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Research Article

IN VITRO ANTHELMINTIC EFFECTS OF FRUIT EXTRACTS OF CITRULLUS COLOCYNTHIS ON LIVER FLUKE *FASCIOLA GIGANTICA* IN BUFFALOES

Rajnarayan Damor and *Gayatri Swarnakar
Department of Zoology, Parasitology Lab., Government P.G. Meera Girls College, MLS University, Udaipur-313001, Rajasthan, India
**Author for Correspondence*

ABSTRACT
The *in vitro* anthelmintic effect of alcoholic fruit extract of *Citrullus colocynthis* and albendazole was investigated against *Fasciola gigantica* parasites. Fruits of *C. colocynthis* were collected from the desert area; Shriganganagar, Barmer, Jaisalmer (Rajasthan) and different concentration 10, 20, 30, 40, 50 mg/ml of *C. colocynthis*fruits extracts were prepared. *In vitro* treated *F. gigantic* with alcoholic and aqueous concentrations of fruit extracts were resulted dose-dependent and exposure time dependent mortality. Alcoholic concentrations of fruit extracts of *C. colocynthis* exhibit mortality 100% at 50 mg/ml in15th hour of exposure time. Aqueous fruit extract mortality rate 80% was exhibited at 50 mg/ml concentration on maximum exposure time 15 hrs. The synthetic drug Albendazole induced 66.67% mortality in 15th hours of incubation at 50 mg/ml dose concentration. Aqueous fruits extract of *C. colocynthis* more effective then synthetic drug albendazole but less effective then alcoholic fruits extract of *C. colocynthis*. Various veterinary drugs have been used to eliminate parasites from cattle but these drugs are unaffordable and inaccessible for poor cattle farmersIn conclusion, the present study revealed that alcoholic fruit extracts of *C. colocynthis* found to be potential sources for novel anthelmintic and justify their ethno-veterinary use.

Keywords: *Fasciola gigantica, Anthelmintic, Citrullus colocynthis, Mortality*

INTRODUCTION
Fascioliasis has been recognized as an important helminthic disease of livestock. This disease is mainly caused by *Fasciola gigantica* in domestic ruminants in India (Dalton, 1999). Fascioliasis is a significant livestock problem yearly an estimated US$ 2 to 3 billion are foregone due to weight loss, reduction in meat, wool and milk production, fertility in domestic animals and at present there is no Vaccine available for the prevention of fasciolosis. Fasciolosis is mainly caused by *F. gigantica* in buffaloes (Meaney *et al.*, 2002; Schweizer *et al.*, 2005; McManus and Dalton, 2006; Gupta *et al.*, 2012; Pandya *et al.*, 2015; Swarnakar and Damor, 2016). *F. gigantica* infections are produce major health problem and economic losses particularly poor livestock owners and dairy industry in Udaipur, India. *F. gigantica* is large and flatworm tropical liver fluke parasites belonging to the trematodes and occurs in the biliary ducts of liver and gall bladder of buffaloes.
Synthetic anthelmintic drugs are currently used for the treatment of *F. gigantica*. Synthetic drugs are not easily available in some of the remotest rural areas and have some serious disadvantages such as development of drug resistance in 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 unwanted adverse effect such as abdominal discomfort, nausea, vomiting, diarrhea, drowsiness vertigo, rashes is common in domestic buffaloes. The emergence of several problems due to synthetic drugs has encouraged the search for new compounds for better use of existing drugs.
Various medicinal plants have anthelmintic properties offer an alternative source can solve these problems and more acceptable to the native users. Many scientists were screened and *in vitro* antihelmintic effects of different indigenous medicinal plants against *F. gigantica* to develop safer, cheaper, eco-friendly and effective remedy for fasciolosis in ruminants (Kushwaha *et al.*, 2004; Githori *et al.*, 2006; Jabbar *et al.*, 2006; Hegazi *et al.*, 2007; Shehab *et al.*, 2009; Saowakon *et al.*, 2009;

Jenthilakhan *et al.*, 2010a and b; Ebedi *et al.*, 2011; Jeyathilakan *et al.*, 2012; Sumaia *et al.*, 2012; Shalaby *et al.*, 2012; Tansatit *et al.*, 2012; Kumar *et al.*, 2014 and Anuracpreeda *et al.*, 2017).

The experimental medicinal plant *Citrullus colocynthis* commonly known as bitter apple, kharatumba, occurs in Jaisalmer, Barmer, Jodhpur and Shriganganagar of Rajasthan. The fruit of *C. colocynthis* had been used medically since ancient times to treat constipation, oedema, cancer, jaundice, inflammation, ulcer, diabetes and urinary diseases, antibacterial, antimicrobial, antifungal property and antileishmanial (Nmillar *et al.*, 2000; Kumar *et al.*, 2008; Huseini *et al.*, 2009; Dallak, 2011; Amrouche *et al.*, 2011; Jeyanthi and Christy, 2011; Baloch *et al.*, 2013 and Zaid *et al.*, 2013). The anthelmintic efficacy of *Citrullus colocynthis* found to be positive on *Haemonchus contortus* and worm *Pheretimaposthuma* (Ullah *et al.*, 2013; Talole *et al*., 2013 and Kumar *et al*., 2014). Effect of aqueous and alcoholic fruits extracts of *C. colocynthis* on amphistome *Orthocoelium scoliocoelium* (Swarnakar *et al.*, 2015). Preliminary phytochemical screening of the plant showed the presence of large amounts of carbohydrate, protein, separated amino acid, tannins, saponins, phenolics, flavanoids, flavone, glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponarin, cardicglycoloids, trace elements and many other chemical groups (Al-Snafi, 2016 and Vinaykumar *et al.*, 2016).

