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Efficacy of an indigenous Siddha herbal anthelmintic medicine NPK (Naaku Poochi Kolli Kudineer Chooranam) in comparison with chemical anthelmintics Albendazole and Ivermectin in the chemotherapy of Syphaciosis in laboratory rats and mice colonies

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Abstract
In this study, the efficacy of the Siddha herbal anthelmintic Naaku Poochi Kolli Kudineer Chooranam (NPK) was assessed for the treatment of pinworm infections (Syphaciosis) in Wistar rats, Swiss albino mice and Balb C and mice maintained in the Laboratory Animal Medicine Unit, Directorate of Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India. The study was conducted for a period of six months from February to August 2014. A total of 160 weaned male and female rats and mice (80 each) found positive for pinworm infection in perianal tape test were used for the study. Anthelmintics used were Ivermectin, Albendazole and NPK and were administered by oral gavage for 5 days to Wistar rats and in drinking water for 5 days to Wistar rats, Swiss Albino mice and Balb C mice. Post treatment tape tests revealed that oral gavage medication with both ivermectin and albendazole caused a rapid reduction in egg count immediately following treatment with no eggs on day 14 and day 28 after treatment. NPK caused a rapid increase in egg count following treatment and then a decline in egg count was observed from day 14 post administration. In case of drinking water administration of anthelmintics, ivermectin and albendazole caused rapid and complete reduction in egg count whereas NPK caused an initial increase in egg counts several folds followed by a decline in egg count and this increase in egg count was more pronounced in female animals compared to males. Overall observations revealed that NPK was equally effective to Ivermectin and Albendazole in treatment of Syphaciosis in rats and mice.

Keywords: Syphaciosis, rodents, herbal treatment, Naaku Poochi Kolli Kudineer Chooranam (NPK)

1. Introduction
Infection with pinworms (Syphaciosis) is one of the common helminthic infections affecting laboratory rodents. Pinworms are found in the caecum and colon of rats and mice. These worms belong to Superfamily Oxyuroidea, Family Oxyuridae and Genera *Syphacia* and *Aspiculuris* [1, 4, 7]. The three common pinworms affecting laboratory rats and mice are *Syphacia obvelata*, *Syphacia muris* and *Aspiculuris tetraptera*. Multiple infections and retroinfections among rats and mice are common as these worms have direct life cycles and eggs from female worms hatch on the host body itself [4]. Besides, aerosolization of eggs contributes to high environmental contamination. *Syphacia obvelata* has been reported to be zoonotic [6] as well as more common in immunocompromised young males. Syphaciosis is very common in specific pathogen free colonies as well [7]. Syphaciosis has untoward effects on growth, behaviour, intestinal physiology and immunology of laboratory rodents [3]. Heavy infections cause rectal prolapse, catarrhal enteritis, intestinal impaction, sticky stools, intussusception, liver granuloma and perianal pruritus. Pinworm infection is a major hindrance to the use of rodents in research. Affected animals have an elevated Th2 cytokine profiles which may mislead the results of immunologic studies [5]. Higher operational cost involved in

treatment and decontamination of the lab animal facility as well as surveillance and eradication warrants strategies of treatment of pinworm infection in order to maintain a healthy stock for research purposes. The commonly used drugs in the control and management of pinworm infection are Ivermectin, Albendazole, Praziquantel, etc. These drugs may have interference on the experimental results obtained from the animals treated with these drugs as they have longer half-lives and the possibility of adverse effects [7]. On the other hand, herbal based alternative anthelmintic such as Naaku Poochi Kolli Kudineer Chooranam (NPK) is effective and used safely in humans in India. As this drug has not been tried in pinworm infections of laboratory rodents, the present study was undertaken to compare the efficacy of NPK with other standard drugs such as Ivermectin and Albendazole for the treatment and control of pinworm infections in laboratory rats and mice.

2. Materials and Methods

The present study was carried out in laboratory rats and mice maintained at the Laboratory Animal Medicine Unit, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram Milk Colony, Chennai, India with the approval of the Committee for the Purpose of Control and Supervision of Experiments on Animals of TANUVAS (Approval No 1614/DFBS/B/2014).

