



***In vivo* Anthelmintic Activity of Ethanolic Leaf Extract of *Senna italica* on Rats with *Hymenolepis diminuta* Infection**

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AM and MS performed all the experiments. Authors TA, AS and AM supervised the work. Authors AM, MS and LGG helped with the literature search, manuscript drafting and statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

In spite of the effectiveness of most anthelmintic agents in use, there is still need to identify more due to their unwanted side effects. Hence, the need to develop more that can be safe for all, cheap and available even in our localities. In the light of the current need for the use of traditional plants in the treatment of parasitic diseases, we have assessed the anthelmintic effect of *Senna italica* and aimed to investigate the therapeutic activity on *Hymenolepis diminuta* infection. We described *in vivo* studies evaluating the anthelmintic effects of the leaf extract at different concentrations in a murine infection model. Phytochemical analysis of the plant extract has shown the chemical components available in the compound. There was a significant ($p < 0.05$) reduction in mean egg/gram (EPG) in one of the treatment groups which was observed to be similar to that of the treatment using conventional anthelmintic agent (Albendazole). A fluctuating but insignificant faecal

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worm-egg count was observed in the other experimental groups. The results obtained suggest a likelihood of its future use as an anthelmintic agent. Our next plan is to establish animal experiments with different types of helminth infection in order to have the full anthelmintic coverage by the agent, hopefully, before it will be validated for use as a human therapeutic agent.

Keywords: Anthelmintic; ethanolic leaf extract; *Senna italica*; rats; *Hymenolepis diminuta*.

1. BACKGROUND

Helminths infections are among the major ailments affecting humans and livestock in many tropical and sub-tropical regions of the world [1]. These diseases which are currently referred to as Neglected Diseases of Neglected Populations, cause enormous hazards to the health of people, particularly, children, by contributing to malnutrition, anaemia and retarded growth [2]. Although there are several drugs effective against intestinal helminths, the fact remains that about one-third of the world's population still lacks regular access to essential drugs, with the figure rising to over 50% in many poor countries [3].

The conventional drugs used in the treatment of helminthes infection include Albendazole and mebendazole which have been the drugs of choice for the treatment of helminthiasis, despite their associated gastrointestinal side effects and resistance side effect by the parasite [4]. In this connection, traditional medicines, based largely on herbs and trees, offer a major and accessible source of health care to people living in developing countries [5]. Medicinal plants have been used as a major source of therapeutic agents by human beings for thousands of years and continued to be so globally [6].

In many regions of the world, *Senna italica* is cultivated for medical and commercial purposes [7]. In traditional medicine species have been well known for their laxative and purgative properties and for the treatment of skin diseases [8]. In Malawi, the leaves, pods and seeds of *Senna italica* are mostly used in traditional medicine and also root infusion is used to treat diarrhea in infants [9]. The dried leaves and pods of Senegal senna are traded for medicinal uses. Dried, powdered leaves are traded internationally from Egypt or India as hair conditioner [8].

Unfortunately, experimental evidence of the anthelmintic activity of the plants is still lacking. The phytochemical component of the plants responsible for the activity has also not been investigated. Information obtained from this

research will help in improving the control of helminth infection. It may also provide a cheaper, effective, less toxic and more affordable means of helminth infection control. This study is aimed at determining the anthelmintic activity of the *Senna italica* plant leave extract in the treatment of *Hymenolepis diminuta* infection in a rat model.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The *Italian Senna* was obtained from Wurno Local Government, Sokoto State. The plant was botanically authenticated at the herbarium of the Botany unit, Usmanu Danfodiyo University, Sokoto.

2.2 Preparation of Plant Material

The *Senna italica* leaves were air dried under shade and ground using pestle and mortar and the powder was stored in air-tight container according to the method of [10], for further analysis.

2.3 Preparation of Ethanolic Extract

The ethanolic extract was obtained using the cold maceration method as described by [11]. The extract was labeled appropriately and stored in plastic container until required.

2.4 Phytochemical Analysis

The extracts were evaluated qualitatively for the presence of flavanoids, tannins, saponins, alkaloids, glycosides, cardiac glycosides, volatile oils and steroids [12,13,14].

2.5 Source of Experimental Animals

Hymenolepis diminuta infected Wistar rats (8 weeks old) were obtained from Biological Garden, Department of Biological Science, Usmanu Danfodiyo University Sokoto.

2.6 Experimental Design

The rats were divided into five (5) groups. Each group consists of five rats.

