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# Role of Neem (*Azardirachta indica*) as a Plant extract Dewormer for *Ancylostoma caninum* Infection in Mice

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## Abstract

*Gastrointestinal parasite is serious threat to the productivity of livestock in developing nation. The major mechanism of controlling nematode parasite of livestock has been limited to the use of synthetic dewormer. Several plant products have been exploited for their dewormer activity. Neem has been shown to possess many medicinal properties including dewormer property. The purpose of this experiment was to study the dewormer activity of neem against A. caninum in infected mice. Two groups of mice were infected with A. caninum infective larvae. Before infection one group of mice were given neem extract at dose level of 0.2ml/ mouse. One group of mice served as non treated group. The dewormer activity was determined by larval reduction, mast cell and eosinophil cell level. Neem extract were highly effective in reducing the number of A. caninum. Larval reduction showed that the number of larvae reduced was higher in the treated and infected group compared to the infected group within 72 and 96 hours after challenge infection. Mast cell result suggest that on day 16 and 24 in mice infected with A. caninum larvae developed higher mastocytosis in comparison to treated and infected group. Decline level of eosinophil cell recorded on day 16 and 24 in treated and infected group when compared with infected group. The result suggests that the number of larvae correlated with number of mast cells and eosinophil cell and a potential role of neem extract as a dewormer activity against A. caninum in mice.*

**Keywords**: Dewormer activity, azardirachta indica, mice, parasite.

## Introduction

Gastrointestinal nematode infections affect 50% of the human population worldwide, and cause great morbidity as well as hundreds of thousands of deaths. The prevalence of intestinal nematode infections is apparently very high and on a global scale these infections cause severe health problems in man and domestic animal, especially in developing countries. In domestic animals gastro- intestinal infections are invariable accompaniments of high density stocking and intensive production, and are responsible for enormous economic losses. Therefore there is an increasingly urgent need to develop alternative or supplementary methods of nematode control. These methods fall into 5 categories: grazing management, biological control, nutrient, vaccination and genetic approaches. The severity of disease and the loss of production depend upon the intensity of infection, immunity of the host and its relative nutritional status[1,2]. The use of sustainable, integrated parasite control system, using scientifically proven non chemical methods and limited use of drugs is being considered to insure animal health and food safety. In dogs, *A. caninum* is the most common hookworm and causes the worst disease. Synthetic drugs are expensive and not easily available to people and also show various side effects in host body. Several plant products have been exploited for their dewormer activity. Thus a better and less expensive is dewormer for the benefit of people infected with parasite. Neem belongs to the family of Meliaceae. The neem tree, *A. indica* is known for its medicinal properties and has been recommened for use against gastrointestinal nematodes[3,4], include the dewormer property. In the present study we tested the neem extract against infection of *A. caninum* in mice.

## Material and Methods

**Cultural techniques of *A. caninum* larvae: -** Infective filliform larvae of *A. caninum* were obtained by the Petri dish method of Sen K.G., Joshi U.N. and Seth D.[5].

**Experimental Animal:** The Swiss albino mice, *Mus musculus albinus* was selected as an experimental animal for the present studies.

**Source and Collection of *A. caninum* larvae:** Faecal sample were collected from dog experimentally infected with a pure strain of *A. caninum.*

**Preparation of dose:** Inoculums of 0.2ml per mouse was orally administered with a suitable syringe sized.

**Method for counting of larvae:** The number of actively motile larvae counted by dilution method of Scott J.A.[6].

**Plant Extract:** This is commonly known as "Neem". *Azardirachta indica* is used throughout the tropics against various ailments including helminth parasites.

**Larval recovery in various organs in mice:** Mice from both groups (control and experimental) were scarified 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.

**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.

**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 Alizadeh H. and Wakelin D.[7].

**Statistical Analysis:** Statistical analysis were done following student't' test[8].

## Results and Discussion

Our results showed a maximum reduction of larvae (341) in treated group when compared with infected group (50) at 72 hours after challenge infection, where as highest reduction of larvae was observed at 96 hours after challenge infection in treated group (391) and infected group (200). Our results suggest significant reduction of larvae in treated group when compared with infected group (figure 1).

Maximum mast cell level (1146) was recorded in mice treated group and (1710) infected group on day 16 post infection. Suddenly a decreased mast cell level 146 and 1097 treated and infected group respectively on day 24 after challenge infection. However these is a significantly variation in mast cell level between treated and infected group (figure 2).