However, no research work has been carried out so far on *in vitro* anthelmintic effects of medicinal plants *Citrullus colocynthis* (Kharatumba) on *Fasciola gigantica* by light and electron microscope. Therefore, it has been decided to undertake the work on "*In vitro* anthelmintic effects of fruit extract *Citrullus colocynthis* on adult *Fasciola gigantica* in buffaloes of Udaipur, India".

The present research work would be significant because it will help to prepare less costly, eco-friendly, anthelmintic and veterinary herbal drugs to replace the synthetic drugs which are currently in use. The current research work provide knowledge for fasciolosis treatment and chemotherapeutic as well as phytothereputic measures. Findings of the study will improve socioeconomic condition of the cattle farmers of Udaipur by removing pathogenic liver fluke *Fasciola gigantica* parasites with the treatment of medicinal plants.

MATERIALS AND METHODS

Collection of Parasites

Live liver fluke *Fasciola gigantica* parasites were collected from the liver of freshly slaughtered buffaloes *(Bubalus bubalis)*, at the local zoo abattoir in Udaipur. *F. gigantica* parasites were washed several times in tap water and then it was transferred into 0.9 % physiological saline.

Preparation of Fruit Extracts

Fresh *Citrullus colocynthis* fruits were collected from the desert area; Shriganganagar, Barmer, Jaisalmer (Rajasthan). Seeds were separate from the pulp of fruits. Then, pulp was put to dry and pulverize with grinder into a powder.

The powder was refluxed in 70% alcohol for 72 hrs at 60° C, and occasional stirring with a glass rod manually at regular intervals. After 72 hrs the macerates solutions were filtered in separate flasks using a Whatman no. 4 filter paper. Than centrifuged at x10000 g for 15 min and the supernatant was dry until a constant dry weight of each extract was obtained. Then, dried plant extracts were reconstituted in the respective solvents (alcoholic) using 10% DMSO.

Experimental Design

Fasciola gigantic parasites were maintained in 0.9% physiological saline and divided into four groups with five parasites in each group (Three replicate in each group). The first group of parasite was untreated used as control *Fasciola gigantica*. Second group of the parasites was treated with synthetic drug albendazole. Third group of the parasites were given *in vitr*o treatment with alcoholic fruit extracts of *Citrullus colocynthis.* Last fourth group of the parasites were given *in vitr*o treatment with aqueous fruit extracts of *Citrullus colocynthis.*

Control Group: The First group of parasites was a control group. The control experiments were prepared by incubating the worms in the phosphate buffered saline (PBS) containing 10 % DMSO (Dimethyl sulphoxide) for 15 hours.

In vitro Treatment with Synthetic Drug Albendazole: Second group of parasites were treated with synthetic drug Albendazole (Methyl 5-propylthio 2- benzimidazolecarbamate) at the concentration of 10, 20, 30, 40, 50 mg /ml for 15 hours.

In Vitro Treatment with Alcoholic Fruit Extracts of C. Colocynthis.: Third group incubated in concentration 10, 20, 30, 40, 50 mg/ml of alcoholic extracts of fruit pulp of *Citrullus colocynthis* with a volume of 30 ml in the petri dish for 15 hours.

In Vitro Treatment with Aqueous Fruit Extracts of C. Colocynthis.: Fourth group incubated in concentration 10, 20, 30, 40, 50 mg/ml of aqueous extracts of fruit pulp of *Citrullus colocynthis* with a volume of 30 ml in the petri dish for 15 hours.

Evaluation of Anthelmintic Activity of Albendazole, Aqueous and Alcoholic Fruit Extracts of Citrullus Colocynthis

