Original Article

Screening the Efficacy of Some Traditional Herbal Drugs for Treatment of *Hymenolepis diminuta* Infection in Rats

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Abstract:

Background: *Hymenolepis nana* (human infecting tapeworm) and *H. diminuta* (rodent infecting tapeworm) are currently incriminated to be the cause of non-specific bowel disturbances. They are in most instances resistant to the available anticestodal compounds due to misuse of drugs and probably adaptation of the parasites to the commercially available drugs

Objective: Our objective is to study the toxicity and curative efficacy of different medicinal plants that are candidate for the treatment of tapeworm infections in man.

Methods: Four medicinal plants were tested for their ability to treat *Hymenolepis diminuta* tapeworm infection in rats. These plants are *Amaranthus viridis*, *Cucurbita maxima, Hagenia abyssinica and Balanites aegyptiaca.* Selection of these plants was based on ethnobotanical information. The evaluation of the efficiency of these medicinal plants was based on the "controlled test design", modified from Moskey and Harwood[10]: Following pre-infection screening, and life cycle establishment rats were grouped to six experimental groups for each plant. Stool specimens were collected from all groups, the mean of eggs counts per gram of faeces were counted. The reduction percentage of eggs per gram (EPG) was calculated and time to clear eggs was compared with that of Niclosamide. Niclosamide drug was used in this study as a control treatment[14].

Results: There were no signs of toxic effect on the rats due to administration of any of the tested medicinal plants. *Amaranthus viridis* leavs exhibited a very weak efficacy. It did not reduce eggs in either water or food significantly as compared to the untreated control group ($p > 0.05$). The deparasitization activity of this plant (35%) was not significant. Similarly, *Balanites aegyptiaca* seeds were not effective in treatment of the infection in rats. Egg counts and deparasitization in food and water, were not significantly ($p > 0.05$) different from those of the untreated control group. On the other hand, *Cucurbita maxima* and *Hagenia abyssinica* seeds were very effective in the treatment of *Hymenolepis diminuta* infection in rats. Egg reduction (100%) was highly significant ($p < 0.01$) in food and water as compared to that of the untreated control group of rats (zero%). *C. maxima* seeds in food deparasitized 80% of the worms, while *Hagenia abyssinica* deparasitized 100%.

Conclusion: Our conclusion was that ***Hagenia abyssinica*** was the most active plant of this group in the treatment of *Hymenolepis diminuta* infection in rats.

Key words: *Hagenia abyssinica,* Niclosamide, Deparasitization.

Hymenolepis nana (human infecting tapeworm) and *H. diminuta* (rodent infecting tapeworm) are currently incriminated to be the cause of non-specific bowel disturbances, or other severe symptoms due to intestinal autoinfection and cysticercosis in immunosuppressed persons[17]. Transmission between man and house mice and rats mainly occur through contaminated food or water with infective eggs or by eating accidentally flour beetles (intermediate hosts) infected with cysticercoids[1]. Tapeworms whether, *H. nana,* or *H. diminuta* are in most instances resistant to the available

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anticestodal compounds due to misuse of drugs and probably adaptation of the parasites to the commercially available drugs. Furthermore, both *H.nana* and *H.diminuta* require double the curative dose for *T. saginata*. In Sudan there is no enough literature to document the use of medicinal plants in the treatment of tapeworm's infections[13]. Currently, a number of laboratories world-wide are experimenting on a number of medicinal plants to study their efficacy in treatment of tapeworms as possible alternatives to the conventional treatment[7]. The present study was undertaken to examine the efficacy of four medicinal plants that are envisaged as candidate for the treatment of *H. nana,* and *H. diminuta* infections in man.

Materials and Methods:

Out breed Albino rats (*Ratus norvigicus*) weighing 200-300g were obtained from the Faculty of Pharmacy, University of Khartoum. Both sexes were used in the present Study. Rats used in the herbal treatment trials were infected with *Hymenolepis diminuta* according to the method of Dixon and Arai[5]. Four medicinal plants were tested for the treatment of Hymenolepis diminuta tapeworm infection in rats. These plants are Amaranthus viridis, Cucurbita maxima, Hagenia abyssinica and Balanites aegyptiaca (plats 1, 2, 3& 4).

Plate 1: Amaranthus viridis leafs.

Plate 2. *Balanites aegyptiaca* seeds.

Plate 3. *Cucurbita maxima* seeds.