Group I: Infected rats and treated with standard drugs; Albendazole at 7.5 mg/kg once (positive control).

Group II: Infected and treated with 75 mg/kg of the *Senna italica* extract, the rats were given food and water *ad libitum*.

Group III: Infected and treated with 150 mg/kg of the *Senna italica* extract, the rats were given food and water *ad libitum*.

Group IV: Infected and treated with 300 mg/kg of the *Senna italica* extract, the rats were given food and water *ad libitum*.

Group V: Infected but not treated with the *Senna italica* extract (Negative control), the rats were given food and water *ad libitum*.

2.7 Morphological Identification of *Hymenolepis diminuta*

Fecal smear methods were used for the microscopic detection of eggs. The morphology of the eggs (oval, not operculated and with two coverings; the inner membrane which surrounds the embryo is also without a polar filament and the outer membrane which is thin; also known as the egg shell) observed were then compared with those in standard text and literature [15] for proper identification.

2.8 Preliminary Acute Toxicity Test

The extract was administered orally in the doses of 400, 800, 1600 and 3200 mg/kg, to four rats. The general signs and symptoms of toxicity and mortality rates were observed for 72 hours post administration of the extract as previously documented [16].

2.9 Stool Analysis

Stool analysis for the identification of therapeutic effect of *Senna italica* against *Hymenolepis diminuta* infection was undertaken. Daily worm-egg count using Modified Mc Master Technique [17] was performed before and after treatment of *Hymenolepis diminuta* infected rats using different concentration of the plant extract to determine the success of the treatment.

2.10 Statistical Analysis

Data of worm-egg count was expressed as means (Mean \pm SEM) of independent experiments and were analyzed using the Statistical package for social sciences (SPSS), version 20.0. Results obtained from therapeutic efficacy of *Senna italica* on experimental rats were compared between groups using repeated measures Analysis of Variance (ANOVA), and significant difference ($P \leq 0.05$) were determined using the Dunnett Post Hoc test for multiple comparison.

3. RESULTS

3.1 Phytochemical Composition of *Senna italica*

Preliminary phytochemical analysis showed that, flavonoid, glycosides and tannins were present in large amount (+++), anthraquinone was moderately presents (++) and alkaloids, balsams, cardiac glycosides, saponins, steroids and volatile oil were quite present in trace amounts (+). Some phytochemical components were quantitatively determined. Among which, Steroids and Tannins were found in large amount and others were in moderate amount (Table 1).

Table 1. Quantitative phytochemical composition of *Senna italica* ethanolic extract

Compound	Quantity (%)
Alkaloids	3.3+0.252
Flavonoids	3.1+0.252
Glycosides	0.7+0.238
Saponins	4.0+0.163
Steroids	79.1+6.309
Tannins	96.7+0.163

3.2 Acute Toxicity Study

The leaf extract when orally given to the rats at doubling doses from 400 up to 3,200 mg/kg, showed no mortality or any adverse signs in the animals with regard to body weight, body temperature, food and water in take up to 72 h post treatment.

3.3 Effects of Albendazole against *Hymenolepis diminuta* Infection in Wistar Rats

The mean egg per gram of rats in GROUP 1 before any treatment was 5,440 eggs/gram (Table 2), the rats were treated with 7.5 mg/kg of

Albendazole and observed after each 24 hours up to 168 hrs. It was observed that, after 24 hours (day 1) the mean eggs reduced from 5,440 to 1140 (percentage reduction is 79%) then after 48 hours reduced to 200 (percentage reduction is 96%) and lastly to 0.00 (percentage reduction is 100%) after 72 hours, the presence eggs were checked up to 168 hours (day 7) and the egg number remained 0.00 (percentage reduction is 100%).

3.4 Effects of *Senna italica* Extract at 75 mg/kg against *Hymenolepis diminuta* Infection in Wistar Rats

The mean egg per gram of rats in GROUP 2 was 3,500 egg before treatment (Table 3). The rats were then treated with *Senna italica* ethanolic extract at 75 mg/kg while egg count per gram of their faeces was examined daily for seven (7) days post-treatment, at first day of treatment (24 hour) the eggs reduces from 3,500 to 3,300 egg, (percentage reduction is 5.7%) the eggs also reduce at the second day of treatment (48 hour) from 3,300 to 3,280, (percentage reduction is 6.3%) the eggs however increase at day 3 (72 hour) from 3,280 - 3,300 egg, (percentage reduction is 5.7%), it also increase from 3,300 - 3,320 egg at 96 hour (day 4), (percentage reduction is 5.1%) there is a decrease at day 5

(120 hour) from 3320 – 3300 egg, (percentage reduction is 5.7%) while as for the day 6 and 7 (144 and 168 hours) there is an increase in the eggs from 3,300 - 3,500 egg, (percentage reduction is 0.0%). There was no significant decrease in the egg throughout the period of seven (7) days.