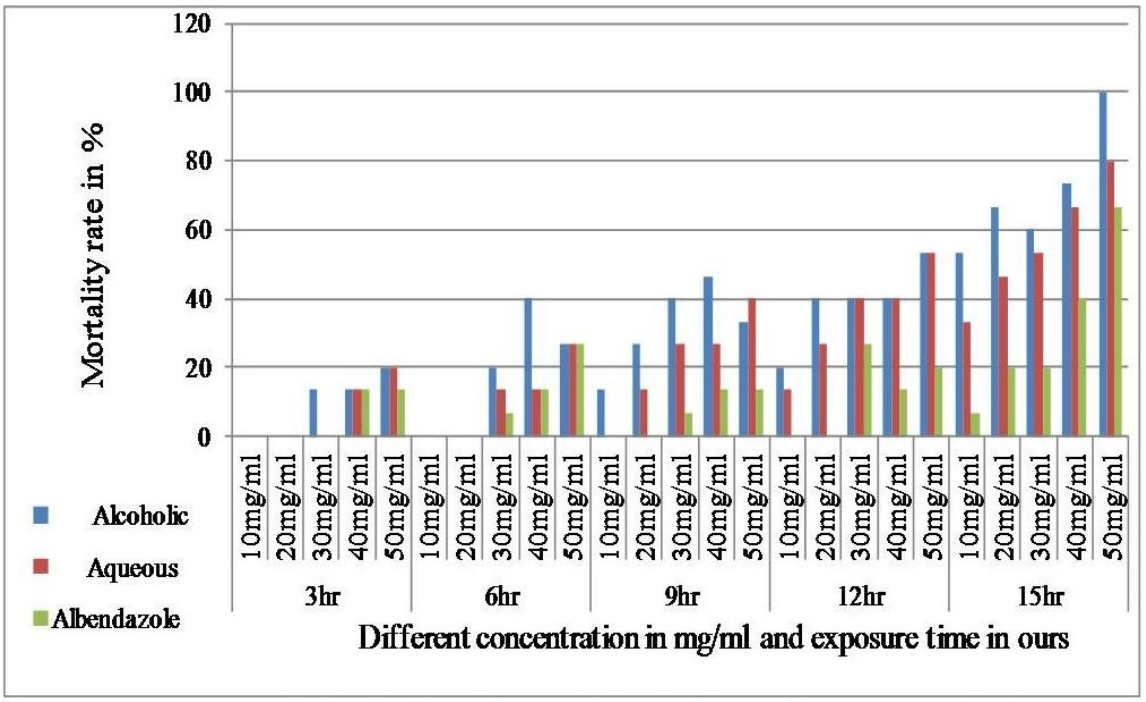
50 ml of each concentration of the anthelmintic effect of aqueous and alcoholic plant extracts of *C. colocynthis* and synthetic drug were applied to a group 5 worm maintained in 50ml of selected medium and 2ml of the sterilizing solution. The experiment was performed in three replicates at the optimal temperature (37°c) and pH 7.4. All experimented worms were examined after 3, 6, 9, 12 and 15 hr and mortality was calculated for each experiment. Mortality was determined visually by activity and by mechanical stimulation using a dissecting needle after 3, 6, 9, 12 and 15 hr and worm mortality was evaluated by observation under magnifying glass or after being removed from the experimental medium and dipped in slightly warm water and on gentle stimulation, worms confirmed by mortality.

Statistical Analysis

The dead worms of *Fasciola gigantica* were counted in each experimental set and the percentage of average mortality was calculated.

RESULTS AND DISCUSSION

Result



Graph 1. *In vitro* antihelmintic effect of alcoholic and aqueous fruits extract of *C. colocynthis* and albendazole on *F. gigantica*

Table 1: Different Concentration of Aqueous Fruits Extract of *Citrullus colocynthis* Shows Antihelmintic Effects on *Fasciola gigantica*

Exposure Time in Hrs	Aqueous C. Colocynthisin mg/ml	Mean	±STD	Mortality Rate %	CV%
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
3hr	30mg/ml	0.00	0.00	0.00	0.00
	40mg/ml	0.67	0.58	13.33	**86.60**
	50mg/ml	1.00	1.00	20.00	100.00
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
6hr	30mg/ml	0.67	0.58	13.33	86.60
	40mg/ml	0.67	0.58	13.33	86.60
	50mg/ml	1.33	0.58	26.67	43.30
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.67	0.58	13.33	86.60
9hr	30mg/ml	1.33	0.58	26.67	43.30
	40mg/ml	1.33	0.58	26.67	43.30
	50mg/ml	2.00	1.00	40.00	50.00
	10mg/ml	0.67	0.58	13.33	86.60
	20mg/ml	1.33	0.58	26.67	43.30
12hr	30mg/ml	2.00	1.00	40.00	50.00
	40mg/ml	2.00	1.00	40.00	50.00
	50mg/ml	2.67	0.58	53.33	21.65
	10mg/ml	1.67	0.58	**33.33**	**34.64**
	20mg/ml	2.33	0.58	46.67	24.74
15hr	30mg/ml	2.67	0.58	53.33	21.65
	40mg/ml	3.33	0.58	**66.67**	**17.32**
	50mg/ml	4.00	**1.00**	**80.00**	**25.00**

Different Concentration of Aqueous Fruits Extract of Citrullus colocynthis Shows Antihelmintic Effects on Fasciola gigantica

The *in vitro* antihelmintic effect of *C. colocynthis* aqueous fruits extract has been shown in table 1. Highest mortality rate 80% and coefficient variation (CV) 25% were exhibited at 50 mg/ml aqueous fruit extract concentration on maximum exposure time 15 hrs. 66.67% mortality rate exhibited on 40 mg/ml same exposure time this mortality rate induced prelatic effect on the experimental parasites near to death of the parasites. Whereas lowest mortality rate 13.33% and

coefficient variation (CV) 86% were exhibited at 40 mg/ml aqueous fruits extract concentration on minimum exposure time 3 hrs.