Plate 4. *Hagenia abyssinica* seeds.

Selection of these plants was based on ethnobotanical information. They were given at a concentration of 25% (w/w or v) in food and water. The evaluation of the efficiency of the four medicinal plants in the treatment of Hymenolepis diminuta infections in rats was based on the "controlled test design", modified from Moskey and Harwood10. Rats were grouped into six groups for each plant. Following commencement of treatment, fresh

faeces were collected from each rat and eggs (plate. 5) per gram faeces were counted using Stoll´s technique for counting eggs by using McMaster Slide.

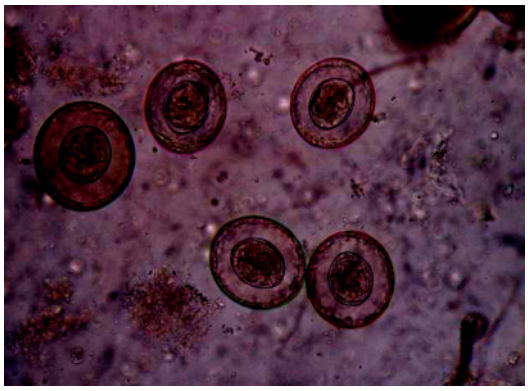

Plate 5. Eggs of Hymenolepis diminuta were counted for assessment of the treatment.

The mean of eggs counts per gram of faeces was plotted against days8.Time to clear eggs was compared with that of Niclosamide and the reduction percentage of eggs per gram (EPG) was calculated. All rats were sacrificed at day 15 of treatment. The intestine of each rat was removed and opened longitudinally in a shallow tray (Petri dish) containing normal saline. Searching for adult and small worms was made using a technique similar to that described by Hopkins and Subramanian9. The percentage of efficacy was calculated, (plate, 6).

a)

b)

Plate 6: The intestine of albino rat was removed and opened longitudinally in Petri dish (a) and was gently scraped (b).

Results:

There were no significant (P> 0.05) differences in egg counts in all experimental groups at day zero of treatment. The egg count at day zero in each group was considered as the 100% egg count. Thereafter each drop or increase in egg counts was calculated as percentage increase or decrease from day zero value.

Niclosamide drug reduced the egg counts in day 4 to zero in food and water. From this day onwards, there were no eggs detected until the end of the study period. Percentage egg reduction was significantly different (P< 0.01) from that of the untreated control rats. There were no worms or cysticercoids recovered from the whole intestine (100% deparasitization), and it was significantly (P< 0.01) different from the deparasitization% of the untreated control rats. Activity of Niclosamide drug in food or water was 100%. On the other hand, following the commencement of treatment with *Amaranthus viridis* leaves in food and water, this plant did not show any activity in food. Also, the mean eggs counts reduction in water (26%) did not decrease significantly (P> 0.05) compared with Niclosamide treatment. The deparasitization% of this plant in food was 20% and in water was 35%, it was significantly (P< 0.01) different from the control group. However this shows that *Amaranthus viridis* has a weak activity against *H. diminuta* infection in rat. Similarly the treatment with *B. aegyptiaca* seeds flesh and kern did not show any significant decrease in egg counts in either food or in water treatment groups. This is also significantly different (P< 0.01) as compared to the Niclosamide treatment. *B. aegyptiaca* seeds flesh in food deparasitized only 9% of the worms while in water it had no effects on the worm burden. However the kern in both food and water did not show any deparasitization. This plant is not effective in the treatment of *H. diminuta* in rat, compared with the commercial drug Niclosamide. While treatment with *Cucurbita maxima* seeds in food and water, decreased the mean egg counts by day 6 to100 %, *C. maxima* seeds in food deparasitized 80% of the worms. While *C. maxima* seeds in water deparasitized only 40%. *Cucurbita maxima* in food can be considered as a significantly (P< 0.01) effective in the treatment of *Hymenolepis diminuta* infections in the rat after a long period of treatment. The treatment with *Hagenia abyssinica* seeds in food and water

reduced the egg counts to 100% by day 7 (P< 0.01). Moreover, the deparasitization% in food was 100% (P< 0.01), while in water was 85% (P< 0.01). It was concluded that *H. abyssinica* seeds 25% (w/w or w/v) in either food or water efficiently treat *Hymenolepis diminuta* infections in rats similar to the commercial drug, Niclosamide (Figures 1&2).

