminthic diseases caused by trematode parasites liver fluke, *Fasciola gigantica* and *Fasciola hepatica* among domestic livestock and livestock holders in India. The definite hosts of liver fluke *F. gigantica* are cows, goats, sheep, and buffaloes, which have an impact on the weight loss, reduction in quality and quantity of growth rate, meat, wool, and milk production, and also reduce fertility in domestic animals. At present, a

vaccination is not available for the prevention of fasciolosis. This parasite generates significant health problems and major economic losses for the livestock industry and dairy industry (Khanjari *et al.*, 2014; Swarnakar and Damor, 2016; Musotsi *et al.*, 2017; Abah *et al.*, 2019; Sah and Sah, 2019; Parvin *et al.*, 2020; Lalor *et al.*, 2021). Various anthelmintic synthetic drugs are currently being used to control *F. gigantica* infections. Synthetic drugs are not easily available in some of the

remotest rural areas and have some serious disadvantages, such as the development of drug resistance after continuous use, adverse drug reactions, or residual effects. The continued usage of current anthelmintic drugs is also posing a major problem of drug resistance in *F. gigantica* as well as an unwanted adverse effect. The emergence of several problems due to synthetic drugs has encouraged the search for new compounds for better use of existing drugs. A variety of medicinal plants produce several bioactive phytochemicals that have effective anthelmintic properties. Therefore, the use of medicinal plants as anthelmintics or anthelminthics offer an alternative source that can solve these problems and is more acceptable to the native users. Various scientists screened the *in vitro* anthelmintic efficacy of different indigenous medicinal plants against *F. gigantica* and other worms to develop a safer, cheaper, eco-friendly, and effective remedy for fasciolosis in ruminants (Jeyathilakan *et al.*, 2012; Sumaia *et al.*, 2012; Swarnakar *et al.*, 2014, 2015; Ullah *et al.*, 2017; Anuracpreeda *et al.*, 2017; Mahardika *et al.*, 2017; Damor and Swarnakar, 2018; Vanda *et al.*, 2020; Kumar, 2021; Sen *et al.*, 2021; Vishwakarma *et al.*, 2021).

The experimental medicinal plant *Cassia fistula* belongs to the family Caesalpiniaceae, commonly known as Amaltas. It is used in the Ayurvedic system of medicine for various ailments. *C. fistula* is widely used in the traditional medicinal system of India and has been reported to possess hepatoprotective, anti-pyretic, anti-inflammatory, antitussive, antifungal, antibacterial, antioxidant and antihelminthic activity (Danish *et al.*, 2011; Kadhim *et al.*, 2016; Satpute *et al.*, 2017; Wahyuni *et al.*, 2019; Nayabaniya *et al.*, 2020; Saeed *et al.*, 2020).

However, no studies on the *in vitro* anthelmintic effects of medicinal plants like *Cassia fistula* on *Fasciola gigantica* have been conducted so far. Therefore, it was decided to carry out the work on *in vitro* anthelmintic activity of *Cassia fistula* against pathogenic liver fluke *Fasciola*

gigantica. The present research work would be significant because it would help to prepare less costly, eco-friendly, anthelmintic and veterinary herbal drugs to replace the synthetic drugs that are currently in use. The current research work provides knowledge for fasciolosis treatment and chemotherapeutic as well as phytotherapeutic measures.

Materials and Methods

Parasite collection:

Live *Fasciola gigantica* were collected from the infected part of the liver and bile duct of a freshly slaughtered domestic goat at the local meat market of Udaipur, India and kept in 0.9% physiological saline. *F. gigantica* parasites survived 2-4 h in physiological saline (Figs. 1A, B).

Collection of leaves of plants and authentication:

Fresh leaves of *Cassia fistula* were collected in Udaipur, Rajasthan. Dr. Asha Arora, Department of Biotechnology, B. N. University, Udaipur, identified and authenticated the plant *C. fistula*. The herbarium sheet was prepared and deposited in the department for future reference and the accession number is BNU/BIOT/2021-22/1136 (Figs. 1C, D, E).

Preparation of plant extracts:

Fresh leaves of *Cassia fistula* were dried and pulverized with a grinder into fine powder. Leaves of the plant were extracted by taking 20 g of each sample in 160 ml of water and ethanol (separately) in a 250 ml flask. After 72 h, the macerated solutions were filtered in separate flasks using filter paper. Then it was centrifuged at x10000g for 15 min and the supernatant was dried. Then the dried plant extract was reconstituted in the aqueous and alcoholic solvents using 10% DMSO. The extract of *Cassia fistula* leaves was stored at 4°C for further use.