Perianal tape test was performed on rats and mice to screen for pinworm infection. Positive rats and mice were segregated into males and females and again divided into groups of five for each drug and regimen with appropriate picric acid marking (head, body, tail, right forelimb and unmarked) and kept in separate cages. Thus a total of One hundred and sixty positive weaned male and female rats and mice (80 each) were used for the study. Animals were kept in appropriate sized polycarbonate cages with labels containing information such as group number, sex, number of animals, treatment details and date of initiation and termination of the study. Autoclaved Corn cobs were used for bedding. The room was maintained at 22+2ºC and 50+5% relative humidity, and at a 12/12 h light/dark cycle. The air was automatically ventilated 15–20 air changes per hour through HEPA filter in order to maintain the particle counts less than one lakh per cubic foot of size 5.0µ and larger. Pellet rodent food and autoclaved reverse osmosis purified water in drinking bottles were given *ad libitum*. Cages, bedding, steel wire tops and bottles were changed every third day. The animal room was disinfected twice daily.

Anthelmintics used were Ivermectin (Silvotin, 0.1% @ 2.5 mg/kg, Sihil Pharma Ltd.), Albendazole (Albomar, 25 mg/ml @ 2.5 mg/kg, Virbac Animal Health India Pvt. Ltd.) and NPK (Naaku Poochi Kolli Kudineer Chooranam, @ 10 ml/kg of the extract, SKM Siddha & Ayurveda). It is a brown coloured powder and contains *Butea monosperma* (13.33%), *Nigella sativa* (13.33%), *Embelia vibes* (13.33%), *Tachyspermum ammi* (13.33%), *Operculina turpethum* (13.33%), *Cassia aungustifolia* (13.33%), *Foenuculam vulgare/Pimpinella anisum* (13.33%) and *Picrorhiza kurroa* (6.66%).

For the study, 5 g of NPK was taken and mixed in 300 ml of water, and it was boiled and reduced to 30 ml. The mixture was brought to room temperature then strained and supernatant was administered at the dosage of 10 ml/kg body weight daily. Control group was administered with drinking water (vehicle). Dose volume administered was 10 ml/kg body weight for all the three anthelmintics, and administered by oral gavage for 5 days to Wistar rats or in drinking water for 5 days to Wistar rats, Swiss Albino mice and Balb C mice. All medications were freshly prepared and administered immediately after preparation. Tape tests were taken one day pre-dosing and on day 1, 14 and 28 post dosing and screened for pinworm eggs. Body weight was measured before dosing and on day 1, 14 and 28 after dosing. Monitoring of cage wise daily feed intake and water consumption was done. Statistical analysis of data was done by using one way ANOVA.

3. Results

The rats and mice positive for pinworm infection were identified by the presence of pinworm eggs on the cellophane tape when it pressed at the perianal region. Infection with *Syphacia obvelata* was found in mice and *Syphacia muris* was found in rats in the present study and there was no mixed infections found in rats and mice. Eggs of *Syphacia obvelata* were thin shelled, banana shaped, elongated with a left-right asymmetry. The average size of eggs was 130.79 x 44.17 µm in Swiss albino mice and 123.46 x 39.99µm in Balb C mice. Eggs of *Syphacia muris* were thin shelled, ellipsoidal and flattened on one side. The average size of eggs of *Syphacia muris* was 79.60 x33.38 µm which was found in Wistar rats (Fig. 1, 2).

