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Anthelmintic effect of Natural Plant (*Carica papaya*) extract against the Gastrointestinal nematode, *Ancylostoma caninum* in Mice

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Abstract

Infection with gastrointestinal nematode have severe consequences for the health of millions of people worldwide, and cause serious economic losses in live stock farming. Synthetic drug have been considered the most effective way of controlling parasite infections. But these drugs are expensive and sometime unavailable to people and show the side effect hence anthelmintic offer a simple, cheap, cost effective method of controlling parasites with no side effect. The purpose of this experiment was to study the anthelmintic activity of Carica papaya extract against Ancylostoma caninum in infection in mice. Two experiments were setup for this study, in experiment no. 1, two groups (A and B) and experiment no. 2, three groups (A, B and C) of mice were taken for larval recovery and mast cell & eosinophil counts respectively. Group A mice were treated with plant extract (Carica papaya) 0.2 ml/ mouse, on day -14 and -7 day before challenge infection and on day 0 mice were challenge with 500 A. caninum larvae. Group B mice were challenge only with dose of 500 Ancylostoma caninum larvae. Group C served as a non treated control. Results of plant extract treated mice clearly demonstrated a reduction of larvae in group (A) when compared with group (B) of mice. Large number of mucosal mast cell observed on day 16 in all groups. Eosinophil levels were markedly reduced in 24 days after challenge infection in all groups. The results suggest a potential role of Carica papaya extract as an anthelmintic activity against intestinal nematodes infection.

Key words: - Anthelmintic activity, *Carica papaya*, Mice, *Ancylostoma caninum*

Introduction

Nematode infection threatens the health and welfare of livestock and compromises the efficiency of livestock production. Nematodes are possibly the major disease challenge facing ruminants[1]. Essentially all grazing livestock are exposed to infection. There are four species of hookworms that infect dogs (*Ancylostoma braziliense, Ancylostoma caninum, Ancylostoma tubaeforme* and *Uncinaria stenocephala*). In dogs, *A. caninum* is the most common hookworm and causes the worst disease. Despite the fact of development of anthelmintic resistance[2-6] in parasites of high economic significance, chemotherapy is still the most widely used option for the control of helminthes. However, many farmers in the developing countries are unable to afford synthetic anthelmintic for their livestock. In this scenario, the farmers depend on time- honoured, centuries- old, affordable and accessible treatments for parasites. Intestinal nematodes are ubiquitous parasites of man and domestic animal. In man such infections are common in countries where climatic and sociological conditions favour transmission. In domestic animals gastrointestinal infections are invariable accompaniments of high density stocking and intensive production, and are responsible for enormous economic losses. The main symptom of the disease is anaemia, accompanied by hydreamia, sometimes oedemas, general weakness and emaciation. Hookworm infection is one of the most prevalent and clinically significant communicable diseases of humankind affecting up to one fourth of the world population. New synthetic anthelmintic drugs or vaccines are unlikely to be available in the near future, so alternative strategies for the control of these parasite infections are urgently required. Several medicinal plants have been used in (as anthelmintic) the treatment of GI nematode infection in developing countries e.g. pineapple (*Ananas comosus;*[7], fig (*Ficus species;*[8], A*framomum sanguineum, Dodonea angustifolia, Hildebrandtia sepalosa, Myrsine africana, Rapanea melanophloeos, Spigelia anthelmia, kiwi fruit (Actinidia, Chinensis), Hagenia abyssinica*, etc. The use of medicinal plants for the prevention and treatment of gastrointestinal parasitism has its origin in ethno veterinary medicine. In a recent experimental study, papaya was shown to anthelmintic activity against patent *Ascaridia galli* [9]. Among the earliest and most widely used have been plants which contain proteolytic enzymes of the cysteine catalytic class such as papaya[10]. The present study was therefore, carried out to the anthelmintic activity of *Carica papaya* extract infected with gastrointestinal nematodes *A. caninum* larvae in mice.

Materials and Methods

Source and Collection of *A. caninum* larvae: - Faecal sample were collected from dog experimentally infected with a pure strain of *A. caninum* and this served as the donor animal throughout the study.

Experimental Animal: - The Swiss albino mouse, *Mus musculus albinus* was selected as an experimental animal for the present studies. Originally they were brought from the College of Veterinary Science and Animal Husbandry; Mhow. Mice were kept, bred and maintained is the animal house under ideal condition of light, temperature, ventilation and food.

