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STUDIES ON ANTHELMINTIC ACTIVITY OF *CARICA PAPAYA* SEED EXTRACT ON METACERCARIA OF *EUCLINOSTOMUM HETEROSTOMUM*

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ABSTRACT
The present study proved that the effect of *Carica papaya* seed extract as an anthelmintic in the metacercaria of *Euclinostomum heterostomum*. The metacercaria of *Euclinostomum heterostomum* is a parasite in the liver, gills, kidney and the internal organs of the fresh water fish *Channa punctatus*. For the assay a specific concentration of papaya seed extract is prepared by adding Tyrode solution to the drug. Here glycogen content, total protein content and total lipids were estimated in the metacercaria. The study revealed that the decreased levels of glycogen and protein levels was observed in Praziquantel treated and papaya seed extract treated metacercaria when compared to control but any difference was not found in total lipids in control, in Praziquantel and in papaya seed extract trated metacercaria. Hence, the results were indicates due to withstand the stress condition the parasite is trying to get more energy, which leads to decreased levels of glycogen and proteins levels. The total experiment was done by taking Praziquantel as a reference drug.

KEY WORDS: Anthelmintic, *Carica papaya*, Glycogen, Proteins, Lipids, Metacercaria.

INTRODUCTION

Medicinal plants have been identified and used traditionally throughout the world from the beginning of the human civilization (Kalesraj, 1975). These medicinal plants contain active principles which are highly potent against parasites (Tylor *et al*., 1994). Parasites cause a quantum of health hazard and economic losses to both human and animals Nadkarni *et al*. (1976). A number of plants possessing anti-parasitic properties are mentioned as phytochemical, and their mode of action on parasites for example Saponins -affect the permeability of the cell membrane of parasites, Isoflavones- inhibit the enzymes of glycolysis and glycogenolysis and disturb the Calcium ions, homeostasis activity in the parasites. The antihelmintic activity of *Terminalia arjuna* bark was studied by Bachaya *et al*. (2009) in eggs, larvae and adult of *Haemonchus contortus*. The antihelmintic activity of oil of *Allium sativum* against *Heterakis gallinae* and in *Ascaridia galli* was studied by Nagaiah *et al*. (2000) and in *Haemonchus contortus* studied by Iqbal *et al*. (2001) and in eggs of *Ascaridia galli* invitro by Chybowski *et al*. (1997). Sutton G A and Haik R (1999) observed the anthelmintic activity of alcoholic extract of Allium sativum against *Cotylophoron cotylophorum*. Parasites causes a quantum of health hazard and economic loss to both human and animal (Tandon *et al*. 2007). The aqueous and ethanol extracts of *Cucurbita maxima* seeds exhibits effective anthelmintic activity against *Fasciolopsis buski*, and in *Ascaris lumbricoides* and in *Hymenolepis diminuta*, was reported by Tandon *et al*. (2007). These herbal drugs are available in low cost, and show less side effects. By observing all these evidences I have selected *Carica papaya* seeds as an antihelmintic against the progenetic worms of *Euclinostomum heterostomum*, in Invitro condition.

MATERIALSANDMETHODS

a) Collection of Fish Specimens:

In present investigation, I have chosen *Channa punctatus*, which is the primary host of the parasite of *Euclinostomum heterostomum. Channa punctatus* is most edible and commonly available fish in Mahabubnagar, for collecting parasites. The fishes were collected from the lower lake of the Koil Sagar reservoir, and, it is 40 kilometers distance away from Mahabubnagar. They were brought to the laboratory and examined morphologically and internally for the occurrence of helminth parasites.

b) Collection of Parasites (metacercaria of *Euclinostomum heterostomum*):

The host fish *Channa punctatus* was collected for two years at regular intervals from 2009-2011. Fishes were dissected out in physiological saline (0.75% Nacl solution) for collecting helminth parasites (metacercaria of *Euclinostomum heterostomum*). Encysted worms were collected from the liver, gills and internal organs of the fish *Channa punctatus.* The encysted metacercaria of *Euclinostomum heterostomum* were teased from the host tissue mechanically excised in tyrode solution.

