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Antihemintic and Hematological Changes of Natural Plant Carica papaya Seed Extract against Gastrointestinal Nematode *Hymenolepis nana*

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Abstract
The purpose of this experiment was to study the anthelmintic activity of *Carica papaya* seeds against *Hymenolepis nana* in infected mice. In this experiment we use three groups (A, B, and C) of mice, group (A and B) were infected with eggs of *Hymenolepis nana*, PCV, Hb and W.B.C counts respectively. Group A mice were treated with *Carica papaya* seed extract (0.1ml /mowe) blood and fecal samples were collected on day 0, 7 and 14 after administration of treatment to examine change in blood packed cell volume PCV Hb, W.B.C, fecal egg counts (FEC). Group C served as a no infected group (negative control). FEC of group A was lower than the control group, PCV, Hb and W.B.C increased after 7days then return to normal value after 14days. The histopathological study showed papaya repair the tissue of intestine and return the villa to normal value.
The results recommended using the C.papaya as an aid to control gastrointestinal nematodes infection.
Keywords: Anthelmintic activity *Carica papaya*, *Hymenolepis nana,* mice, gastrointestinal nematode.

1. INTRODUCTION

Hymenolepiasis is a disease caused by *Hymenolepis diminuta* (rat tapeworm) and *Hymenolepis nana* (dwarf tapeworm) *(*Waugh*, et al., 2006). Hymenolepiasis* has a high prevalence in populations in tropical and subtropical climates characterized by poor hygiene and *poverty (*Melhorn*, , et al., 2008;* Roberts*, 2008)..Hymenolepis nana,* is a cosmopolitan intestinal cestode helminthes of the warmer climates, whose entire life-cycle is completed in the bowel, so infection can persist for years if left untreated *(*Hardy*, et al., 1941). Hymenolepis nana* is the most common cause of all cestode infections and is found globally. In temperate zones, its incidence is high in children and institutionalized groups. *H. nana* infections are typically asymptomatic, but heavy infections by *H. nana* can cause headaches, weakness, anorexia, abdominal pain, and diarrhea *(*Chitchang*, et al., 1985)*. *H. nana* is the only cestode capable of completing its cycle without an intermediate host. Infection is most commonly acquired from eggs in the feces of another infected individual, which are transferred by contamination in food *(*Smyth*, et al., 1989)*. .
Since ancient times, plants and herbal preparations have been used as medicine. Traditional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of diseases successfully including antibacterial and anthelmintic, anti-inflammatory etc*(*Ajaiyeoba*, et al., 2001)*. *Carica papaya*, belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases *(*Munoz*, , et al., 2000;* Mello *et al.,, 2008).* Originally derived from the southern part of Mexico, *C. papaya* is a perennial plant, and it is presently distributed over the whole tropical area. Many scientific investigations have been conducted to evaluate the biological activities of various parts of *C. papaya*. *(*Seigler*, et al., 2002)*. Anthelmintic properties have been reported for latex and seeds from *C. papaya* when used in humans and mice*(*Okeniyi*, et al., 2007)*. The anthelmintic efficacy of Papaya might be due to presence of proteolytic enzyme such as papain, chymopapain and lysozymes in the latex as well as in leaves (Dakpogan*, et al., 2005)*.

In the present study, an attempt has been made to screen and evaluate the anthelmintic activity of *C. papaya* against *H.nana*.

2. MATERIALS AND METHODS

Preparation of the *C.papaya* seeds extract

The seeds were collected freshly from the market of Maskat / Oman, washed with clean water to remove dirt. The seeds were sundried and later grinded into powdery forms. The *C.papaya* seed powders were weighed (75g), and blended into liquefaction in 150ml of distilled water, the mixture was then centrifuged at 1500rpm. The supernatant was filtered through sterile filter papers into conical flask as the study extract. One milliliter of the filtrate is expected to contain 0.5g (500mg /ml).