Table 2: *In Vitro* antihelmintic Effect of Alcoholic Fruits Extracts *C. colocynthis* on *F. gigantica* with Different Concentration

Exposure Time in Hrs	Alcoholic *C. Colocynthisin* mg/ml	Mean	±STD	Mortality Rate %	CV%
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
3hr	**30mg/ml**	0.67	0.58	**13.33**	86.60
	40mg/ml	0.67	0.58	13.33	86.60
	50mg/ml	1.00	1.00	20.00	100.00
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
6hr	30mg/ml	1.00	1.00	20.00	100.00
	40mg/ml	2.00	1.00	40.00	50.00
	50mg/ml	1.33	0.58	26.67	43.30
	10mg/ml	0.67	0.58	13.33	86.60
	20mg/ml	1.33	0.58	26.67	43.30
9hr	30mg/ml	2.00	1.00	40.00	50.00
	40mg/ml	2.33	0.58	46.67	24.74
	50mg/ml	1.67	1.15	33.33	69.28
	10mg/ml	1.00	0.00	20.00	0.00
	20mg/ml	2.00	0.00	40.00	0.00
12hr	30mg/ml	2.00	1.00	40.00	50.00
	40mg/ml	2.00	1.00	40.00	50.00
	50mg/ml	2.67	0.58	53.33	21.65
	10mg/ml	2.67	0.58	**53.33**	21.65
	20mg/ml	3.33	0.58	66.67	17.32
15hr	30mg/ml	3.00	1.00	60.00	33.33
	40mg/ml	3.67	0.58	**73.33**	15.75
	50mg/ml	5.00	0.00	**100.00**	0.00