3.5 Effects of *Senna italica* Extract at 150 mg/kg against *Hymenolepis diminuta* Infection in Wistar Rats

Rats in GROUP 3 had mean egg per gram of their faeces was 2,560 egg before any treatment was instituted (Table 4). They were then subjected to treatment with the *Senna italica* ethanol extract at 150 mg/kg, the mean eggs per gram of faeces at first day of treatment (24 hour) reduce from 2,560 – 1,840, (percentage reduction is 28.1) it also drop down from 1,860 – 1,140 at day 2 (48 hour) (percentage reduction is 55.5) there is also a decrease in the mean of eggs from 1,140 - 640 at day 3 (72 hour) (percentage reduction is 75) while at day 4 (96 hour) it also decrease from 640 - 480, (percentage reduction is 81.2) the EPG level was monitored for day 5,6 and 7 and observed that it only reduce from 480 - 420 for the rest of the days (120,144 and 168 hour) (percentage reduction is 83.6).

Table 2. Therapeutic effect of albendazole against *H. diminuta* infection in wistar rats

Days	Mean ± SEM eggs before treatment with Albendazole	Mean ± SEM of eggs after treatment with Albendazole (7.5 mg/kg)	Reduction of eggs (%)
DAY 1	5,440.0+1,175.8	1,140+756	79
DAY 2	5,440.0+1,175.8	200+200	96
DAY 3	5,440.0+1,175.8	0.00+0.00	100
DAY 4	5,440.0+1,175.8	0.00+0.00	100
DAY 5	5,440.0+1,175.8	0.00+0.00	100
DAY 6	5,440.0+1,175.8	0.00+0.00	100
DAY 7	5,440.0+1,175.8	0.00+0.00	100

Table 3. Therapeutic effect of *Senna italica* leaf extract at 75 mg/kg against *H. diminuta* infection in wistar rats

Days	Mean ± SEM of eggs before treatment	Mean ± SEM of eggs after treatment with 75 mg/kg of the extract	Reduction of eggs (%)
DAY 1	3500.0+1334.9	3300+1342.0	5.7
DAY 2	3500.0+1334.9	3280+1337.3	6.3
DAY 3	3500.0+1334.9	3300+1342.0	5.7
DAY 4	3500.0+1334.9	3320+1335.4	5.1
DAY 5	3500.0+1334.9	3300+1330.7	5.7
DAY 6	3500.0+1334.9	3500+1510.2	0.0
DAY 7	3500.0+1334.9	3500+1510.2	0.0

Table 4. Therapeutic effect of *Senna italica* leaf extract at 150 mg/kg against *H. diminuta* infection in wistar rats

Days	Mean \pm of eggs before treatment	Mean \pm of eggs after treatment with 150 mg/kg of the extract	Reduction of eggs (%)
DAY 1	2560+329.5	1840+374.9	28.1
DAY 2	2560+329.5	1140+222.7	55.5
DAY 3	2560+329.5	640+136.3	75.0
DAY 4	2560+329.5	488+177.2	81.2
DAY 5	2560+329.5	420+233.2	83.6
DAY 6	2560+329.5	420+233.2	83.6
DAY 7	2560+329.5	420+261.1	83.6

3.6 Effects of *Senna italica* Extract at 300 mg/kg against *Hymenolepis diminuta* Infection in Wistar Rats

The mean egg per gram of rats in GROUP 4 before any treatment was 4,000 eggs/gram (Table 5), the rats were treated with 300mg/kg of *Senna italica* ethanolic extract and observed after each 24 hours up to 168 hrs. It was observed that, after 24 hours (day 1) the mean eggs reduced from 4,000 to 820 epg (percentage reduction is 79.5%) then after 48 hours reduced from 820 to 260 epg (percentage reduction is 93.5%) and lastly reduces from 260 to 0.00 epg after 72 hours (day 3) (percentage reduction is 100%). The presence eggs were checked up to 168 hours (day 7) and the egg number remained 0.00 epg (percentage reduction is 100%).