At 16 days eosinophil cell level was recorded in (354000) treated and (470500) infected groups. On day 24 decline level recorded in mice treated (112000) and (350000) infected group. Increased no. of eosinophil cells correlated with migration of larvae to the muscles. Larvae were eliminated or destroyed by the neem plant extract. Eosinophil levels were markedly reduced in treated group when compared with infected group. A great difference observed in treated and infected group (figure 3).

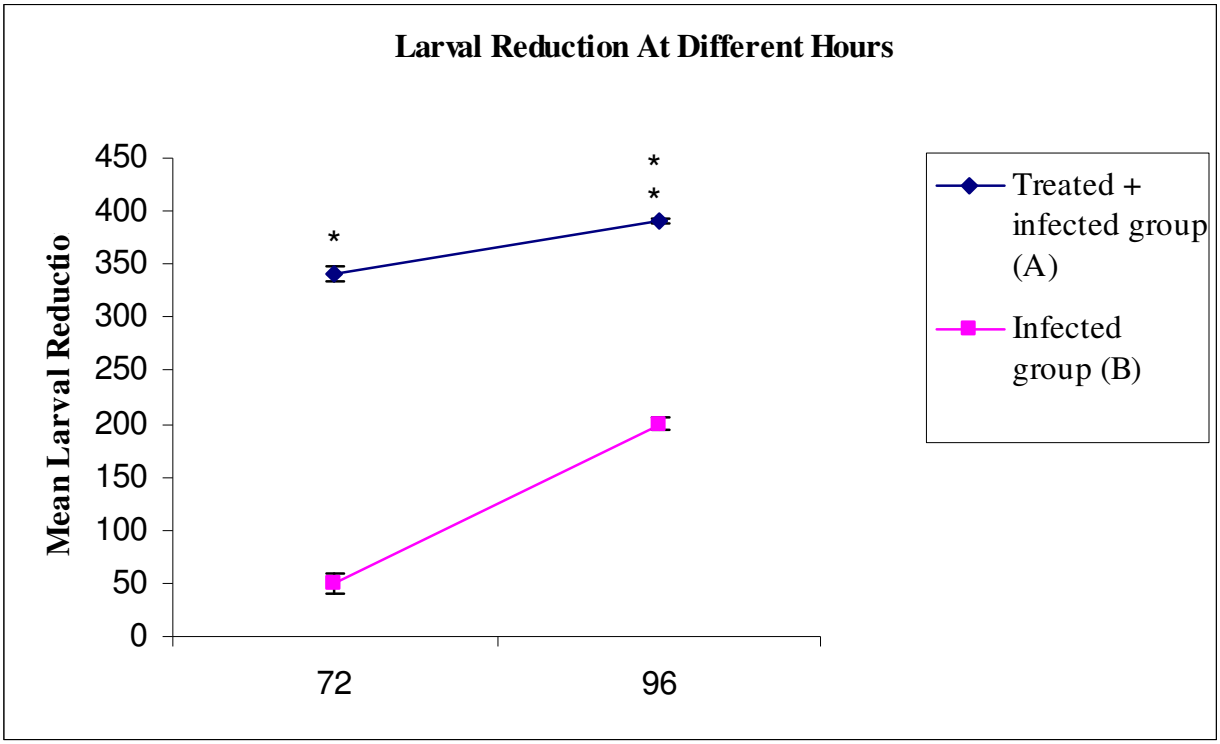


**Figure-1**
**Larval reduction from group (A) and (B) of mice. Significance of difference from group (A) and (B). (* p < 0.05, ** p < 0.01; Student's't' test)**