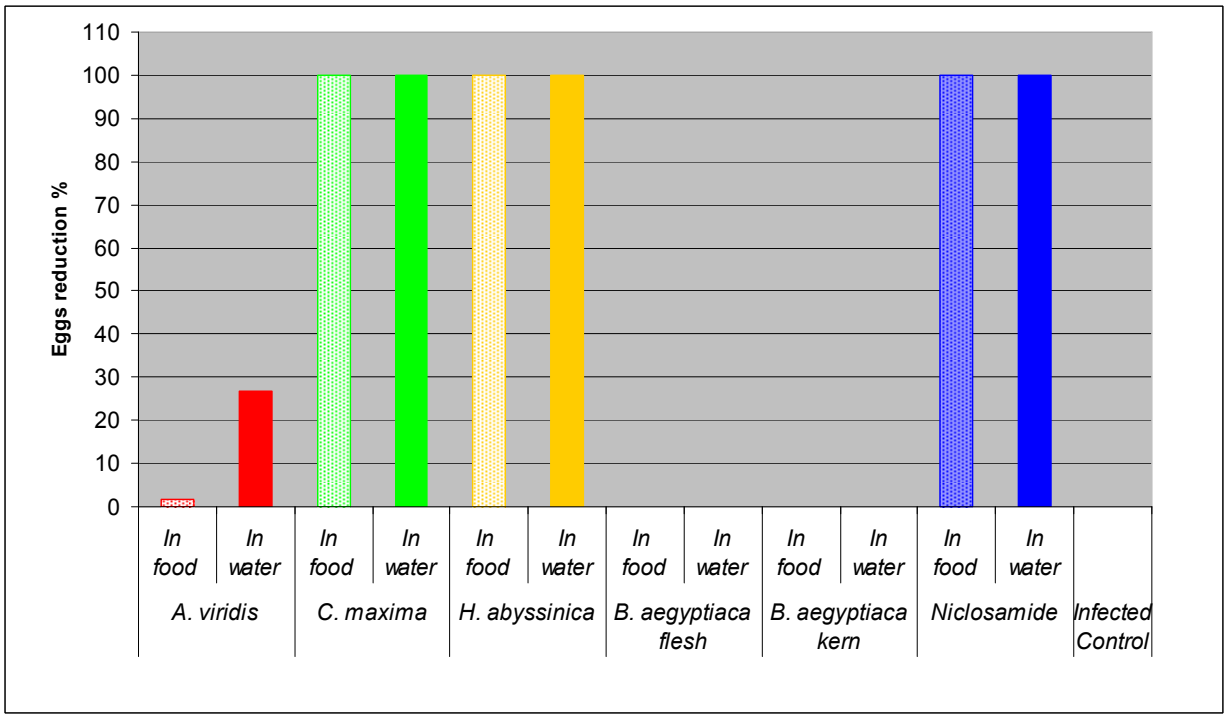

Fig 1:Summary of the results of Eggs reduction% 15 days post treatment of *H. diminuta* infections with 4 crude plants, 25% (w/w) in food and 25%(w/v) in water.