Experimental design:

In vitro anthelmintic activity was studied by the *in vitro* petri dish method (Githiori *et al.*, 2006). The *in vitro* anthelmintic effect of alcoholic and

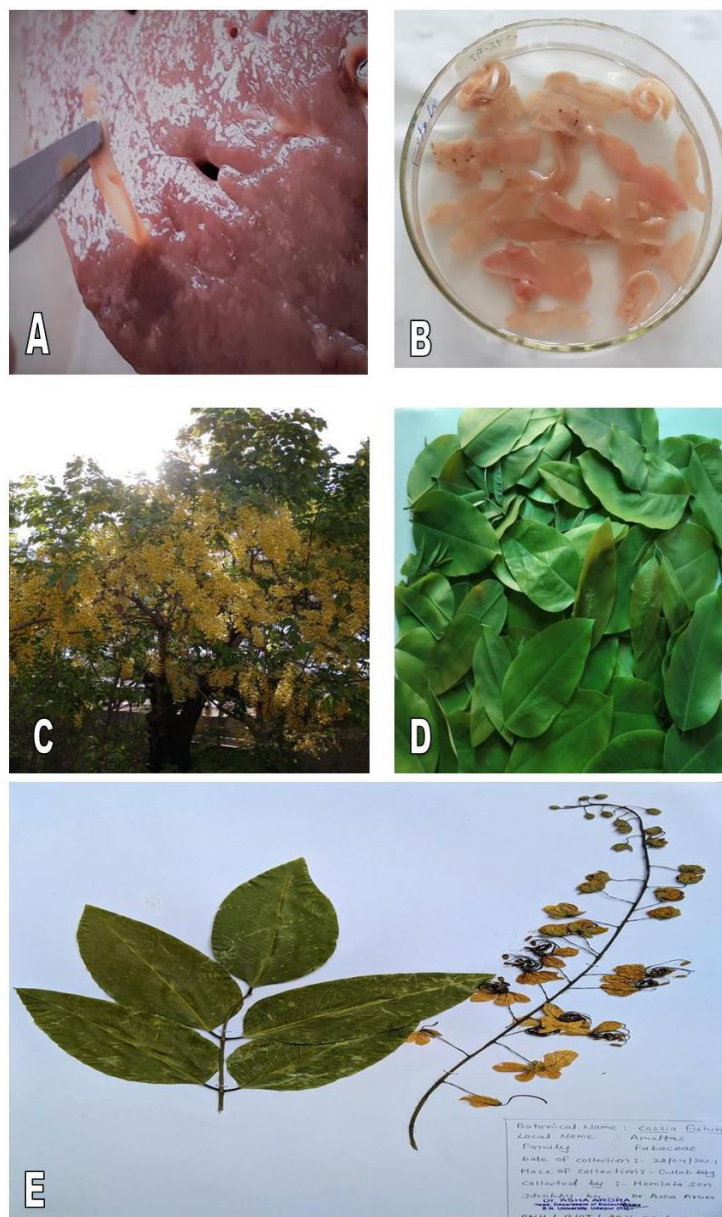


Fig. 1: (A) Liver fluke attached with infected liver, (B) *Fasciola gigantica* in saline solution, (C) Plant *Cassia fistula*, (D) Leaves of *C. fistula*, (E) Authentication of plant *C. fistula*.

aqueous *Cassia fistula* leaf extracts was evaluated in comparison with the albendazole drug. A group of five *F. gigantica* was given 10 ml of each concentration (10, 20, 30, 40, and 50 mg/ml) of the alcoholic and aqueous *Cassia fistula* leaf extracts and albendazole drug. The experiment was performed in three replicates at the optimal temperature (37°C) and pH 7.4. The mortality and

motility of liver fluke parasites were examined after 2, 4, 6, 8 and 10 h. The dead parasites were examined visually and mechanically stimulated using a dissecting needle. Motility was scored using the following criteria: - score 3 indicated whole-body movement; score 2 indicated movement of only a portion of the body; score 1 indicated immobility but not death; and score 0

indicated death. The dead parasites were counted in each experimental set and the percentage of average mortality was calculated according to the following formula:

$$\text{Mortality rate} = \frac{\text{Total number of dead flukes}}{\text{Total number of experimental flukes}} \times 100$$

Results

The anthelmintic effects of different concentrations of albendazole, alcoholic and aqueous leaf extracts of *Cassia fistula* against *Fasciola gigantica* are shown in Table 1 and Figure 2. A high concentration of albendazole of 50 mg/ml showed a 73.33% mortality rate after 10 h of exposure to *F. gigantica* parasites. On the other hand, the lowest concentration of 10 mg/ml at 2 h of exposure time did not induce any mortality, whereas 50 mg/ml albendazole concentration at the same exposure time showed 20% mortality.

