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COMPARATIVE EFFICACY OF ALBENDAZOLE, MEBENDAZOLE AND NEEM LEAF EXTRACT IN HELMINTH INFECTIONS IN RAT MODELS.

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Abstract

Comparative efficacy of Albendazole, Mebendazole and neem leaf extract (*Azadirachta indica*) against *Vampirolepis nana, Hymenolepis diminuta* and *Syphacia muris* infections in laboratory rat strain Long-Evans (*Rattus norvegicus* Berkenhout, 1769) was evaluated. After treating with the drugs, egg reduction rate and cure rate from the worm infection were observed. Mebendazole had greater efficacy in terms of cure rate against *H. diminuta,* but in terms of egg reduction rate in *H. diminuta* infection , Albendazole had greater efficacy. The relative efficacy of Albendazole and Mebendazole were similar in terms of cure rate for *S. muris* infection (33.33%) . In case of *H. diminuta,* both Albendazole and Mebendazole had nearly similar efficacy in terms of cure rate (20% vs. 25%). Albendazole had greater efficacy in terms of cure rate and egg reduction rate against *V. nana* than Mebendazole (25% vs. 16.67% and 28.57% vs. 10%). In case of *S. muris* infection, Mebendazole showed the higher egg reduction rate than Albendazole (20.09% vs 14.52%). Neem leaf extract had better efficacy on egg reduction rate in case of *S. muris* infection than Albendazole and Mebendazole. The relative efficacy of the neem extract was higher than that of the administred chemotherapy.

Introduction

Disease caused by helminth parasites pose significantly health problems in veterinary medicine. Long-Evans is one of the important strains of laboratory rat strain (*Rattus norvegicus* Berkenhout, 1769) found in most of the animal houses in Bangladesh. Throughout the world, proper antihelminthic drugs are used for controlling or for eradication of parasites.

D'Silva(1) observed the course of infection of *S. muris* in the laboratory rat. The course of infection or pinworm *Syphacia muris* in Wister rat was reported. The worm is commonly found in animal breeding houses where the infection is maintained by auto-infection. Whether rats are caged singly, or in groups, it was shown that infections were similar. Worm burdens build up rapidly for a period of time and then become regulated.

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The transmission of parasitic disease involves three major factors: the source of infection, the mode of transmission, and the presence of susceptible host. The combined effect of these factors determine the dispensability and prevalence of the parasite at any given time and place. Since parasitic disease often tends to run a chronic course with a few or no symptoms, an infected individual may become a carrier without source of infection to others. The morbidity of the infection and the dynamics of transmission are influenced by the intensity of the helminth parasite population.[2, 3]

There are very limited number of endo-parasites that are host specific to both rats and man. The best way of prevention is to maintain a clean environment; for that, to prevent rat's exposure to other animal's excrement, treatment of any external parasites promptly. The complex life cycles of these parasites take place in different organs of the rat body, no drug can effectively treat all parasites.[4]

D'Silva[5] showed, *S. muris* Yamaguti (Nematoda: Oxyuroidea*)* oviposition and host behavior. The rat pinworm *S. muris* Yamaguti, 1935 follows a circadian rhythm to deposit its eggs on the peri-anal region. Most of the eggs are laid in the day time with a pick occurring around noon. The rhythm is dependent upon the behavior of the rat which is itself influenced by the lighting regime in the environment. Rats are nocturnal animals and normally feed and defecate at night. Adult female worms of *S. muris* therefore synchronize eggs release during the day with the rest period the rat so as to avoid the loss of eggs in the feces at night.

Infestation of intestinal helminthiasis is more common in the animal house due to the combination of some factors, such as, gathering of more rats in a single case, poor food supply that means essential food elements protein, carbohydrate, fat and other food ingredients were not properly supplied, unhygienic environment of the room, inadequate ventilation, non-sterilized water supply, improper handling by lab-attendant due to lack of proper health education and ignorance.[6] Improvement of room environment, food and hygiene are probably the best approach to reduce the prevalence of helminthiasis in animal houses, but large scale chemotherapy also plays an impotent role in disease control.

Materials and Methods

Twenty four adult laboratory rats of the strain Long-Evans (*R. norvegicus)* were randomly selected from the animal house of the Institution of Food Science and Technology (IFST) of Bangladesh Council of Scientific and Industrial Research (BCSIR) for the experiment from August 2005 to January 2006 and from February 2006 to August 2006. Mean weight of rats was 275.50 gm (215 - 335 gm) and mean length from the snout to tail was 38.5 cm (32 - 45 cm). The experimental rats were reared in cages. During the rearing period essential food and water were supplied. To ensure proper growth, the

food supplied contained protein, carbohydrate, fat, major vitamins and important minerals in specific quantities.

Total 280 stool samples were examined of which 163 were positive for helminthiasis and upon them the follow-up study was conducted. The stool samples were collected in the morning between 10 a.m. and 12 a.m. and were carried as soon as possible to the laboratory of Parasitology Branch, Department of Zoology, University of Dhaka.