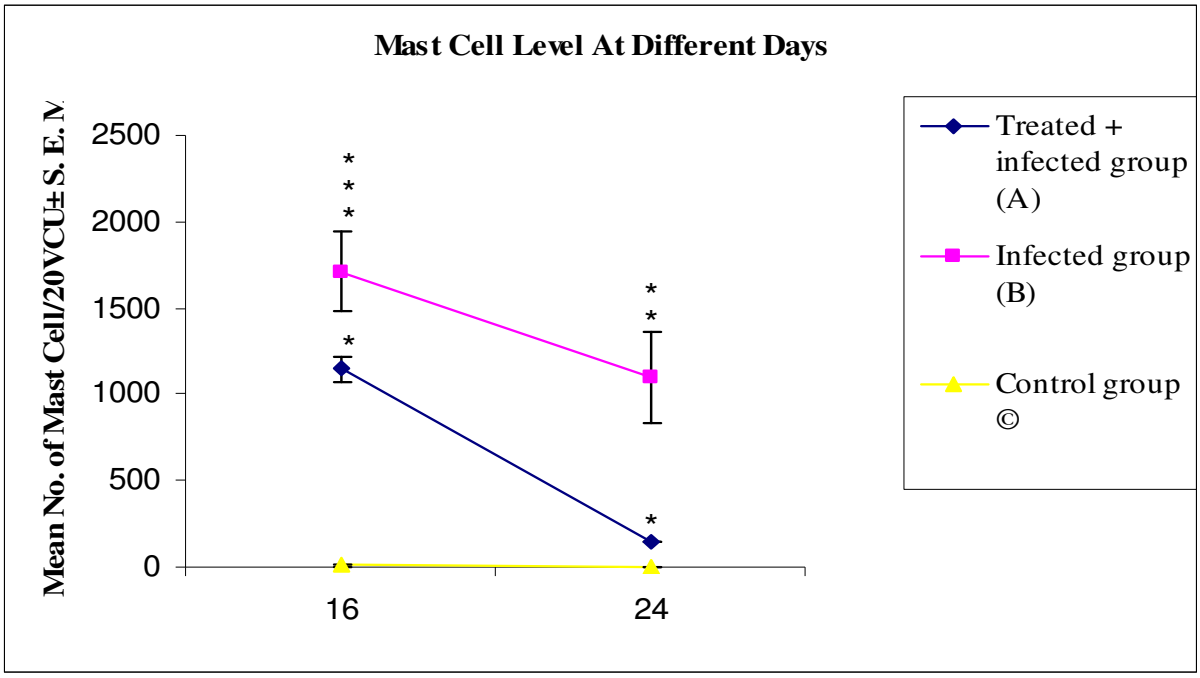


**Figure- 2**
**Mean no. of mucosal mast cell level per 20 villus cript units (V.C.U.) from group (A), (B) and (C) of mice. Significance of difference from group (A), (B) and (C). (* p < 0.05, ** p < 0.01, *** p < 0.001; Student's't' test)**