The aqueous extracts of *C. fistula* leaves showed the highest mortality rate of 93.33% at concentration of 50 mg/ml after 10 h of exposure time. No mortality is shown after 10 mg/ml concentration of leaf extract after 2 h exposure time. The same exposure time also showed a mortality rate of 26.66% at a concentration of 50 mg/ml (Table 1; Fig. 2).

The highest mortality rate of 100% in the experiment was achieved with the alcoholic extract of *C. fistula* leaves at 50 mg/ml of concentration at 8 h of exposure time. Whereas the same highest mortality rate (100%) was also found in 10 to 50 mg/ml concentrations at 10 h of exposure time. The lowest mortality rate was 13.33% noticed at 10 mg/ml concentration of *C. fistula* leaves extract after 2 h exposure time (Table 1; Fig. 2).

Discussion

The present *in vitro* studies on the effect of *Cassia fistula* against *Fasciola gigantica* elucidated the anthelmintic potential of *C. fistula*. The mortality of parasites was identified by their visual and physical touch. It was found that *C. fistula* was effective against *F. gigantica*. The study showed

that aqueous and alcoholic leaf extracts of *C. fistula* caused more mortality than the synthetic drug albendazole and higher concentration were more effective than lower concentrations. It was also evident that alcoholic extracts were more potent than aqueous extracts. These results indicated the highest toxic effects of alcoholic extracts in comparison to albendazole and aqueous extracts at lower concentrations and lesser exposure times.

The present study indicated that plant-sourced medicine can be considered a promising alternative treatment. It has been reported that *Citrullus colocynthis* effects on *Orthocoelium scoliocoelium* (Swarnakar *et al.*, 2015) and *F. gigantica* (Damor and Swarnakar, 2018). *Gastrothylax crumenifer* (Jogpal *et al.*, 2021) has anthelmintic effects on trematode parasites. At 1 and 3 mg/ml concentrations, the essential oils *Allium sativum* (garlic) and *Piper longum* (Indian long pepper) were found to have antihelmintic effects on the liver fluke *Fasciola gigantica*. Methanolic peel extracts of *Dioscorea bulbifera* showed anthelmintic activity on *Pheritima posthuma* and *F. gigantica* at concentrations ranging from 10 to 100 mg/ml. The peel was more potent at 100 mg/ml (Singh *et al.*, 2009; Adeniran *et al.*, 2013). After 6 h of incubation in 100 and 200 g/ml, *F. gigantica* showed mortality after exposure to *Terminalia catappa* L. Ethanol extracts of *Ocimum sanctum* Linn. leaves caused mortality against *F. gigantica*. LC₅₀ of ethanol extract of *Ocimum sanctum* Linn. leaves was 7.9% at 4 h, 3.7% at 6 h, 1.8% at 8 h, and 0.8% at 10 h, and the LC₉₀ was 8.4% at 10 h. Alcoholic extract of *Potentilla fulgens* showed toxicity against *F. gigantica* and *P. fulgens*. The *Starchytarpeta jamaicensis* L. tea at a concentration of 10% showed anthelmintic activity comparable to the anthelmintic drug Albendazole (Anuracpreeda *et al.*, 2017; Mahardika *et al.*, 2017; Vanda *et al.*, 2020; Fahlevi *et al.*, 2021; Kumar 2021).

Phytochemical screening of *C. fistula* revealed several bioactive compounds. *C. fistula* is an important source of tannins, flavonoids, linoleic, glycosides, oleic, stearic, and carbohydrates. Its

Table 1: *In vitro* anthelmintic effects of albendazole, aqueous and alcoholic *Cassia fistula* extract on *Fasciola gigantica*.

Exposure time in h	Leaves extracts of <i>Cassia fistula</i> (mg/ml)	Mortality in %		
		Albendazole	<i>Cassia fistula</i> Aqueous extracts	<i>Cassia fistula</i> Alcoholic extracts
2 h	10 mg/ml	0.00	0.00	13.33
	20 mg/ml	0.00	6.67	20.00
	30 mg/ml	6.67	13.33	26.67
	40 mg/ml	13.33	20.33	33.33
	50 mg/ml	20.00	26.66	46.67
4 h	10 mg/ml	0.00	6.67	0.00
	20 mg/ml	6.67	20.00	33.33
	30 mg/ml	20.00	26.00	46.67
	40 mg/ml	26.67	33.33	53.33
	50 mg/ml	33.33	40.00	73.33
6 h	10 mg/ml	6.67	20.00	40.00
	20 mg/ml	20.00	33.33	53.33
	30 mg/ml	26.67	46.67	60.00
	40 mg/ml	33.33	53.33	73.33
	50 mg/ml	53.33	60.00	86.67
8 h	10 mg/ml	20.00	33.33	73.33
	20 mg/ml	40.00	40.00	80.00
	30 mg/ml	46.67	53.33	86.67
	40 mg/ml	53.33	73.33	93.33
	50 mg/ml	60.00	80.00	100.00
10 h	10 mg/ml	26.67	40.00	100.00
	20 mg/ml	40.00	60.00	100.00
	30 mg/ml	60.00	73.33	100.00
	40 mg/ml	66.67	86.67	100.00
	50 mg/ml	73.33	93.33	100.00