Collected samples were examined microscopically through "Formol-Ether concentration Technique" which is recommended as the best overall technique for the concentration of parasitic eggs/ova in feces.[7] Egg negative stool results were noted and no further investigation was carried out. In rats, the doses of the drugs were applied according to their body weight. In the present study, the experimental rats were grouped into three groups. In each group, 8 rats of different weight groups were present. First group treated with Albendazole, second group with Mebendazole and the third group with granules of neem leaf extract. The granules were made by drying the paste (grined) of neem leaves.

The doses of these drugs applied was 0.5 mg/rat. In the present study, Albendazole was given daily for 5 days, Mebendazole was given daily for 3 consecutive days and neem leaf extract was given daily morning before taking meal for 2 consecutive days.

The stool examinations were repeated to through the "Formol-Ether concentration Method" on the 21st day after administrating the drugs and the results were noted.

Results and Discussion

Total 280 faeces samples were examined to determine the rate of infection in *V. nana, H. diminuta* and *S. muris* and also to find out the efficacy of anthelmintic drugs with the efficacy of neem leaf extract against Hymenolepiasis and Oxyuiriasis infections. Out of 280 stool samples, 62 from the weight group 215 - 245 gm, 75 from the weight group 245-275 gm and 105 from the weight group 275-300 gm and 38 from the weight group 305-335 gm of experimental rats were collected. The prevalence of single and mixed infections in Long-Evans is presented in Table 1. After 21 days treatment, evaluation of anthelmintic drugs and neem leaf extract was based on two parameters: percentage of cure rate and percentage of egg reduction or egg reduction rate as estimated by egg counts. A total of 22 for *Vampirolepis nana,* 17 cases for *Hymenolepis diminuta* and 16 cases for *Syphacia muris* were subjected to clinical trial with Albendazole, Mebendazole and neem leaf extract randomly (Table 2).

After 21 days, the treated rat with Albendazole showed stool negative for ova of *Vampirolepis nana* in 25% cases, for ova of *Hymenolepis diminuta* in 20 and 33.33% for ova of *Syphacia muris.* The rats treated with Mebendazole showed stool negative for ova of

Table 1. Prevalence of helminth parasite single and mixed infections in Long-Evans (*Rattus norvegicus* Berkenhout 1769) according to weight groups of the host.

(a) Single infection

Weight group (g)	Total no. of stool samples	*Vampirolepis nana*		*Hymenolepis diminuta*		*Syphacia muris*	
		No. of worm collected	Prevalence (%)	No. of worm collected	Prevalence (%)	No. of worm collected	Prevalence (%)
215-245	62	25	40.32	06	09.67	9	14.51
245-275	75	9	12	08	10.66	18	24
275-305	105	10	9.52	45	42.85	15	14.28
305-335	38	2	5.26	12	31.57	4	10.52

(b) Mixed infection

Weight group (gm)	Total no. of stool samples	*V. nana* + *H. diminuta*		*V. nana* + *S. muris*		*S. muris* + *H. diminuta*		*V.nana* + *H. diminuta* + *S.muris*	
		No. of worm collected	Prevalence (%)	No. of worm collected	Prevalence (%)	No. of worm collected	Prevalence (%)	No. of worm collected	Prevalence (%)
215 - 245	62	8	12.9	27	43.54	5	8.06	00	00
245 - 275	75	5	6.66	8	10.66	22	29.33	15	20
275 - 305	105	20	19.04	10	9.52	40	38.09	40	8.57
305 - 335	38	6	15.79	2	5.26	10	26.31	00	00

Vampirolepis nana in 16.67% cases, for ova of *Hymenolepis diminuta* in 25% cases and 33.33% cases for ova of *Syphacia muris.* The rats treated with neem leaf extract showed stool negative for ova of *Vampirolepis nana* in 12.5 %cases, for ova of *H. diminuta* in 25% cases and 28.57% cases for ova of *Syphacia muris.* Table 1b.

The egg reduction rates for ova *V. nana* were 28.57% for Albendazole, 10% for Mebendazole and only 6.16% for neem leaf extracts, for ova *H. diminuta* were 13.76% for Albendazole, 12% for Mebendazole and only 8.26% for neem leaf extracts, for ova *S. muris* were 14.52% for Albendazole 20.09% for Mebendazole and 5% for neem leaf extracts (Table 3).

Table 2. Efficacy of Albendazole, Mebendazole and neem leaf extract after 21 days of treatment against *Vampirolepis nana, H. diminuta* and *S. muris* infection.