PHOTOGRAPHIC PLATE – 1

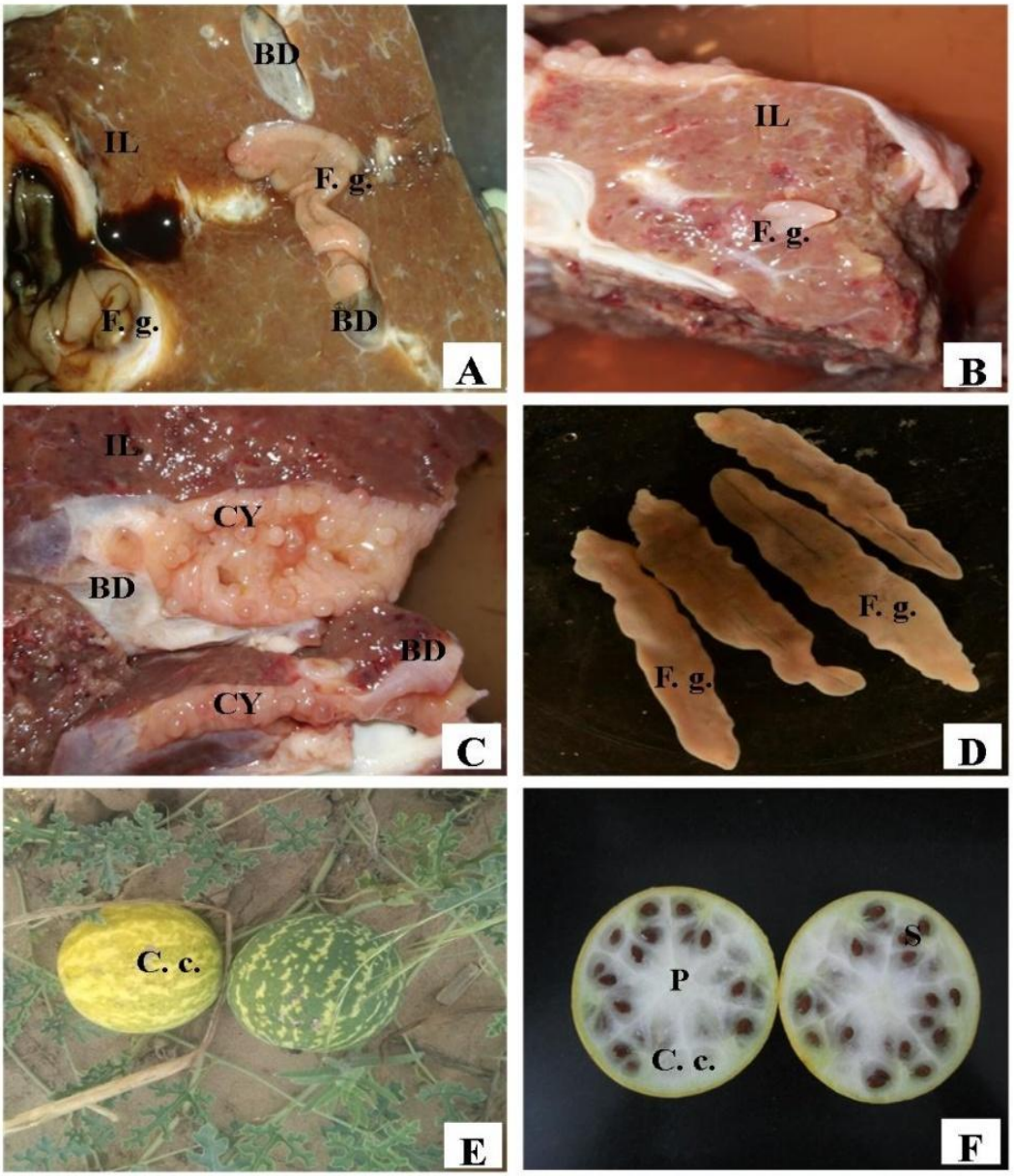


Figure 1: A: Infected Liver (IL) with *Fasciola gigantica* (F.G.); B: *F. gigantica* (F. G.) in Bile Duct (BD) of Infected Liver (IL); C: Cyst (CY) Formation in Bile Duct of Infected Liver by *F. Gigantica* (F. G.); D: F. *gigantica* (F. G.) in Saline Solution; E: Plant of *Citrillus colocynthis* (C. C.) with Fruits; F: *Citrillus colocynthis* (C. C.) Fruits with Seeds (s) and Pulp (P)

Different Concentration of Alcoholic Fruits Extract of Citrullus Colocynthis Shows Antihelmintic Effects on Fasciola Gigantica

The *in vitro* antihelmintic effect of *C. colocynthis* has been shown in table 2. The highest mortality rate 100 % and coefficient variation (CV) 0% were observed at 50 mg/ml fruits extract concentration on highest exposure time 15 hrs. 73.33 % mortality rate and coefficient variation (CV) 15.75 revealed on 40 mg/ml and at same exposure time this mortality induced prelatic affect on the experimental parasites near to death of the parasites. Whereas lowest mortality rate 13.33% and coefficient variation (CV) 86% were

exhibited at 30 mg/ml aqueous fruits extract concentration on minimum exposure time 3 hrs.10 mg/ml and 20 mg/ml fruit extracts concentration on 3 hrs and 6 hrs respectively did not showed any mortality.

Table 3: *In Vitro* Antihelmintic Effect of Synthetic Drug Albendazole on *Fasciola gigantica* with Different Concentrations

Exposure Time in Hrs	Albendazole in mg/ml	Mean	±STD	Mortality Rate %	CV%
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
3hr	30mg/ml	0.00	0.00	0.00	0.00
	40mg/ml	0.67	0.58	**13.33**	**86.60**
	50mg/ml	0.67	0.58	13.33	86.60
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
6hr	30mg/ml	0.33	0.58	6.67	173.21
	40mg/ml	0.67	0.58	13.33	86.60
	50mg/ml	1.33	0.58	26.67	43.30
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
9hr	30mg/ml	0.33	0.58	6.67	173.21
	40mg/ml	0.67	0.58	13.33	86.60
	50mg/ml	0.67	0.58	13.33	86.60
	10mg/ml	0.00	0.00	0.00	0.00
	20mg/ml	0.00	0.00	0.00	0.00
12hr	30mg/ml	1.33	0.58	26.67	43.30
	40mg/ml	0.67	0.58	13.33	86.60
	50mg/ml	1.00	1.00	20.00	100.00
	10mg/ml	0.33	0.58	**6.67**	**173.21**
	20mg/ml	1.00	1.00	20.00	100.00
15hr	30mg/ml	1.00	1.00	20.00	100.00
	40mg/ml	2.00	1.00	**40.00**	**50.00**
	50mg/ml	3.33	0.58	**66.67**	**17.32**

Different Concentration of Synthetic Drug Albendazole Shows Antihelmintic Effect on Fasciola gigantica

The *in vitro* antihelmintic effect of different concentration of albendazole showed on *F. gigantica* (Table 3). 66.67 % mortality rate and coefficient variation (CV) 17.32 were observed at high concentration of albendazole of 50 mg/ml at 15 hrs exposure time. 10 mg/ml exhibited 6.67% mortality rate and coefficient variation (CV) 173.21% at same exposure time (15 hrs). Whereas 6.67% mortality rate were observed at 30 mg/ml on 6 hrs and 9 hrs exposure time and 10 mg/ml concentration also showed 6.67% mortality rate. 10 mg/ml and 20 mg/ml concentration did not produce any mortality rate on 3, 6, 9 and 12 hrs of exposure time of the test exposure parasites.

Comparison of antihelmintic effect of *in vitro* different concentration of synthetic drug albendazole, alcoholic and aqueous fruits extract of *C. colocynthis* has been shown graphical representation in graph 1. Aqueous fruits extract of *C. colocynthis* more effective then synthetic drug albendazole but less effective then alcoholic fruits extract of *C. colocynthis*. Highest mortality rate in alcoholic fruits extract of *C. colocynthis* on *Fasciola gigantica*.

Discussion

In the present study the antihelmintic effect of the different concentration of aqueous and alcoholic extract of *Citrullus colocynthis* were evaluated on pathogenic liver fluke *Fasciola gigantica*. Results were compared with synthetic drug albendazole at different concentration. This study showed that *C.*

colocynthis have higher antihelmintic effect on *F. gigantica*. Both the alcoholic and aqueous fruits extract of *C. colocynthis* exhibited dose dependent mortality of *F. gigantica* by paralyzing them or causing their death within the incubating period.