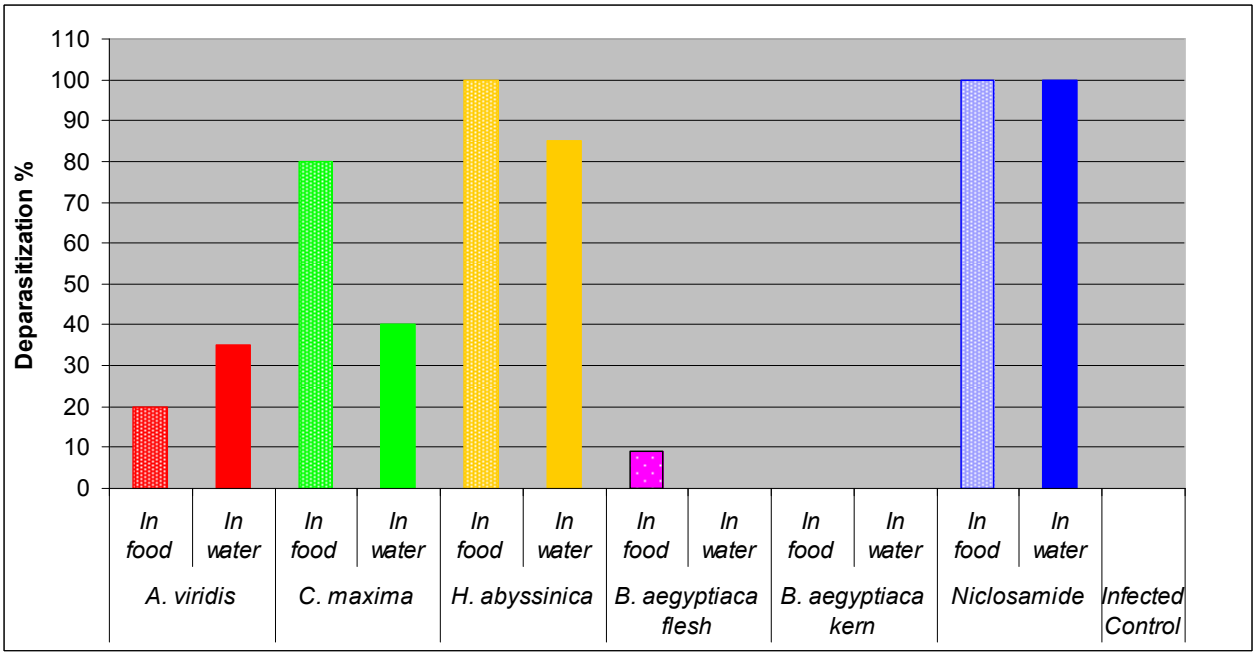

Fig 2:Summary of the results of deparasitization % activity 15 days post treatment of *H. diminuta* infections with 4 crude plants, 25% (w/w) in food and 25%(w/v) in water.

Discussion:

Hymenolepis diminuta infection in rat is of high prevalence. Studies regarding *H. diminuta* can easily be conducted in rats. Detection of *H. diminuta* in rats needs more sensitive technique[11].

Niclosamide drug is a synthetic compound used for the treatment of tapeworms infections[14]. It was used in this study as a reference treatment. It reduced egg counts by 100% and with a 100% deparasitization. These findings were previously reported by Williams and Heim[15].

In our study *Amaranthus viridis* leaves 25% (w/w&v) in food and water did not reduce the mean egg counts of *Hymenolepis diminuta* significantly ($p > 0.05$) from day zero value. It has somehow low deparasitization activity in food (20%) and water (35%). Batish found that the volatile oil of *Amaranthus viridis* had an antihelmintic activity[2].

Our study showed that throughout the treatment with *B. aegyptiaca* seeds, the mean eggs counts of *Hymenolepis diminuta* did not significantly ($p > 0.05$) deviate from the pretreatment value. Similarly, the deparasitization percentage of *B. aegyptiaca* seeds in food or water did not change from the pretreatment value. Contrary to our findings, Gnoula found that *Balanites aegyptiaca* seeds showed anticestodal activity in vitro[6]. He used the crude aqueous extract while we used the crude plant seeds.

Following the treatment period with *Cucurbita maxima* seeds in food or water, the mean egg counts of *Hymenolepis diminuta* was decreased significantly ($p < 0.01$). Post treatment, egg counts were reduced by 100% at day 7. Similarly, the deparasitization percentage in food or water groups was significant ($p < 0.01$) when examined at day 15 post treatment. These results are similar to Diaz who found that 23g of pumpkin seed in 100 ml of distilled water has an anthelmintic effect in rat, which resulted in complete deparasitization[18].

The efficacy of *Hagenia abyssinica* (kosso) as anthelmintic depends on the dosage and the health of the patient[4]. In the present study the treatment with *Hagenia abyssinica* seeds in food or water showed that the mean egg counts of *Hymenolepis diminuta* were decreased significantly ($p < 0.01$). By days 7 and 8 post treatment, there was a 100% reduction in egg counts. Similarly, the treatment in food or water groups significantly ($p < 0.01$) deparasitized all of the worms (100%) at day 15 post treatment. The present study suggests that *Hagenia abyssinica* seeds at a concentration of 25% (w/w or v) in food or water were very effective in treatment of *Hymenolepis diminuta* in rats. These findings are in agreement with Bekele, who reported that *Hagenia abyssinica* has a very effective anticestodal activities[3].