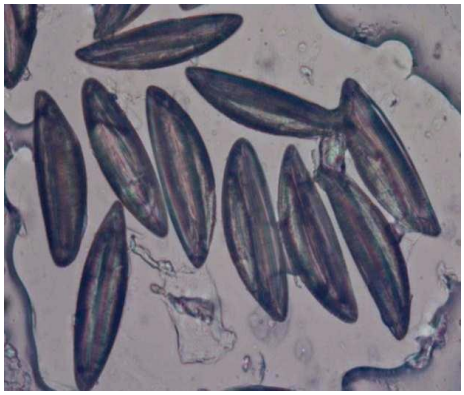

Fig 1: Eggs of *Syphacia obvelata*

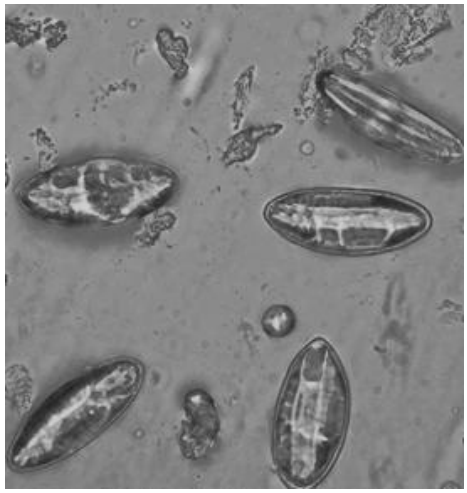

Fig 2: Eggs of *Syphacia muris*

In male Wistar rats, after administration of the anthelmintics by oral gavage for five days, Ivermectin group showed no egg on tape test from day 1 till day 28 post dosing indicating the complete elimination of the eggs except for one animal. Eggs were found in only one animal on day 1 post dosing whereas none of the animals were positive for eggs on days 14 and 28 post dosing. Albendazole was also found to be almost equally effective but reinfection occurred after four weeks since eggs

were observed on day 28 post dosing. Three animals were found to be positive on day 1 post dosing whereas none were positive on day 14 post dosing. But on day 28, two animals were positive for eggs indicating that reinfection has occurred. NPK showed a variable effect by reduction in the egg counts on day 1, increase on day 14 and decrease on day 28. The high variability in the data is due to the variation in the egg counts in all five animals on day 1 post dosing whereas 1 and 2 animals showed positive to eggs on day 14 and 28 post dosing, respectively. Drastic reduction in egg count in NPK treated male rats was observed on day 28 post dosing (Table 1).

Table 1: Efficacy of anthelmintics administered by oral gavage in male Wistar rats (n=5/group)

Group	No. of animals positive pre-dosing	No. of animals positive post dosing		
Males		Day 1	Day 14	Day 28
Control	5 (37, 120)	5 (74, 200)	3 (86, 250)	3 (53, 150)
Ivermectin	5 (30, 70)	1 (1, 1)	0 (0,0)	0 (0,0)
Albendazole	5 (49, 120)	3 (2, 2)	0 (0,0)	2 (17, 30)
NPK	5 (108, 400)	5 (39, 100)	1 (-, 400)	2 (6, 7)

Values given in the parenthesis are mean of egg counts in positive animals, and highest egg count observed among the positive animals

In female Wistar rats, after administration with Ivermectin by oral gavage for five days, total elimination of worms was observed from day 1 till day 28 post dosing as indicated by a negative tape test for eggs. None of the animals were positive for eggs on day 1, 14 and 28 post dosing. Albendazole was also found to have equal efficacy like Ivermectin but reinfection seems to occur in two animals in which eggs were observed on day 28 post dosing with no egg counts on days 1 and 14 post dosing. NPK drastically increased egg counts on day 1 post dosing whereas reduction in number of eggs was observed on days 14 and 28 post dosing (Table 2).

Table 2: Efficacy of anthelmintics administered by oral gavage in female Wistar rats (n=5/group)

Group	No. of animals positive pre dosing	No. of animals positive post dosing		
Females		Day 1	Day 14	Day 28
Control	5 (13,25)	4 (144,400)	4 (113, 400)	5 (40, 80)
Ivermectin	5 (10, 17)	1 (-, 4)	0 (0,0)	0 (0,0)
Albendazole	5 (13, 20)	0 (0,0)	0 (0,0)	2 (55, 100)
NPK	5 (18, 30)	5 (106, 200)	5 (60, 191)	3 (84,150)

Values given in the parenthesis are mean of egg counts in positive animals, highest egg count observed among the positive animals

Comparing the mean egg count on day 28 post dosing, Ivermectin was found to have the highest efficacy followed by Albendazole and NPK. Mean egg count on day 28 post dosing was 0, 55 and 84 in Ivermectin, Albendazole and NPK treated groups, respectively.

The anthelmintics were mixed in drinking water (Ivermectin (Silvotin, 0.1% @ 2.5 mg/kg), Albendazole (Albomar, 25 mg/ml @ 2.5 mg/kg) and NPK (Naaku Poochi Kolli Kudineer Chooranam @ 10 ml/kg of the extract) in order to expose the animals continuously throughout the treatment period. Ivermectin and Albendazole treated rats of both sexes showed no pinworm egg output on day 1, 14 or 28 post dosing. Similar effects were also observed in Albendazole treated group. NPK treated rats showed reduced pinworm eggs on tape test in males and none of the animals were positive for pinworm eggs on day 14 and 28, post dosing. In female rats treated with NPK showed drastically increased pinworm egg counts on day 1followed by a reduction on day 14 post dosing and none of the animals were positive for eggs on day 28 post dosing (Table 3 and 4).