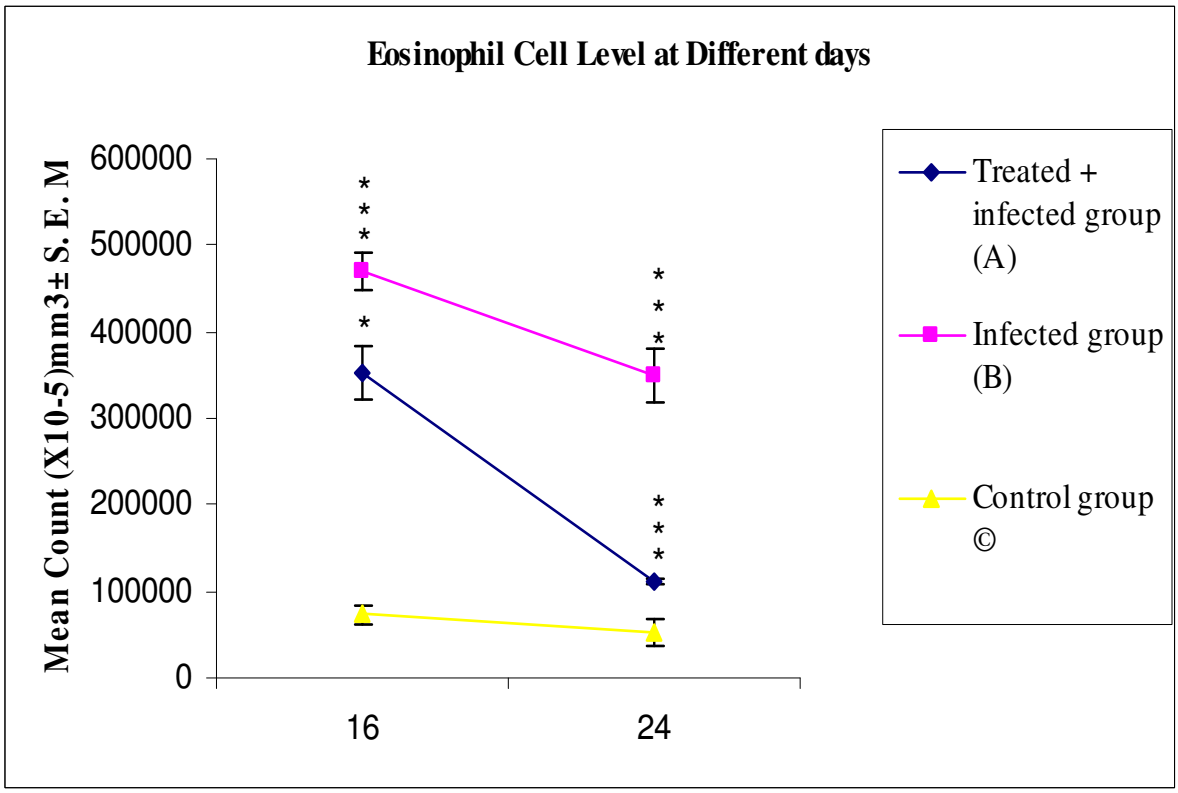


**Figure-3**
**Eosinophil cell level from group (A), (B) and (C) of mice, Significance of difference from group (A), (B) and (C), (* p < 0.05, *** p < 0.001; Student's't' test)**

Synthetic drugs have long been considered the most effective way of controlling parasite infections. However, these drugs are expensive and sometimes unavailable to smallholder's farmers and pastoralists in developing countries. Similarly, other control options, such as 'dose and move' or rotational grazing, may not be readily practiced by many smallholders farmers due to limited lend size, or by many pastoralists due to communal land ownership. Studies of the anthelmintic efficacy of neem[9-13] have been reported with varying results.

Presence of the larvae showed that the number of parasites was significantly higher in the infected group compared to treated group. It was observed that this plant extract is beneficial against nematode parasite in mice. Infected group of mice showed a much slower rate of larvae expulsion in comparison to treated group. Similar to the effectiveness of Neem in lowering the worm count [14] also reported reductions in worm burdens of Neem[15,16] found that there was a reduction in faecal egg counts and worm burdens in animals with Neem. Most significantly, it is apparent that high pathogenic, *Ancylostoma caninum* appears particularly sensitive to the intake of Neem by the animal.

One of the most important cells involved in such responses is the mast cells a well defined component of helminth induced inflammation. The accumulation of mucosal mast cells (MMC) is a characteristic and well defined response to infection with intestinal nematodes[17]. The nematode *Ancylostoma caninum* provides a good model for such correlative studies. Increased number of mast cell level recorded in infected group when compared with treated group. This is in agreement with[18] observations where there was a slight increase of mast cells in infected mice. Pharmacological investigation should be conducted on neem in order to understand the active dewormer principles possessed by this plant. Mast cell population slightly decrease in treated group. Decreased number of mast cell also reported by Dehlawi M.S. and Wakelin D.[19].

Eosinophils have been shown to be potent effector cells for the killing of helminthes parasites *in vitro* culture. 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. In humans and rats, there is evidence that eosinophil may be important role in host defence against helminthes such as *Schistosomes*[20,21]. Mechanisms of parasite killing by eosinophils are widely studied and are often implicated in mediating resistance to parasitic infection. Mice with treated and infected group administered show the increase number of eosinophil cell level. Increased eosinophil production response to challenge infection by the nematode *Ancylostoma caninum*. Larva being trapped in eosinophil reached inflammatory foci in the lungs or the skin. Similar mechanisms act against hook worm's larval migration in immunized mice[22]. Later as the antigenic stimulus subsides due to larval rejection or migration from the intestine[23], the number of eosinophils declined in plant extract treated and challenge with *A. caninum* larvae.

There is a marked variation in the capacity to elicit eosinophil response, as there was in all other parameters of the immune responses generated by adult worms[24]. Our results showed that the mice treated with neem extract and challenge with *A. caninum* larvae dewormer activity was much higher then the mice of infected group. The aim of this study was to observe the effectiveness of neem as a possible natural dewormer agent for the parasites.

## Conclusion

On the basis of this study, it is observed that the effect of gastrointestinal nematode (*Ancylostoma caninum*) was significantly reduced by the devormer activity of plant extract, Neem (*Azardirachta indica).*

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