The study exhibited that aqueous and alcoholic fruit extract of *C. colocynthis* caused more mortality than synthetic drug albendazole and higher concentration are more effective than lower concentration. It was also evident that aqueous extract was less potent than alcoholic extracts. Some other researchers were also worked on anthelmintic activity of *C. colocynthis* on different parasites. Similar studies were also done by various scientists on *in vitro* effects of various alcoholic and aqueous medicinal plant extracts. A large number of medicinal plants have been claimed to have anthelmintic activities in traditional system of medicine and are also utilized by ethnic groups worldwide such as *Allium sativum*, *Lawsonia inermis* and *Opuntia indica, B. egyptiaca, Cymbopogan nardus, Arecacatechu*, *Erythrina indica, Zingiber officinale, Caparis decidua*, *Moringa olifera* Ivermectin, *Nigella sativa* and *Azadirachta indica* were observed in comparing with albendazole and triclabendazole on adult *F. gigantica* and other helminth parasites include *Haemonchus contortus*, *Moniezia expansa* (Kushwaha *et al.,* 2004; Githori *et al.*, 2006; Jabbar *et al.,* 2006; Hegazi *et al.,* 2007; Shehab *et al.,* 2009; Saowakon *et al.,* 2009; Jenthilakhan *et al.,* 2010a and b; Ebedi *et al.,* 2011; Jeyathilakan *et al.,* 2012; Shalaby *et al.,* 2012; Sumaia *et al.,* 2012; Tansatit *et al.,* 2012; Massoud *et al.,* 2012 and Shareefa *et al.,* 2014). In India, traditional medicinal plants *Ferula asafoetida* and *Syzygium aromaticum* also show have several medicinal, pesticidel, antioxidant, antiviral, antibacterial, antifungal, molluscicidal and larvicidal properties. the i*n vitro* antihelmintic effect of medicinal plants *F. asafoetida* and *S. aromaticum* on the liver fluke *F. gigantica* (Kumar *et al.,* 2014 and Anuracpreeda *et al.,* 2017).

Antihelmintic properties of medicinal plants were showed on different parasites. Kumar *et al.,* (2014) evaluated the acaricidal activities of the aqueous methanolic extracts of rhizome of *Curcuma longa*, fruit of *C. colocynthis* and seed of *Peganumharmala*. Crude aqueous methanol extracts of the three plants and their combination were found effective against *Rhipicephalus microplus* larvae of ticks (Ullah *et al.,* 2015). The crude oil from *C. colocynthis* fruit showed that the major constituents were mainly the triglycerides, free fatty acids, phospholipids and sterols along with other minor unidentified constituents (Dash *et al.,* 2015). The study exhibited that alcoholic and aqueous fruit extract of *C. colocynthis* caused more mortality than synthetic drug albendazole and higher concentration are more effective than lower concentration. It was also evident that aqueous extract was less potent than alcoholic extracts.

Present research work have similarity with observations of Ullah *et al.,* (2013); Talole *et al*., (2013) and Kumar *et al*., (2014) because they exhibited similar observations of anthelmintic efficacy of *C. colocynthis* on *Haemonchus contortus* and worm *Pheretima posthuma.* The *in vitro* anthelmintic activity of aqueous and alcoholic extract of *C. colocynthis* were observe on amphistome *Orthocoelium scoliocoelium* and found the potential sources for novel anthelmintic and justify their ethno veterinary use (Swarnakar *et al.,* 2015). *C. colocynthis*, *Physalis alkekengi* and *Solanum nigrum* displayed potent *in vitro* antimalarial activity. The existences of anthelmintic and anti plasmodial compounds were detected in *C. colocynthis* plant extracts (Rani *et al.,* 2017 and Haddad *et al.,* 2017).

The alcoholic extract of fruit pulp of *C. colocynthis* could offer a suitable and cheaper alternative for the most expensive anthelmintic as comparison to aqueous extract and synthetic drugs. It showed a potent and progressive effect on the parasites. Consequently, it will help to reduce the occurrence of parasite in the host environment after treatments. The results of this study will be helpful to preparing ecofriendly, less costly anthelmintic veterinary drug, socio-economic upliftment of cattle farmers and scientific documentation of traditional veterinary practices.

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REFERENCES

Al-Snafi (2016). Chemical constituents and pharmacological effect of *Citrullus colocynthis*- a review. *Journal of Pharmecutical* **6**(3) 57-67.

Amrouche AA, Benmehdi H, Mebarki K, Chaoufi A, Saneba A, Lazouni A and Sari DC (2011). Evalution of antifungal activity of some oils from Algerian medicinal plants against *Aspergillusflavus* strain produced aflatoxin. *Journal of Applied Pharmecutical Science* **1**(8) 48-53.

Anuracpreeda P, Chawengkirttikul R, Ngamniyom A and Panyarachun B (2017). The *in vitro* anthelmintic activity of the ethanol leaf extract of *Terminaliacatappa* L. on *Fasciola gigantica*. *Parasitology* **144**(14) 1931-1942.