Cultural techniques of *A. caninum* larvae: - Infective filliform larvae of *A. caninum* were obtained by the petri dish method of [11].

Method for counting of larvae: - The number of actively motile larvae counted by dilution method of [12].

Preparation of dose: - Inoculums of 0.2ml / mouse was orally administered into the stomach with a suitable sized syringe fitted with a blunt 2" 18 gauge feeding needle.

Larval recovery in various organs in mice: - Mice from both groups (A and B) were sacrificed under ether anaesthesia at various intervals according to the experimental design and larval recoveries were made from different organs and parts of body and actively motile larvae counted under a dissecting microscope.

Mast cell count: - A 2 cm length of small intestine taken 10 cm from the pyloric sphincter was fixed in Carnoy's fixative and processed using standard histological techniques. Section cut at 5µm were strained with Alcian Blue, counterstained with Safranin O using the method of 1with the following modification section were strained for 25-30 min in Mayer's haematoxylin, then for 20-25 min in phosphate- buffered Safranin O before processing and mounting in DPX.

Eosinophil count: - Blood sample were collected into heparinized capillary tubes and diluted 1:10 in discombe's fluid with 3% EDTA. Eosinophil counts were made using a haemocytometer and values expressed as number of cells/ ml of blood. To reduce the effect of diurnal variation in eosinophil numbers, counts were made between 08.45 and 10.00h. Control values determined from untreated mice in each experiment were always low.

***Carica papaya:* -** This is commonly known as "Papita". Most widely used have been plants which contain enzyme of the cysteine catalytic class such as papaya. Statistical analysis were done following student't' test [13].

Results and Discussion

The larval recovery was made at 6 hours interval from both experimental and control group of mice. The larvae recovered from mice of experimental group A was (270) and control group B (450) at 6 hours after challenge infection. There was a decreased in number of larvae at 12 hours after infection from group A was (245) and B (425), suddenly a great decreased in larval recovery was observed at 18 hours from group A was (200) and group B (390). Maximum larval reduction was observed at 24 hours from group A (160) and B (365). At 48 hours larvae started migrating to abdominal and thoracic muscles, and also in fore limb and hind limb. The larval recovery from group A (135) and B (330) where as at 72 and 96 hours was from group A (110 and 7) and B (300 and 275) respectively. Fig no. 1

Mast cell response in 8^{th} day after challenge infection was observed in experimental groups A (600) group B (1060) and control group C (11); Where as 12^{th} day after challenge infection in group A (850) group B (1300) and control group C (10). Number of mast cell count decreased at 20^{th} day after challenge infection in comparison to 6 and 12^{th} day in group A (253) group B (1320) and control group C (7). Again number of mast cells great decreased at 24^{th} day after challenge infection in A (126) B (1097) and C (3). Increased number of mast cells was observed on day16 in all groups of mice, in group A (1012), ($p < 0.05$) B (1710), ($p < 0.001$) and control C (8). However there is significant variation in number of mast cells between experimental groups A, B and control C. Fig no. 2

The numbers of eosinophil counts was in experimental group A (282000) group B (392306) and control group C (93701) at 4^{th} day after challenge infection. Increased number of eosinophil was observed on day 8^{th} and 12^{th} day after challenged infection, the eosinophil count was in group A (470500 and 511000), group B (520780 and 564964), and group C (90361 and 87000) respectively, although there is great variation in counts amongst group. Decreased number of eosinophil was observed on day 16^{th}, it was in group A (350000) group B (470500) and control group C (74020). Continuous decreased number of eosinophil was also observed on day 20^{th} and 24^{th} in experimental group A (170000 and 110000) and group B (370700 and 350000) and control group C (66218 and 52513) respectively. Fig no. 3

An anthelmintic is a substance that expels or destroys gastrointestinal worms. The more common name is dewormer or "wormer". Anthelmintics are also called parasiticides, endectocides, nematodcides, parasitic, antiparasitic, and drenches. All anthelmintics essentially kill worms by either starving them to death or paralyzing them. Because worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs. Any disruption in this process results in energy depletion. Interfering with feeding for 24 hours or less sufficient to kill most adult parasites. Parasites will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut. The result of the present experiment clearly demonstrated a reduction of worm burdens in mice receiving *Carica papaya* extract, the larval recovery was (270 within 6 hours and 7 within 96 hours). It is due to the immunity produce by the host.