Table 3: Efficacy of anthelmintics administered by drinking water in male Wistar rats (n=5/group)

Group	No. of animals positive pre dosing	No. of animals positive post dosing		
Males		Day 1	Day 14	Day 28
Control	5 (16,40)	5 (16,60)	5 (46, 90)	5 (64, 100)
Ivermectin	5 (37, 120)	0 (0,0)	0 (0,0)	0 (0,0)
Albendazole	5 (36, 70)	0 (0,0)	0 (0,0)	0 (0,0)
NPK	5 (259, 1000)	3 (58, 100)	0 (0,0)	0 (0,0)

Values given in the parenthesis are mean of egg counts in positive animals, highest egg count observed among the positive animals

Table 4: Efficacy of anthelmintics administered by drinking water in female Wistar rats (n=5/group)

Group	No. of animals positive pre dosing	No. of animals positive post dosing		
Females		Day 1	Day 14	Day 28
Control	5 (72,150)	5 (32,100)	5 (54, 90)	5 (42, 80)
Ivermectin	5 (13, 25)	0 (0,0)	0 (0,0)	0 (0,0)
Albendazole	5 (54, 90)	0 (0,0)	0 (0,0)	0 (0,0)
NPK	5 (28, 80)	5 (224, 400)	3 (8,20)	0 (0,0)

Values given in the parenthesis are mean of egg counts in positive animals, highest egg count observed among the positive animals

In Swiss Albino mice, Ivermectin and Albendazole treated groups showed a complete elimination of pin worm eggs in both the sexes on day 1, 14 or 28 post dosing. Similar effects were also observed in Albendazole treated group. NPK treated animals showed reduced pinworm egg counts in males and females on day14 post dosing, however on day 28, all the animals of either sex showed negative for pinworm eggs (Table 5 and 6).

Table 5: Efficacy of anthelmintics administered by drinking water in male Swiss Albino mice (n=5/group)

Group	No. of animals positive pre dosing	No. of animals positive post dosing		
Males		Day 1	Day 14	Day 28
Control	5 (94,400)	5 (27,100)	5 (34, 100)	5 (64, 120)
Ivermectin	5 (45, 90)	0 (0,0)	0 (0,0)	0 (0,0)
Albendazole	5 (34, 100)	0 (0,0)	0 (0,0)	0 (0,0)
NPK	5 (26, 50)	4 (86, 300)	3 (45,100)	0 (0,0)

Values given in the parenthesis are mean of egg counts in positive animals, highest egg count observed among the positive animals

Table 6: Efficacy of anthelmintics administered by drinking water in female Swiss Albino mice (n=5/group)

Group	No. of animals positive pre dosing	No. of animals positive post dosing		
Females		Day 1	Day 14	Day 28
Control	5 (12,30)	5 (17,30)	5 (50, 90)	5 (64, 120)
Ivermectin	5 (9, 30)	0 (0,0)	0 (0,0)	0 (0,0)
Albendazole	5 (50, 90)	0 (0,0)	0 (0,0)	0 (0,0)
NPK	5 (98, 400)	4 (86, 300)	4 (56,200)	0 (0,0)

Values given in the parenthesis are mean of egg counts in positive animals, highest egg count observed among the positive animals

In Balb C mice, Ivermectin and Albendazole treated groups showed negative for the pinworm eggs in both sexes on day 14 or 28 post dosing. But three animals in female group administered with Albendazole showed positive for pinworm egg on day 1 post dosing. NPK treated animals showed drastically increased pinworm egg counts on day 1 post dosing in both male and female animals followed by reduction and negative for pinworm egg counts on day 28 post dosing (Table 7 and 8).