Baloch N, Nabi S, Kakar AM, Wajid Z and AL-Kahraman YMSA (2013). *In vitro* antileishmanial, antitumor, cytotoxic activities and phytochemical analysis of *Citrullus colocynthis* fruit extract and its fractions. *International Journal of Medicinal and Aromatic Plants* **3**(1) 78-84.

Dallak M (2011). *In vivo* hypolipidemic and antioxidant effect of *Citrullus colocynthis* pulp extract in alloxan induced diabetic rats. *African Journal of Biotechnology* **10**(48) 9898-9903.

Dalton (1999). *Fasciolosis*, (CABI Publication, Wallingford, UK) 17-35.

Dash S, Raj M and Padhi S (2015). Characterization of seed oil of *Citrulluscolocynthis* (L.) *Scholar Research Journal* **3**(18) 188-192.

Ebedi MH, Moustafa AM, Arnaout FK, Degheidy NS, Omer EA, Shalaby HA and EL-Hamed AF (2011). *In vitro* evaluation of anthelmintic efficacy of *Balanites aegyptiaca* on *Fasciola gigantica*. Beenha. *Veterinary Medicinal Journal* **22**(2) 56-67.

Githori B, Athanasiadou S and Thamsborg M (2006). Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Veterinary Parasitology* **139**(4) 308-320.

Gupta A, Dixit AK, Dixit P and Mahajan C (2012). Prevalence of gastrointestinal parasites in cattle and buffaloes in and around Jabalpur, Madhya Pradesh. *Journal of Veterinary Parasitology* **26**(2) 186-188.

Haddad MHF, Mahbodfar H, Zamani Z and Ramazani A (2017). Antimalarial evaluation of selected medicinal plant extracts used in Iranian traditional medicine. *Iranian Journal of Basic Medical Science* **20**(4) 415-422.

Hegazi AG, Faten K, Hady AE and Shlaby HA (2007). Inhibitory effect of Egyptian propolis on *Fasciola gigantica* eggs with referenca to its effect on *Clostridium oedematien* and Cirrelation to chemical composition. *Pakistan Journal of Biological Science* **10**(19) 3295-3305.

Huseini HF, Darvishzadeh F, Heshmat R, Jafarizar Z and Larijani B (2009). The clinical investigation of *Citrullus colocynthis* (L.) schrad fruit in treatment of type II patient; a randomized, double blind, placebo-controlled trial. *Phytotherephy Research* **23**(8) 1189-1199.

Jabbar A, Raza MA, Iqbal Z and Khan MN (2006). An inventory of the ethno botanicals used as anthelmintic in the southern Punjab (Pakisthan). *Journal of Ethnopharmacology* **108**(1) 152-154.

Jeyanthi KA and Christy A (2011). Antioxident effect of *Citrullus colocynthis* on alloxan induced diabetic rat. *International Journal of Pharmaceutical and Biological Archives* **2**(2) 696-701.

Jeyathilakan N, Murali K, Anandaraj A and Abdul BS (2010a). *In vitro* evalution of anthelmintic property of herbal plants against *Fasciola gigantica*. *Indian Journal of Animal Science* **80**(11) 1070-74.

Jeyathilakan N, Murali K, Anandaraj A and Abdul BS (2012). *In vitro* evaluation of anthelmintic property of ethano-veterinary plant extracts against the liver fluke *Fasciola gigantica*. *Journal of Parasitic Diseases* **36**(1) 26-30.

Jeyathilakan N, Murali K, Anandaraj A, Latha BR and Abdul BS (2010b). Anthelmintic activity of essential oils of *Cymbopogan nardus* and *Azadirachta indica* on *Fasciola gigantica*. *Journal of Veterinary Animal Science* **6**(5) 204-209.

Kumar A, Saran G, Bharath KN, Kumar A, Kulashekar KS, Tamoli S and Vamshikrishna N (2014). Evaluation of laxative activity of Easigo (a polyherbal formulation). *International Journal of Traditional Natural Medicine* **4**(1) 1-5.

Kumar P and Singh DK (2014). *In vitro* anthelminticum activity of *Alliumsativum, Ferula asafoetida, Syzygium aromaticum* and their active components against *Fasciola gigantica*. *Journal of Biology and Earth Science* **4**(1) 57-65.

Kumar S, Kumar D, Saroha K, Manjusha, Singh N and Vashishta B (2008). Antioxideny and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. Methanolic fruit extract. *Acta Pharmecutical* **58**(2) 215-220.

Kushwaha DS, Kumar D, Tripati HC and Tandan SK (2004). Effect of some indigenous medicinal plant extracts on *Fasciola giganticain vitro*. *Indian Journal of Animal Science* 74(2) 143-146.

Massoud AM, Shalaby HA, El Khateeb RM, Mahmoud MS and Kutkat MA (2012). Effects of mirazid and myrrh volatile oil on adult *Fasciolagigantica* under laboratory conditions. *Asian Pacific Journal of Tropical Biomedicine* **2**(11) 875-884.