2.1Experimental animals

46 adult healthy male Swiss albino mice, about 4-5 weeks old, weighing 20-25g were maintained under GLP conditions. 36mice were orally infected with about 100 viable eggs of *H.nana* per mouse, these infected mice were maintained until the 6th day of infection to complete the life cycle of the parasite.
On the 16th day, the mice were divided into 2groups (A, B) with 18mice per group. Group A received no medication (normal saline). Group B were administrated orally with the aqueous crude extract of *C.papaya* at

(0.1ml /day). These groups were compared with third group (C) contain 9 noninfected mice which used as control negative.

For all studies, feces and blood were collected on days 0, 7 and 14 after administration of treatment. Blood was collected by the decapitation method*(*Dieterich***, *et al., 1972)***. anti-coagulated with 1.5mg/ml blood of the dipotassium salt of Ethylenediaminetetraacetic acid (EDTA), the total and differential leukocyte counts analyzed using an automatic blood analyzed*(*Lewis***, *et al., 1991)***., and packed cell volume (PCV) was determined by the conventional method. Fecal egg count were performed using a modified *McMaster technique (*Whitlock***, *et al., 1948)***. , also on 14th day the mice were sacrificed and the intestine removed, put in (10%) formalin for histopathological study.

2.2Statistical analysis

Results are expressed as the mean of parameters ± standard error of the mean (SEM), differences between mean were evaluated using the Anova tests.

3.RESULT AND DISCUSSION

Haematological results showed that the PCV mean values for animals in group A (control group negative) was (52- 51.5%)after 0.7 and 14days respectively, while in control group positive group C the PCV decreased between 0 and 14days (45-42%). After treatment with *C.papaya* seed extract this parameter showed increased slightly (45-52%) between 0 and14days.

In the case of Hb (g /dl) the mean values for in group A 17(g /dl) and decrease in group C between 0 and 14days (14.6-13.6) and Hb return increase during this time in group B (14.6-17).

W.B.C. values increase in group C while after treated with *C.papaya* in group B return to normal value 5.75×10^3. The lymphocyte value exhibited increased in group B, C (7×10^3) then decrease during this time period in group B (5.75×10^3). Anova tests showed that when PCV, Hb and lymphocyte W.B.C. compared between groups (B, C) and control group (A), the differences were significant at $p< 0.001$(Table 1).

Parameter	Group	1 day	7 days	14 days
P.C.V. (%)	A	52 ± 0.81	52 ± 0.81	51.5 ±1.29
	B	45 ± 0.95	51 ± 0.81	52 ±0.1
	C	45 ± 0.95	43 ± 0.81	42 ±0.5
HB(g/d)	A	17 ± 0.27	17 ± 0.27	16.8
	B	14.6 ± 0.95	17 ± 0.81	17 ± 0.01
	C	14.6 ± 0.95	14 ± 0.81	13.6 ± 0.5
W.B.C	A	5×10^3 ± 0.81	5×10^3 ± 0.81	5×10^3 ± 0.81
	B	7×10^3 ± 0.81	5.87×10^3 ± 0.25	5.75×10^3 ± 0.95
	C	7×10^3 ± 0.81	7×10^3 ± 0.81	7×10^3 ± 0.81
Lymphocyte	A	61.66 ± 2.88	61.66 ± 2.8	61.66 ± 2.8
	B	68.66 ± 1.15	63 ±1	62 ± 1.5
	C	68.66 ± 1.15	69 ±0	69 ± 0

Table1: Haemogram of experimental group during *Carica papaya* trails

Fecal egg counts showed that the mice in control group heavily shedding eggs in the feces till 14th days ,while in group B after administration of aqueous extracts produced a significant reduction in the fecal egg count after 4days, and the eggs disappear completely after 9days Fig(1).