Table 7: Efficacy of anthelmintics administered by drinking water in male Balb C mice (n=5/group)

Group	No. of animals positive pre dosing	No. of animals positive post dosing		
Males		Day 1	Day 14	Day 28
Control	5 (116,400)	5 (45,200)	5 (36, 80)	5 (89, 100)
Ivermectin	5 (9, 18)	0 (0,0)	0 (0,0)	0 (0,0)
Albendazole	5 (36, 80)	0 (0,0)	0 (0,0)	0 (0,0)
NPK	5 (66, 200)	5 (199, 500)	3 (19,30)	0 (0,0)

Values given in the parenthesis are mean of egg counts in positive animals, highest egg count observed among the positive animals

Table 8: Efficacy of anthelmintics administered by drinking water in female Balb C mice (n=5/group)

Group Balb C mice Drinking water	No. of animals positive pre dosing	No. of animals positive post dosing		
Females		Day 1	Day 14	Day 28
Control	5 (10,27)	5 (10,25)	5 (61, 200)	5 (38, 60)
Ivermectin	5 (5, 10)	0 (0,0)	0 (0,0)	0 (0,0)
Albendazole	5 (61, 200)	3 (4,10)	0 (0,0)	0 (0,0)
NPK	5 (35, 100)	5 (105, 400)	0 (0,0)	0 (0,0)

Values given in the parenthesis are mean of egg counts in positive animals, highest egg count observed among the positive animals

On administration in drinking water for five days continuously, overall efficacy indicated that all three anthelmintics, Ivermectin, Albendazole and NPK were equally effective as there was no pinworm egg observed on tape test until day 28 post dosing. The pattern of reduction in pinworm eggs was found to vary between the three anthelmintics with Ivermectin causing early reduction in egg output followed by Albendazole and NPK.

In case of oral gavage medication with ivermectin to male and female Wistar rats, there was a rapid reduction in egg count immediately following treatment with no eggs on day 14 and day 28 after treatment. Albendazole also caused a rapid reduction in egg count on days 1 and no eggs on day 14 after treatment but on day 28, two animals showed eggs in tape test. NPK caused a rapid increase in egg count following treatment and then a decline in egg count was observed from day 14 post administration. In case of drinking water administration of medication, ivermectin and albendazole caused rapid and complete reduction in egg count after treatment with no eggs found in tape test from day 1 to day 28 post treatment. NPK caused an initial increase in egg counts several folds followed by a decline in egg count and this increase in egg count was more pronounced in female animals compared to males. No significant changes were observed in feed intake, water consumption and body weight. All treatments were well tolerated and no mortality was observed

in any group during and after dosing till day 28 post dosing.

Statistical analysis of the results by one way ANOVA revealed that the variation in egg count in Wistar female rats between treatment groups administered with anthelmintics by drinking water on day 1 post dosing to be highly significant (0.003**) and variation in egg count in male Balb C mice groups administered with anthlemintics in drinking water on day 1 post dosing was significant (0.035*). Similarly and variation in egg count in female Balb C mice groups administered with anthlmintics in drinking water on day 14 and 28 post dosing was significant (0.036*). Regarding body weight the variation in body weight in egg count in both male and female Balb C mice groups administered with anthelmintics in drinking water on day 1 post dosing was significant (0.022* and 0.017*, respectively). No statistically significant difference was observed in other groups regarding egg count and body weight. Feed intake and water consumption also did not show any statistical difference between treatment groups.

4. Discussion

Most popular treatments to eradicate pinworms in rodent colonies consist of avermectins and benzimidazoles, represented by ivermectin and fenbendazole, respectively and drinking water administration of ivermectin is most ideal [2]. An oral ivermectin regimen to eradicate pinworms (*Syphacia* spp.) in laboratory rats and mice showed efficacy when ivermectin was administered in drinking water for 4 days with mean ivermectin doses of 2.9 and 4.0 mg/kg of body weight per day in rats and mice respectively [1]. In another study, efficacy of drinking water administration of ivermectin was analysed and it was concluded that at a dose of 2.5 mg/kg/day over four 5-days periods to rats, a successful control of the infection was achieved [4]. Similar observations were made in the present study where administration of 2.5 mg/kg of ivermectin in drinking water for 5 days effectively reduced the pinworm load as indicated by absence of eggs in tape test till day 28 post dosing. No reinfection was noticed in the treated animals. Ivermectin was also well tolerated by rats and mice. Albendazole is another effective anthelmintic and has been successfully used for treatment and eradication of pinworm infections in laboratory rodents [7]. The present findings corroborate with the above study and albendazole when used at 2.5 mg/kg was equally effective to ivermectin except the fact that reinfection occurred as indicated by presence of eggs in tape test which was evident on day 28 in Albendazole treated group by oral gavage, whereas in drinking water administration, Albendazole was found to be equally effective to ivermectin.