The mechanism of action of the efficacious plant cysteine proteinases (Papaya) is similar, and probably identical, involving digesting and removal of the cuticle. It is evident that

the loss of motility associated with incubation of *H. polygyrus* adult worm in cysteine proteinases occurs whenever the cuticle is damaged, suggesting that these nematodes are sensitive to cuticle removal/ damage. Mean worm recovery of group A treated with plant extract (270 within 6 hour and 7 within 96 hour) were significantly reduced compared with control group B (450 within 6 hour and 275 within 96 hour).

These results support previous studies [14] suggesting that Papaya latex may have potential as an anthelmintic against nematode parasites and too define the mechanisms of its antiparasitic action. 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 [15]. Occurring in tissues throughout the body, mast cells are part of the immune system (defence mechanism of the body) and respond to inflammations, infections, allergies and disease. They can release large amounts of very powerful chemicals including enzymes that break down proteins (proteolytic enzymes), histamine, heparin, prostaglandins and seratonin. Toxic to foreign invaders, such as parasites, these enzymes are released into the body when mast cells are triggered by the immune system. These chemicals are vital to normal body functions, especially immune response. However, they can be very damaging when released in chronic excess, affecting blood pressure, heart rate and other body functions. Because of this, sites where mast cell tumours are surgically removed can sometimes refuse to heal leading to life-threatening diseases, such as gastric ulcers, allergies and internal bleeding. Maximum number of mast cell was observed in all groups A (1012), B (1710) and control C (8), of mice during the entire 16 days period after challenge infection. Mice showed a much slower rate of larvae expulsion and correlated with lower mucosal mastocytosis. Larvae were eliminated or destroyed by plant extract. Anthelminth kill existing parasites and reduce the production of egg. Eosinophils have been shown to be potent effector cells for the killing of helminthes parasites *in vitro* culture. Mechanisms of parasite killing by eosinophils are widely studied and are often implicated in mediating resistance to parasitic infection, especially in infection with specific antibodies. Evidence for the eosinophil as an anti parasite killer cell *in vivo* is limited and may not justify the belief that eosinophils engage and / or kill infective helminthes. Increasing number of eosinophil correlated with migration of larvae to the muscles. The results of present study clearly demonstrated that the eosinophil response of mice was affected by its immune status during *A. caninum* challenge infection. Mice infected orally reached to the intestine, immunity acts against the intestinal stages during a primary infection, and subsequently against tissue stage infection. Larva being trapped in eosinophil reached inflammatory foci in the lungs or the skin. Eosinophil levels were markedly reduced in 24 days after challenge infection. Reduced eosinophil number was also observed in group A (110000), group B (350000) and control group C (52513) on day 24 after challenge infection. Infected mice showed higher levels of eosinophil then treated with plant extract. The data are discussed in terms of eosinophil counts in mice treated with plant extract and challenged with *Ancylostoma caninum* larvae during experimental Ancylostomiasis.

Conclusion

An anthelmintic is a substance that destroys gastrointestinal larvae, it is observed that the number of (*Ancylostoma caninum*) was significantly reduced by the anthelmintic effect of plant extract. Larvae were eliminated or destroyed by the extract, in which mast cells and eosinophil plays important role.