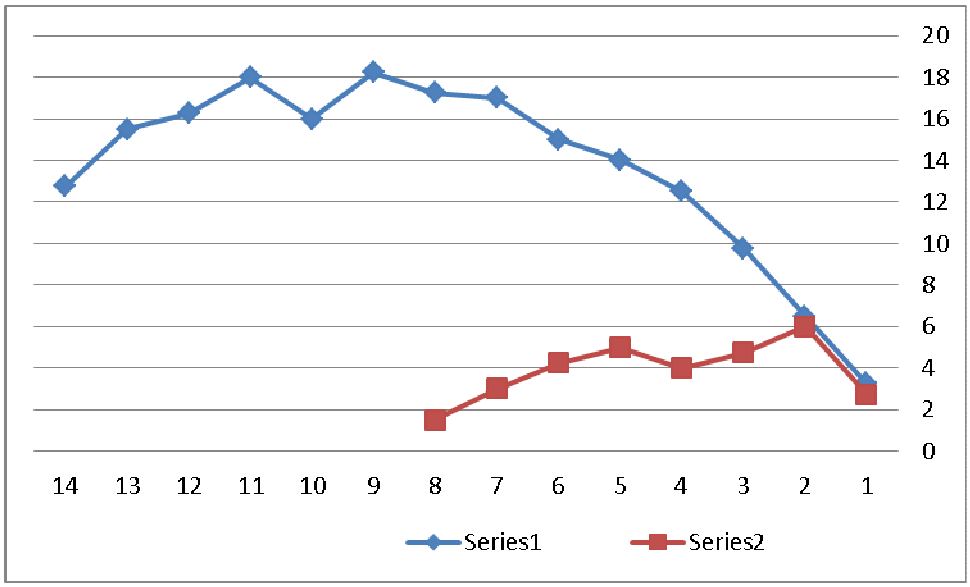

Fig 1:Egg per gram (EPG)level of infestation before and after treatment with papain

3.1Histological study

The histological study showed that *C.papaya* plant was able to re-structure of intestinal tissue (Fig 3) has been able to bring along the length of the villa to normal when compared with control mice (Fig 1), after it suffered from the palace because of parasite and also led to a lack of preparation of goblet cells (Fig 2).

The aqueous extracts administered in this study caused significant reduction in the worm burden of the mice, the reduction of helminthes egg loads observed with aqueous extracts of *C.papaya* maybe attributed to the presence of papain in capable of digesting bacteria and parasitic cells, hence its use an anthelmintic and antibiotics(Fajimi***, et al., 2005)***.Anthelmintic is a substance that expels or destroys gastrointestinal worms, all anthelmintic essentially kill worms by either starving them to death or paralyzing them, because worms have no means of storing energy. The must sat almost continuously to meet their metabolic needs. Parasite will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut. Adu *et al.* ***(2009)***.reported that *C. papaya* has certain chemical components that are of high anthelminthic attributes in poultry with satisfactory efficacy at the dosage of 1200mg/bird.

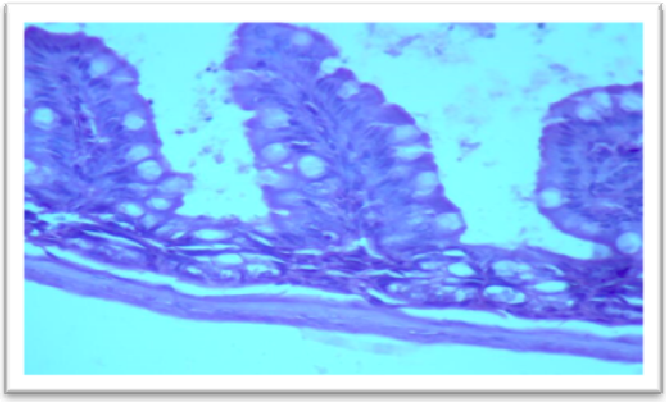

Figure 1. Cross- section of a healthy mice intestine high, narrow intestinal villi with a small number of goblet cells and oligocellular mesenchymal stroma.x400.