NPK showed a varied response by initially increasing the pinworm egg count followed by decrease in egg counts on day 14 and 28. The increase in pinworm egg count on day 1 post dosing was more pronounced in female animals compared to males. In female Wistar rats on day 1 post dosing, the mean egg count was 106 wherein pre-dose mean egg count was only 18. Similarly when NPK was administered in drinking water, the female Wistar rats on day 1 post dosing, the mean pinworm egg count was 224 wherein pre-dose mean pinworm egg count was only 28. Similar effects were also observed on Balb C mice females when NPK was administered in drinking water. But in case of female Swiss Albino mice there was a reduction in egg output after administration of NPK in drinking water.

These results indicate that NPK might have an ecbolic effect on the female pinworms so that they increase the release of eggs to several fold and these effects are found to be host sex dependent where more egg output by worms was observed in female rats and mice as compared to males irrespective of the mode of administration and the reason for the same is unknown. Most of these eggs which were released by pinworms of rats and mice administered with NPK were found to be immature eggs based on the observations made on the characteristics of the egg shell. The shells of the eggs were very thin compared to the matured eggs which were found in normal untreated pinworm infected animals. Therefore these immature eggs were incapable of reinfection in rodents even it is consumed by the animal, this result in the interference in the life cycle of pinworm and may lead to complete eradication of the pinworms in the laboratory rodents. Further studies are required to understand the mechanism of anthelmintic effect of NPK, a Siddha product.

Based on the above experimental results, the herbal formulation NPK showed similar effects to that of the Ivermectin and Albendazole on day 28 at least, and they can be used for the eradication of the pinworm in the laboratory animal facilities without any adverse effects on the host. NPK may not have any influence on the experimental results when the NPK treated animals are used for the research. More studies are required to confirm the ecbolic action of the NPK formulation on the worms. It is concluded that the NPK which is Siddha formulation can be used to control and eradicate the pinworm infections in laboratory rodents and similar effect need to be checked for the other laboratory animal species such as laboratory dogs and monkeys. This report seems to the first of its kind in the use of herbal anthelmintics for treatment of pinworm infections in laboratory rats and mice and therefore comparable studies are unavailable.

5. Conclusion

Based on the observations in the present study, it can be concluded that herbal anthelmintics like NPK can be used in laboratory rat and mice colonies to treat Syphaciosis. This could help to minimize the use of chemical anthelmintics as well as help to avoid the development of chemical anthelmintics especially when they are used for longer periods continuously for deworming against pinworms. Administration of NPK in drinking water to laboratory rodents is easier and effective and can be practiced for treatment of pinworm infections. The herbal medicine was well tolerated and can be therefore a safer alternative dewormer to chemical anthelmintics for pinworm infections in laboratory rodents.

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7. References

1. Klement P, Augustine JM, Delaney KH. An oral ivermectin regimen that eradicates pinworms (*Syphacia* spp.) in laboratory rats and mice. Laboratory Animal Science. 1996; 46:286-290.
2. Lorchem K. LAS Overview: The clinical and

environmental treatment of pinworms and their eggs. Laboratory Animal Science. 2013, 58-61.
3. Lucke R, Hutcheson FAR, Barbezat GO. Impaired intestinal electrolyte transport in rats infested with the common parasite *Syphacia muris*. Digestive Diseases and Sciences. 1992; 37:60-64.
4. Lytvynets A, Langrova I, Lachout J, Vadlejch J, Fucikova A, Jankovska I. Drinking water ivermectin treatment for eradication of pinworm infections. Helminthologia. 2010; 47:233-237.
5. Michels C, Goyal P, Nieuwenhuizen N, Brombacher F. Infection with *Syphacia obvelata* (Pinworm) induces protective Th2 immune responses and influences ovalbumin-induced allergic reactions. Infection and Immunity. 2006; 74:5926-5932.
6. National Research Council, Infectious diseases of mice and rats: a report of the Institute of Laboratory Animal Resources Committee on Infectious Diseases of Mice and Rats. National Academy Press, Washington, D.C. 1991.
7. Pritchett K, Johnston N. A review of treatments for the eradication of pinworm infections from laboratory rodent colonies. Contemporary Topics in Laboratory Animal Science. 2002; 41:31-41