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c) Preparation of Tyrode solution: Nacl 8.00 gm/L, Kcl 0.20 gm/L, and $Cacl_2$ 0.20 gms/L, and $Mgcl_2$ 0.10gms/L, and NaH_2Po_4 0.05gms/L and $NaHCO_3$ 1.00 gms/L and Glucose 1.00 gm/L. After mixing all these components take 5 ml of tyrode solution and add in 100 ml of distilled water, and it becomes 5% of tyrode solution.

d) Preparation of *Carica Papaya* Seed Extract:
The fruits of *C. papaya* were (purchased freshly from the market-Mahaboobnagar) cut open and the seeds were collected, dried on top of laboratory bench and pulverized into coarse powder using a hammer mill. About 400 g of the coarse powder was extracted by cold maceration with 1.0 liter of 70% aqueous methanol and intermittent shaking for 48 h. The extract was filtered with Whatmann #1 filter paper and concentrated *in vacuo* to dryness using rotary evaporator. The yield was calculated and the extract stored in a refrigerator at 4°C. The extract yielded about 3.61 w/w dry matter which was light brown in colour.

e) In Vitro Culture of Metacercaria:
Chick Embryo Extract (CEE) which was prepared by Paul (1960) method. Collected progenetic metacercaria of *Euclinostomum heterostomum* were cultured in CEE media by maintaining 7.2-7.4 pH and 4 ^{O}C in Petri dishes in oven for 6 days. Chick embryo extract: CEE was prepared using 11-day-old chick embryos. All instruments and glass ware used were sterile and the processing of the eggs was done in a sterile cabinet.
1. Each egg was held with its blunt end up in a beaker of suitable size standing in the center of a large Petri dish. A small amount of 95% alcohol was poured over the egg to wet the egg-shell, which was then flamed to hasten drying as well as to ensure sterilization. The blunt end of the egg was carefully cut open with a pair of scissors and the membrane beneath the air space was removed using a pair of forceps. The embryo was held by its neck with the help of a sterile steel hook, taken out of the egg and placed in a dish containing Hank's solution. The embryo was washed again in Hanks' contained in a second dish to free it from adhering blood and egg yolk, and transferred to the barrel of a 10 ml Luer syringe. The tip of the syringe was inserted into a 250 ml centrifuge flask held in a clamp and the embryo was expressed by pressing the plunger. The process was repeated with each egg taking care that each instrument used was sterile. An equal volume of Hanks' BSS was added to the pulp of the embryos in the centrifuge flask, stirred with a sterile glass rod and allowed to stand at room temperature for 30 minutes. The mixture was then centrifuged for 20 minutes at nearly 2000 g at 10°C.The supernatant (CEE_{50}) was dispensed in 10 ml aliquots into screw-top universal containers and stored at -15°C. Before use, the frozen extract/medium was thawed slowly in cold water and the precipitate formed was dispersed evenly.

f) *In Vitro* Anthelmintic Assay:
After 6 days adults worms were transferred in to Petri dishes (3) which contains Hanks' solution. The test worms (*n = 10)* were then kept in Petri dishes containing 4 mg/ml concentrations of papaya seed extract in Hank's solution and put inside an incubator at 37 ± 1°C and PZQ (4 mg/ml) were used as reference drugs. One set of worms (*n = 10),* maintained in Hank's solution, was kept as control.

HBSS Composition per 1mL: Sodium chloride (NaCl) 6.4mg, potassium chloride (KCl) 0.75mg, calcium chloride dihydrate ($CaCl_2$. $2H_2O$) 0.48mg, magnesium chloride hexahydrate ($MgCl_2$. $6H_2O$) 0.3mg, sodium acetate trihydrate ($C_2H_3NaO_2$).

RESULTS
In the present investigation the amount of Glycogen, Protein and Lipid were observed after one hour treatment with Praziquantel and Carica papaya seed extract in the metacercaria of *Euclinostomum heterostomum*.

Table.1. Glycogen content in Metacercaria of *Euclinostomum heterostomum* in Control, Treated with Praziquantel and Papaya seed extract at one hour incubation period.