Hagenia abyssinica seeds can be as effective as the chemical anthelmintics such as: dichlorophen, niclosamide and praziquantel[16]. This study proved the powerful efficacy of *Hagenia abyssinica* in treatment of *Hymenolepis diminuta*. Some ingredients of this plant killed the adult tapeworm and cysticercoids. The mechanism or mode of action of this plant remains to be discovered. However, most anticestodals either stop glucose uptake by the tapeworm or denature the oxidative phosphorylative enzymes or may cause paralytic contraction of the worm or may damage their protective outer surface exposing them to digestion by proteolytic enzymes or having sedative effect that remove or expel the worms with faeces[12]. This plant may have some ingredients that are highly toxic to the tapeworms. However, the active ingredient mode of action and pharmaceutical properties and their effects on other human cestodes remain to be investigated.

Conclusion: Our conclusion was that *Hagenia abyssinica* has no toxic effect and it was the most active plant of this group in the treatment of *Hymenolepis diminuta* infection in rats.

References

1. Andreassen J, Bennet-Jenkins E, and Bryant C. Immunology and biochemistry of Hymenolepis diminuta. Adv Parasitol. 1999;42: 223-275.
2. Batish D. R, Singh H. P, Setia N et al. Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of Eucalyptus citriodora. Department of Botany, Panjab University, India. 2006; 61(7-8): 465-471.
3. Bekele A, Birnie A, and Tengns B. Useful trees and shrubs for Ethiopia. Identification, propagation and management for agricultural and pastoral communities. Technical Handbook. Regional Soil Conservation Unit/SIDA, Nairobi, Kenya 1993; 5: 474.
4. Dharani N. Field guide of common trees and shrubs of East Africa. Struik Publishers, Cape Town 2002; 2:319.
5. Dixon B. R. and Arai H. P. Anthelmintic-induced destrobilation and its influence on calculated drug efficacy in Hymenolepis diminuta infections in rats. J. Parasitol. 1991; 77: 769-774.
6. Pettit G.R, Doubek D.L and Herald D.L. Isolation and structure of cytostatic steroidal saponins from the African medicinal plant Balanites aegyptiaca. J. Nat. Prod., 1991;54: 1491-1502
7. Gulfraz, M, Arshad, N. and Nayyar H. Extraction of Bioactive compounds from Berberis lyceum Royle and Justicia adhatoda L.Int. J. Ethnobotanical leaflet: 2004;6-11.
8. Hansen, A. and Perry, B. (1994). The Epidemiology diagnosis and control of helminth parasites of Ruminants: Ahandbook. ILRAD, Kenya, Page 171.
9. Hopkins CA, Subramanian G, Stallard H. The development of Hymenolepis diminuta in primary and secondary infections in mice. Parasitology. 1972 Jun;64(3):401–412
10. Moskey, HE. and Harwood PD. Methods of evaluating the efficacy of anthelmintics. Amer. J. Vet. Res. 1941;2: 55-59.
11. Parija, S. C., and Srinivasa, H. The neglect of stool microscopy for intestinal parasites and possible solutions. Tropical Medicine and International Health 1999; 4(7): 522-524.
12. Roberson, E. L. (1982). Anticestodal and antitrematodal drugs. veterinary pharmacology and therapeutics. Editor Booth, N.A. and Medonal, L.E. the Iowa state university press/ames. 56(5): 852-863.
13. Silva N, Guyyat H. and Bondy D. Anthelmintics: A comparative review of their clinical pharmacology. Drugs 1997; 53:769–788.
14. Weinbach, E. and Garbus, J. (2000). Mechanism of action of reagents that uncouple oxidative phosphorylation Nature. 1969;221(5185): 1016-8.
15. Williams, M. and Heim, L. (1993). Dissociation Constant (for Niclosamide): Lab Project Number: 41223: FIFRA-D-63-10. Unpublished study prepared by ABC Labs, Inc. p. 31.
16. Woldemariam T. Z, Linley P. A, and Fell A. F. Phytochemical studies on male and female flowers of H abyssinica by column chromatography, thin-layer chromatography and high-performance liquid chromatography. Analytical Proceedings 1990;27: 178–179.
17. Bundy D. A. and Guyatt H. L. The health of school-age children: Report of a workshop. Parasitology today. 1995;11:166-7.
18. Díaz Obregón, D.; Lloja Lozano, L. and Carbajal Zúñiga, V. Preclinical studies of Cucurbita maxima (pumpkin seeds) a traditional, intestinal antiparasitic in rural urban areas, Hospital Nacional Edgardo Rebagliati Martins (HNERM), Lima. 2004; 24(4): 323-327.