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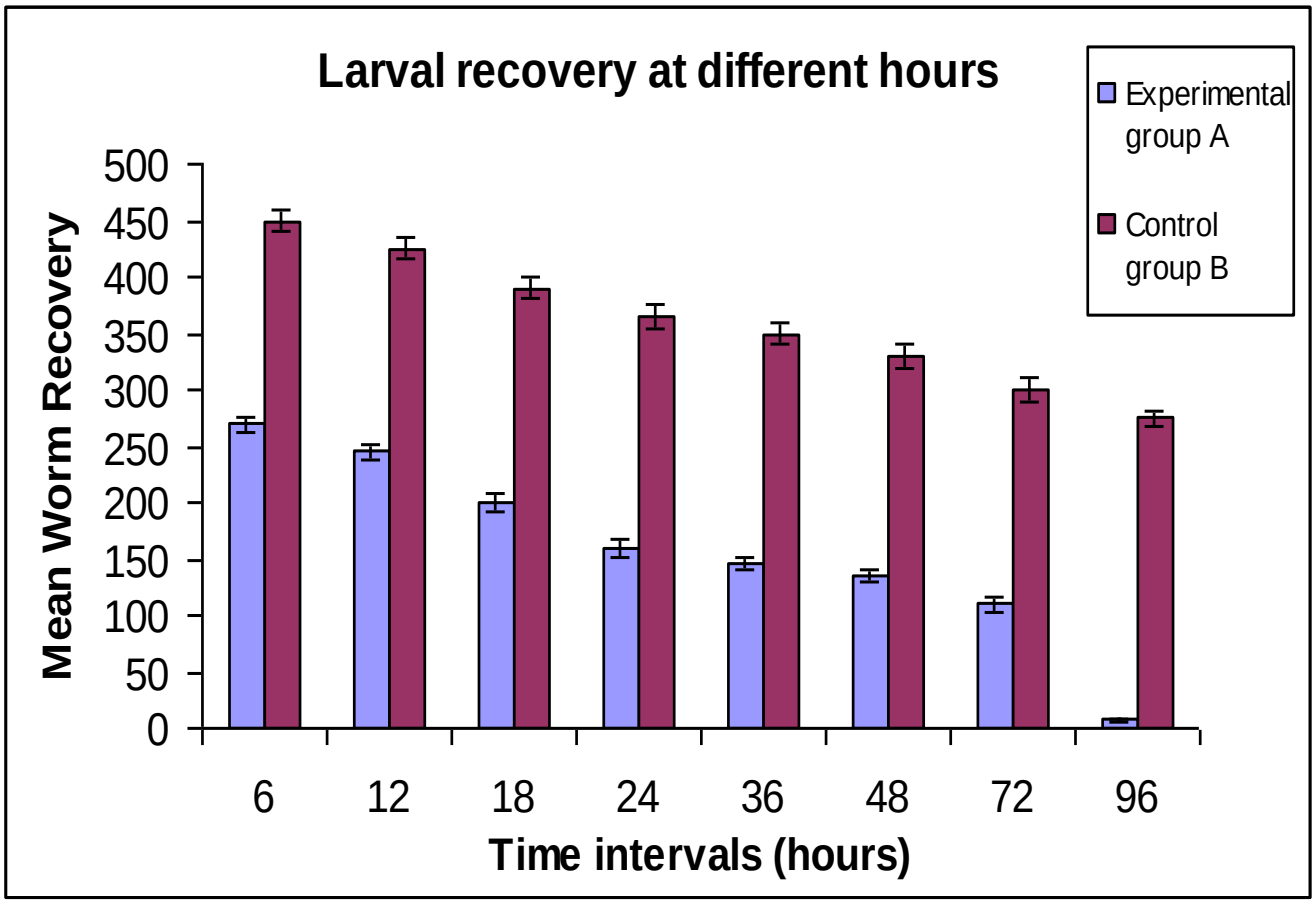

Figure -1
Larval recovery from experimental and control groups of mice. Results from experimental group were compared with those of the control group