Sample	Control	Praziquantel	Papaya Seed extract
	1 hour	1 hour	1 hour
Mean	6.762	6.649	6.076
S.D±	0.023	0.021	0.009

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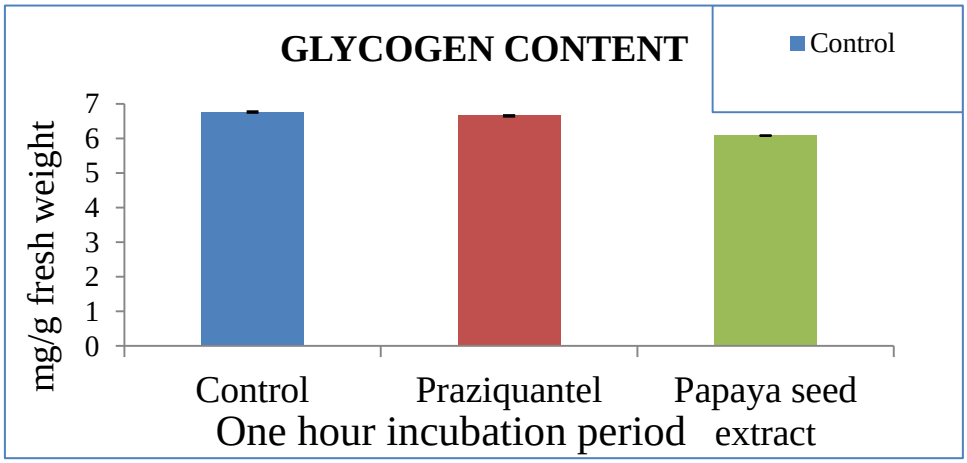
The Glycogen levels were decreased significantly in treated larvae when compared to control. That is from 6.762mg in control followed by 6.649mg in Praziquantel treated and 6.076mg in Papaya seed extract treated metacercaria larva.

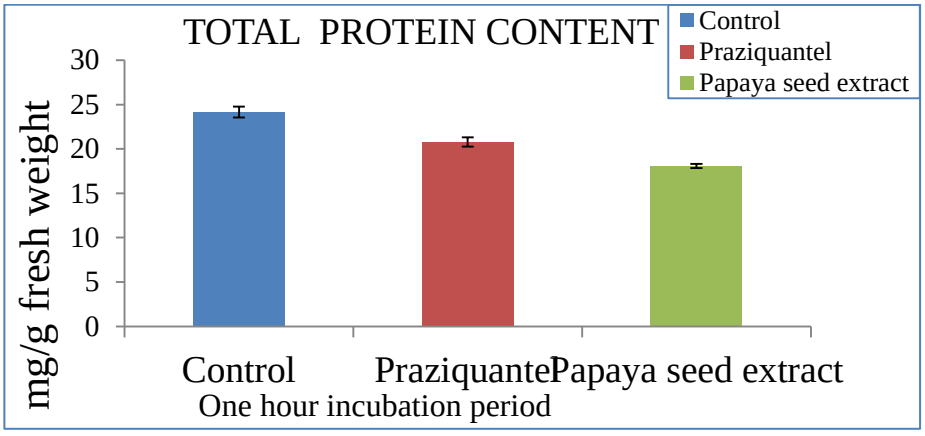
Significant depletion was found in total protein levels when larvae were treated with Praziquantel and Papaya seed extract when compared to control. The decrease was observed as 24.15mgin control, 20.79mg in Praziquantel treated larvae, and 18.09mg in papaya seed extract treated larvae. There was an insignificant decrease was found in total lipid levels in larvae after the treatment also. The values are 21.362mg in control, 21.353mg in Praziquantel treated larvae and 21.353mg in papaya seed extract larvae.



Graph.1. Glycogen content in Metacercaria of *Euclinostomum heterostomum* in Control, Treated with Praziquantel and Papaya seed extract at one hour incubation period.

Table.2. Total Protein content in Metacercaria of Euclinostomum heterostomum in Control, treated with Praziquantel and Papaya seed extract, at one hour incubation period

Sample	Control	Praziquantel	Papaya Seed extract
	1 hour	1 hour	1 hour
Mean	24.15	20.79	18.09
S.D±	0.614	0.521	0.236



Graph.2. Total Protein content in Metacercaria of *Euclinostomum heterostomum* in Control, treated with Praziquantel and Papaya seed extract, at one hour incubation period.