McManus DP and Dalton JP (2006). Vaccine against the zoonotic trematodes *Schistosoma japanicum, Fasciola hepatica* and *Fasciola gigantica*. *Parasitology* **133** 43-61.

Meany M, Fairweather MI, Brennan GP, Ramasamy P and Subramanian PB (2002). *Fasciola gigantica*; Tegumental surface alteration following treatment *in vitro* with the sulphoxide metabolite of triclabendazole. *Parasitological Research* **88**(4) 315-325.

Nmillar R, Gross R, Rchid M, Manteghehim M, Petit P, Tijane M and Ribes G (2000). Insulin tropic effect of *Citrullus colocynthis* fruit extracts. *Planta Medicine* **66** 418-423.

Pandya SS, Hasnani JJ and Patel PV (2015). Morphological and histological identification of *Fasciola gigantica* recovered from liver of infected buffaloes. *International Journal of Research Granthaalayah* **3**(3) 25-31.

Rani A, Goyal A and Arora A (2017). A brief review on *Citrullus colocynthis*- bitter apple. *Archive of Current Research International* **8**(4) 1-9.

Saowkon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V and Sobhon P (2009). *Fasciola gigantica*; anthelmintic effect of the aqueous extract of *Artocarpus lakoocha*. *Experimental Parasitology* **122**(4) 289-98.

Schweizer G, Braun U, Deplazes P and Torgerson PR (2005). Estimating the financial losses due to bovine fasciolosis in Switzerland. *Veterinary Research* **157**(7) 188-193.

Shalaby HA, Ezz AE, Farag TK and Zeina A (2012). *In vitro* efficacy of a combination of Ivermectin and *Nigella sativa* oil against helminth parasites. *Global Veterinary* **9**(4) 465-473.

Shalaby HA, Namaky AE, Khalil FA and Kandil OM (2012). Efficacy of methanolic extract of *Balanites aegyptiaca* fruits on *Toxoceravitulorum. Veterinary Parasitology* **183**(3-4) 386-392.

Shareefa PA, Brennanb GP, Veighb PM, Khana H, Russell M, Morphewc RM, Mousleyb A, Marksb NJ, Saifullaha MK, Brophyc PM, Mauleb AG and Abidia SMA (2014). Time dependent tegumental surface changes in juvenile *Fasciola gigantica* in response to triclabendazole treatment in goat. *Acta Tropicana* **136** 108-117.

Shehab NG, Monem AR, Hassan SE and Toaleb NI (2009). Botnical study of *Meriytadenhamii* seem and its anthelminthic activity against *Fasciola gigantica*. *Journal of Egyptian Society of Parasitology* **39**(1) 269-288.

Sumaia AA, Mohammed GF and Gameel AA (2012). *In vitro* adulticidal efficacy of albendazole, *Capparis decidua* stems and *Moringaoleifera* leaves against *Fasciola gigantica*. *SUST Journal of Science Technology* **13**(2) 59-67.

Swarnakar G and Damor R (2016). Surface topography of *Fasciola gigantica* (Trematoda: digenea) from infected liver of buffalo (*Bubalus bubalis*) in Udaipur, India. *International Journal of Advance Pharmecutical Science* **7**(2) 3056-3059.

Swarnakar G, Kumawat A and Swarnakar P (2015). Effect of aqueous and alcoholic fruit extract of *Citrullus colocynthis* on amphistome *Orthocoelium scoliocoelium*. *International Journal of Advance Pharmaceutical Science* **6**(2) 2790-2796.

Talole BB, Baheti DG and More PA (2013). *In vitro* helmintholytic activity of leaves of *Citrullus colocynthis*. *International Journal of Pharmacy and Chemistry* **3**(2) 240-243.

Tansatit T Sahaphong S, Riengrojpitak S, Viyanant V and Sobhond P (2012). *Fasciola gigantica*; the *in vitro* effect of artesunate as compared to triclabendazole on the 3 week old juvenile. *Experimental Parasitology* **131** 8-19.

Ullah S, Khan MN, Sajid MS, Iqbal Z and Muhammad G (2015). Comparative efficacies of *Curcuma longa*, *Citrullus colocynthis* and *Peganumharmala* against *Rhipicephalus microplus* through modified larval immersion test. *International Journal of Agriculture Biology* **17**(1) 216-220.

Ullah S, Khan MS, Sajid MS and Muhammud G (2013). Comparative anthelmintic efficacy of *Curcuma longa*, *Citrullus colocynthis*, *Peganum harmala*. *Global Veterinary* **11**(5) 560-567.

Vinaykumar T, Eswarkumar K and Roy H (2016). Evaluationof *Citrullus colocynthis* fruit pulp in streptozotocin induced diabetic rats. *International Journal of Pharmecutical Research and Health Science* **4**(2) 1136-1142.

Zaid KHA, EL-Wakil H, EL-Hussein A, Jomma S and Shohayeb M (2013). Evaluation of the molluscicidal activity of *Punica granatum*, *Calotropis procera*, *Solanum incanum* and *Citrullus colocynthis* against *Biomphalaria arabica*. *World Applied Science Journal* **26**(7) 873-879.