Parasitic infection	Treatment received	Total no. of patient	No. of stool negatives cases	Cure rate (%)
V. nana	Albendazole	8	2	25
	Mebendazole	6	1	16.67
	Neem leaf extract	8	1	12.5
H. diminuta	Albendazole	5	1	20
	Mebendazole	4	1	25
	Neem leaf extract	8	2	25
S. muris	Albendazole	6	2	33.33
	Mebendazole	3	1	33.33
	Neem leaf extract	7	2	8.57

Table 3. Comparative egg reduction/gm rate due to the efficacy of Albendazole, Mebendazole and neem leaf extract after 21 days of treatment against *V.. nana, S. muris* and *H. diminuta.*

Treatment	Parasitic infections	No. of eggs/gm before treatment	No. of eggs/gm after treatment	Egg reduction rate (%)
Albendazole	*V. nana*	4200	3000	28.57
	H. diminuta	1832	1580	13.76
	S. muris	1280	1060	14.52
Mebendazole	*V. nana*	950	855	10
	H. diminuta	2000	1760	12
	S. muris	1120	895	20.09
Neem leaf extract	*V. nana*	260	244	6.16
	H. diminuta	2060	1890	8.26
	S. muris	1400	1190	15

In 1980, a study was done by Islam *et al.*[8] on incidence of hleminthic infections and comparative study of pyrantel pamoate with Levamisole and Mebendazole in hospital patients at Barisal, Bangladesh. Spatafora and Platt[9] found, the prevalence of *V. nana* 18.5% with an intensity of 47.00 + 63.3 in *Rattus norvegicus* from Maymont Park, Virginia. They also found two other species, *Nippostrongylus brasiliensis* and *Heterokis spumosa,* with a prevalence of 63 and 85.2%, respectively. But, these two species *N. brasiliensis* and *Heterokis spumosa* which were not found in Long-Evans strain.

Sen[10] observed the incidence of helminth infection and to determined the comparative efficacy of a single 400 mg dose of Albendazole, 600 mg dose of Mebendazole and neem extract leaf extract in the treatment of round worm, hookworm and whipworm infestations.[2] Bhuiyan *et al.*[11] reported seven helminth parasites from *Rattus Linnaeus* and *Bandicota bengalensis* Gray of which only *V. nana* was found in Long-Evans. Abu-Madi *et al.*[12] studied the infections of urban brown rat (*Rattus norvegicus*) population of Qatar. They found *H. diminuta* (167.6%), increasing with host age but not in relation to host sex or there was no seasonal variation.

Khanum and Arefin[13] reported five species of helminth parasites from the laboratory mice, *Mus musculus.* Among them, three were cestodes, *V. nana, Raillietina celebensis* and *Dipylidium canium* and two were nematodes, *Heterokis spumosa* and *Syphacia obvelata.* Except *V. nana* no other helminth remains common with the present study and the representative of the genus *Syphacia* were understandably different as they are highly host specific.[3] Alam *et al.*[14] observed three types of parasites in the Long-Evans strain. Among them two were cestodes, *V. nana* and *H. diminuta.* Another one is nematode, *S. muris.*

Huerkamp[15] on rats in a large complex of a research institute and applied Fenbendazole treatment and successfully eradicated *Syphacia muris* from all rats. It has been claimed that Praziquantel in a single dose as the current treatment of choice for hymenolepisis. Niclosamide is also used to treat infection with adult worms. Humans are susceptable to infections with *Vampirolepis nana*, since autoinfection can occur, a heavy parasite load may quickly develop. Good hygiene, public health and sanitation programs, elimination or isolation of rats help to prevent the spread of hymenolepisis. Oxyuriasis, pinworms are a persistent problem in well managed animal colonies. The pinworms that commonly infect laboratory colonies include *Syphacia muris.* The recommended drug of choice for *S. muris* is Fenbendazole. Fenbendazole treated feed has been successful in treating rats, present authors efforts must be made to remove ova from the environment to prevent reinfection.

The prevalence of infection was found highest in caecum (95.83%) and intensity of infection was highest in anterior part of intestine (51). The intestinal worms *Vampirolepis nana, Hymenolepis diminuta* and *Syphacia muris* may exhibit any, or all of the following signs such as diarrhoea, blood tinged stool, distended abdomen, weight loss, listlessness,

pruritus (itching) in and around the rectum and rectal prolapsed. However, sign and symptoms may not be apparent in an otherwise healthy rat.[16]

The histological observation showed that ht e the presence of parasites within the organs causes hazard in the host in many ways. Heavy infection to the intestine may cause perforation and thereby physiological function might be abnormal. Damage of villi tissues may cause many abnormalities in metabolism.

The major goal of periodical large scale treatment in reduction of worm load below pathogenic levels, furthermore, a decrease of the worm burden in the treated population may bring a reduction in environmental contamination and possible affecting the transmission pattern.[5,9] Only de-worming by antihelminthic drugs and antihelminthic plant products cannot control helminthiasis, rather other related factors like the food preparation and maintenance of the environment around the raring animal that are responsible for endmicity of intestinal parasites in all over the animal houses must be looked for.

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