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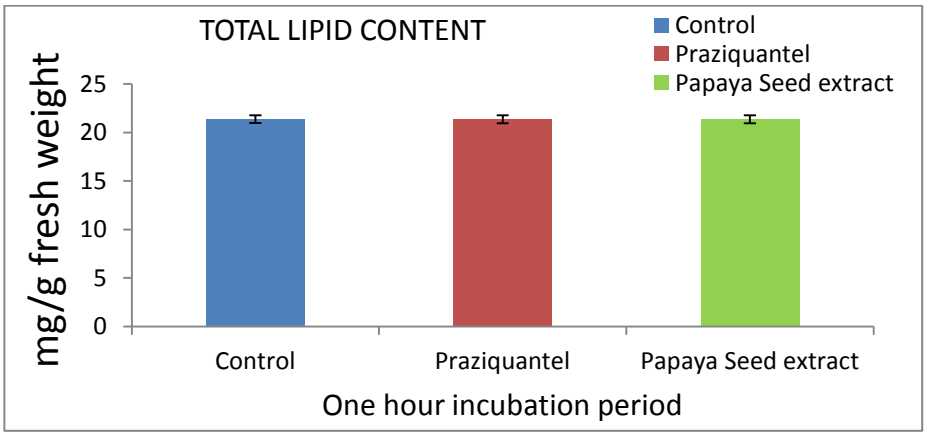
Table.3. Total Lipid content in Metacercaria of *Euclinostomum heterostomum* in control, treated with Praziquantel and Papaya seed extract, at one hour incubation period

Sample	Control	Praziquantel	Papaya Seed extract
	1 hour	1 hour	1 hour
Mean	21.362	21.353	21.353
S.D±	0.404	0.401	0.401



Graph.3 Total Lipid content in Metacercaria of *Euclinostomum heterostomum* in control, treated with Praziquantel and Papaya seed extract, at one hour incubation period.

DISCUSSION

The metacercaria of *Euclinostomum heterostomum* when treated with papaya seed extract and Praziquantel at one hour incubation period showed decreased glycogen content compared to the control. The treated groups showed reduced glycogen content when compared to controls. This may be because the worm becoming stress when treated with the papaya seed extract and is not able to take nourishment from the surrounding medium. Hence the energy supplied through the glycogen reserves so the treated groups showed reduced glycogen content when compared to controls**.** Rajakumari (1989) observed depleted levels of glycogen in *Schistosoma mansoni* and *Heteropneustes fossilis* exposed to an organochlorine insecticide. Vijayavel *et al*. (2006) noticed depleted value of glycogen in Haemonchus contortus of Oreochromis mossambicus under monocrotophos and ammonium chloride stress condition. Crestani *et al*. (2005) observed decrease in glycogen content in trematode larva of mussels with clomazone herbicide. Tripathi *et al*. (2003) reported decreasing tend of glycogen content in *Clinostomum complanatum* of *Channa punctata* during the administration of organophosphates.

The total protein content also decreased in metacercaria of *Euclinostomum heterostomum* compared to Praziquantel and control. This is due to which the rapid decrease in the worms and are becoming sluggish, to withstand this proteins of the worms may be entering into energy cycle due to this reason the protein content decreased in treated worms compared with control worms. These digestive enzymes can break down the peptide bonds and involve in the formation of amino acids it can be studied in only few trematodes, in the intestinal caeca of *Schistosoma mansoni* was studied by Rogers (1940). There was not much difference in total lipid content of treated and control groups. This may be the worms are not used lipids as the energy source during the stress conditions.

CONCLUSION

Due to the strong effect and highly destructive nature of the *Carica papaya* seed extract against the metacercaria of *Euclinostomum heterostomum*. Results conclude that the *Carica papaya (CPE)* seed extract is more effective and accurate and highly preferable when compared to the Praziquantel. CPE is acting only on parasitic worms in 4 mg/ml concentration, at one hour incubation period. Finally I want to conclude that this herbal antihelmintic drug that meets with needs of small hold farmers, and is available in the simplest formulation. This herbal drug is more applicable to control all vertebrate trematode parasites.

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