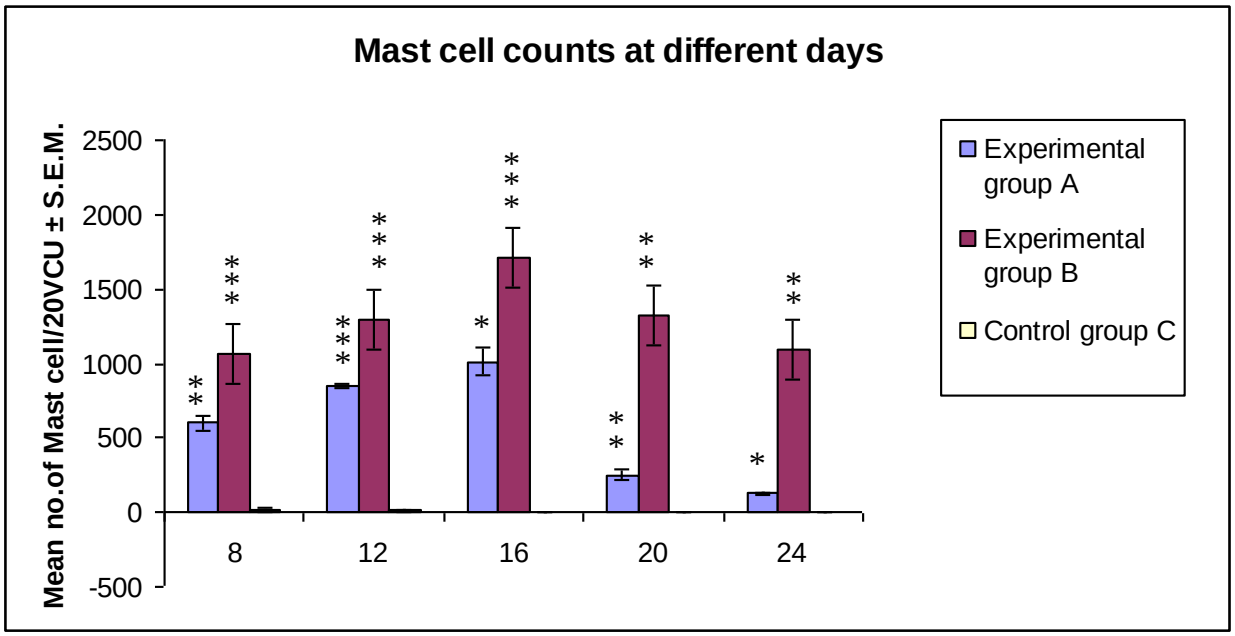

Figure -2

Mean no. of mucosal mast cells per 20 villus cript units (V.C.U.) from experimental and control groups of mice. Results from the experimental groups were compared with those of the control group. Significance of difference from experimental and control groups. (* p < 0.05, ** p < 0.01, *** p < 0.001; Student's't' test)

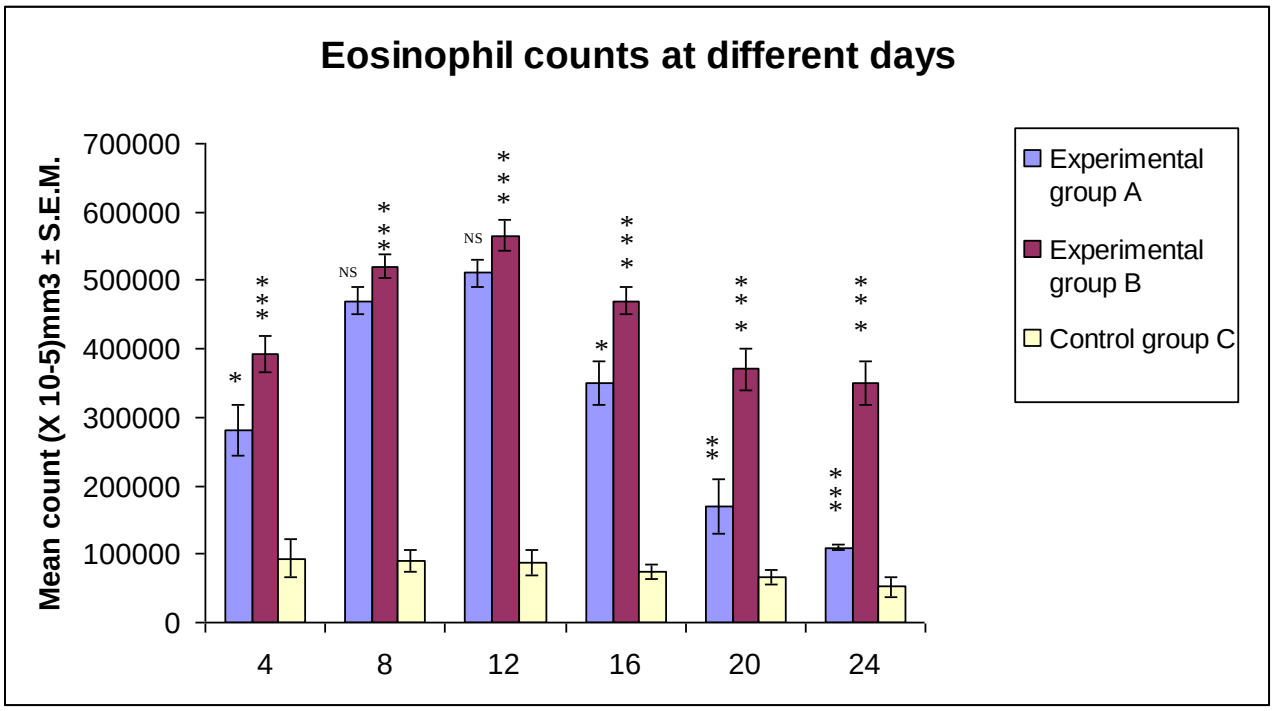

Figure – 3

Eosinophil response from experimental and control groups of mice. Results from the experimental groups were compared with those of the control group. Significance of difference from experimental and control groups. (* p < 0.05, ** p < 0.01, *** p < 0.001, NS- Non significant; Student's't' test)