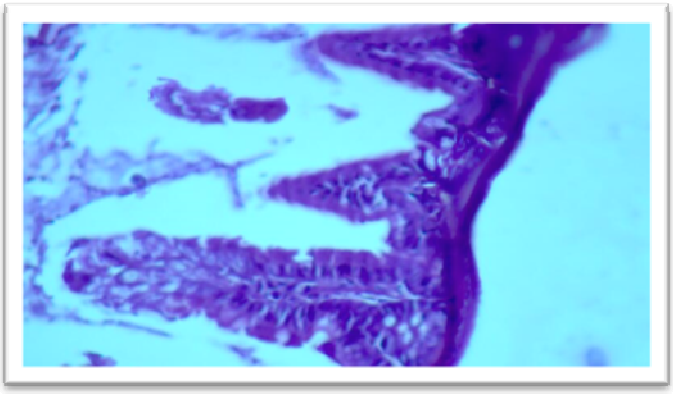

Figure 2. Cross- section of infected mouse's intestine shows shortening of intestinal villi with atrophy of the villi and depletion of goblet cells.x400.

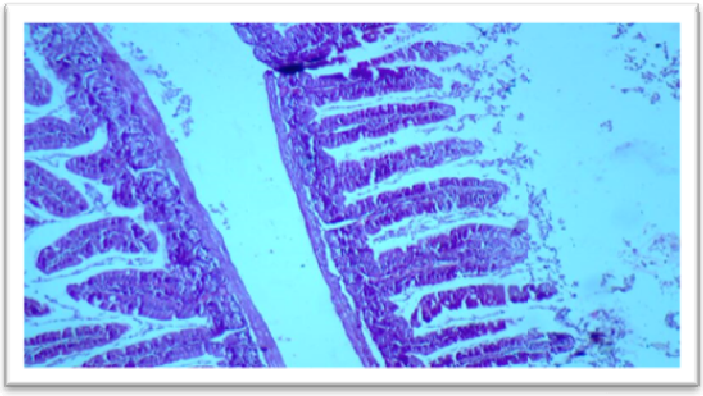

Figure 3. Section of treated mouse's intestine showing normal like appearance of height of villi and presence of goblet cells few in number.x250

The mechanism of action of the efficacious plant cysteine proteinases (papaya) is similar, and probably identical involving digesting and removal of the cuticle. Our results support previous studies *(*Mursof***, et al., 1991)***.* suggesting that papaya may have potential as an anthelmintic against nematode parasites and too define the mechanisms of its antiparasitic action.

Worm counts of *Hymenolepis nana* in mice decreased in response to 1-2g*/*kg papaya seeds for three consecutive days *(*Lamtiur***, et al., 1945)***.*,* also showed the reduction percentage esin (EPG_egg per gram) for papaya seeds treatment *Hymenolepis deminuta* in rats, were very high which is 96.8% for 0.6g. kg dose level and 96.2% for 1.2g kg. Dose level *(*Sapaat ***et al., 2012)***.

Fetterer *et al.*(1978) found an increase in the immunity to *Hymenolepis nana* infection by transfer from spleen cells, lymphocytes are essential for the repair mechanism, while Burke *et al.*(2009) reported there was no indication that administration of papaya seed led to a reduction in gastrointestinal in lambs.

When papaya latex given in water at dose level of 2, 4, 6and 8g */*kg for treated mice infected with 100 *Heligmosomoides polygurus* found after 3days papaya latex an antiparasitic efficacy of 55.5, 60.3, 67.9 and 84.5 respectively*(*Satrija***, et al., 1995)***.* also Ameen *et al.* (2012).showed that the powered and aqueous extract of *C.papaya* after its administration, produced a significant increase in packed cell volume, red blood cells, hemoglobin concentration lymphocyte counts and significant decrease in eosinophil counts. Shaziya and Goyal(2012) found *Carica papaya* extract treated mice clearly demonstrated a reduction of larvae for *Ancylostoma caninum*, also eosinophil levels were markedly reduced in 24hr after treatment.

Keiichi *et al.*(2008)suggest that the oral administration of the fermented papaya preparation to mice may have a therapeutic potential for the prevention of contact hypersensitive immuno-response, also Melissa *et al.*(2010). found no morphological alteration in the integrity of the intestine mucosa after 3months administrated for Wister rats.

The use of plant material for the management of nematode population is apparently effective and environmentally friendly compared to synthetic nematicides.

4.CONCLUSION

From the result it is conclude that the delicious *Carica papaya* fruit can use it as anthelmintic drug, but we need to conduct research to detect and extract the active substance and use it in treatment.

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