leaves contain glycosides, free rhein, sennosides A and B, isofavoneoxalic acids, and oxyanthraquinone derivatives. *C. fistula* is widely utilised in traditional medicine against various diseases (Saeed *et al.*, 2020; Mwangi *et al.*, 2021).

The present study suggested that the alcoholic extracts of *C. fistula* could offer a suitable and cheaper alternative anthelmintic or anthelminthic in comparison to aqueous extracts and synthetic

drugs. Consequently, it will help to reduce the occurrence of the parasite in the host environment after treatments. *C. fistula* alcoholic extract has significant anthelmintic activity. Statistical analysis determined significant anthelmintic activity between the concentration and time of exposure of the above plant extracts. The results of the present study would be supportive of less costly, eco-friendly, anthelmintic veterinary drugs,

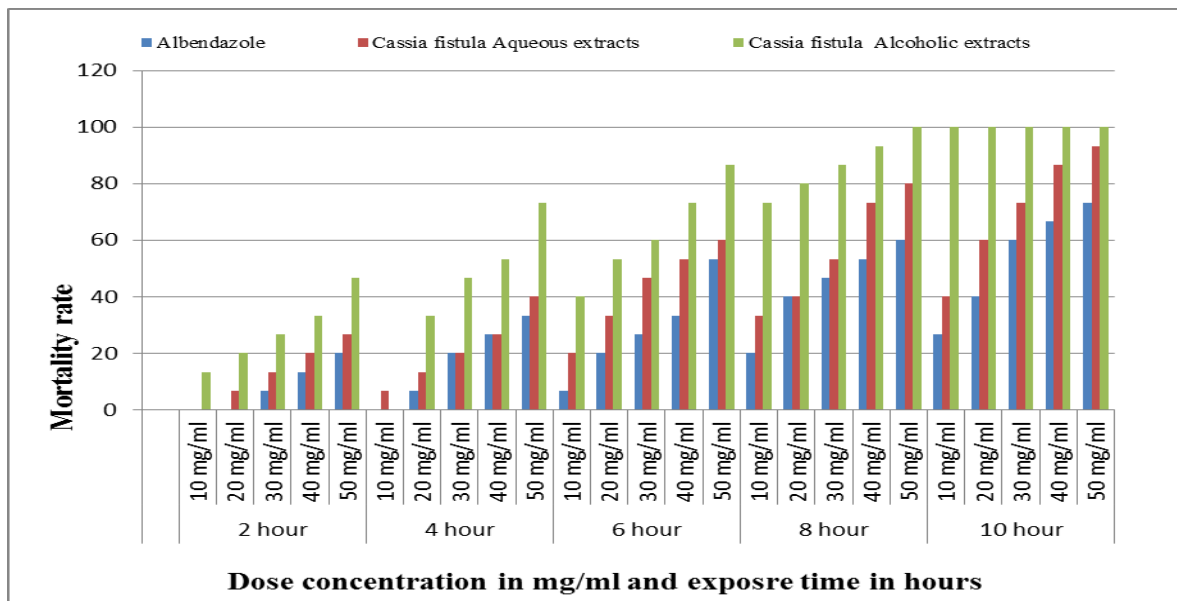


Fig. 2: A graphical representation is showing *in vitro* anthelmintic effects of albendazole, aqueous and alcoholic *Cassia fistula* extract on *Fasciola gigantica*.

improving the socio-economic condition of livestock holders.

Conclusion

The study showed that aqueous and alcoholic leaf extracts of *Cassia fistula* caused more mortality than the synthetic drug albendazole and higher concentrations were more effective than lower concentrations. It was also evident that alcoholic extracts were more potent than aqueous extracts. The present research work would be significant because it would help to prepare less costly, eco-friendly, anthelmintic, and veterinary herbal drugs to replace the synthetic drugs that are currently in use. The current research work provided knowledge for fasciolosis treatment and chemotherapeutic as well as phytotherapeutic measures.

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