3.7 Mean Egg per Gram of Faeces from Rats Infected with *Hymenolepis diminuta* But Not Treated with Any of the Therapeutic Agents Used in the Study (Negative Control)

Rats in group 5 were infected but not treated with either of the therapeutic agents to serve as negative control while egg count per gram of their faeces was examined daily for seven (7) days post-observation. They had an EPG of

3,300 before any observation which had increased from 3,300 to 3,500 at the first and second day of observation (24 and 48 hours), (percentage reduction is -6.0%). The eggs were also increased at day 3,4,5,6 and 7 (72, 96, 120, 144 and 168 hours) from 3,500-3,640 epg (percentage reduction is -10.3%). There was no decrease in the EPG throughout the period of seven (7) days (Table 6).

In order to compare the EPG from rats of the different groups, those treated with Albendazole (GROUP 1) at 7.5 mg/kg has shown significant decrease similar to those treated with 300 mg/kg (GROUP 4) of the *Senna italica* extract. A statistically significant difference ($P < 0.05$) was observed in EPG between groups and among days post-treatment for GROUP 1 and 4 compared to the negative control (GROUP 5). In order to compare epg from rats of different groups; Negative control (Group 5), rats treated with albendazole at 7.5 mg/kg Group 1 (positive control) and the rats treated with ethanolic extract of *Senna italica* at 75, 150 and 300 mg/kg in Group 2, 3 and 4 respectively. The comparison between Mean egg per gram of faeces of different groups of rats infected with *Hymenolepis diminuta* and treated with either of the therapeutic agents in different concentrations (Table 7).

Table 5. Therapeutic effect of *Senna italica* leaf extract at 300 mg/kg against *H. diminuta* infection in wistar rats

Days	Mean \pm SEM of eggs before treatment	Mean \pm SEM of eggs after treatment with 300 mg/kg of the extract	Reduction of eggs (%)
DAY 1	4000+789.3	820+326.1	79.5
DAY 2	4000+789.3	260+193.9	93.5
DAY 3	4000+789.3	0.00+0.00	100
DAY 4	4000+789.3	0.00+0.00	100
DAY 5	4000+789.3	0.00+0.00	100
DAY 6	4000+789.3	0.00+0.00	100
DAY 7	4000+789.3	0.00+0.00	100

Table 6. Daily mean egg-count of rats infected with *H. diminuta* but not treated (negative control)

Days	Mean ± SEM of eggs before treatment	Mean ±SEM of eggs after observation ± SEM	Reduction of eggs (%)
DAY 1	3300+1330.7	3500+1510.2	-6.0
DAY 2	3300+1330.7	3500+1510.2	-6.0
DAY 3	3300+1330.7	3640+1455.5	-10.3
DAY 4	3300+1330.7	3640+1455.5	-10.3
DAY 5	3300+1330.7	3640+1455.5	-10.3
DAY 6	3300+1330.7	3640+1455.5	-10.3
DAY 7	3300±1330.7	3640±1455.5	-10.3

Table 7. Mean egg per gram of faeces from the different groups before and after treatment with Albendazole and *Senna italica* extract using different concentrations (mean ± SEM)

Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
TRM								
Albendazole	5440+1175.8	1140+756.0	200+200.0	0.00+0.0	0.00+0.0 ^c	0.00+0.0 ^e	0.00+0.0 ^g	0.00+0.0 ⁱ
Extract at								
75 mg/kg	3500+1334.9	3300+1342.0	3280+1337.3	3300+1342.0	3320+1335.4	3300+1330.7	3500+1510.2	3500+1510.2
150 mg/kg	2560+329.5	1840+374.9	1140+222.7	640+136.3	480+177.2	420+233.2	420+233.2	420+261.5
300 mg/kg	400+789.3	820+326.1	260+193.9	0.00+0.0 ^b	0.00+0.0 ^d	0.00+0.0 ^f	0.00+0.0 ^h	0.00+0.0 ^j
0.0 mg/kg	3300+1330.7	3500+1510.2	3500+1510.2	3640+1455.5 ^{a,b}	3640+1455.5 ^{c,d}	3640+1455.5 ^{e,f}	3640+14455.5 ^{g,h}	3640+1455.5 ^{i,j}

Means with the same superscript are significantly different ($P < 0.05$), TRM = treatment

4. DISCUSSION

Intestinal parasitic infections continue to be a cause of major concern to human and animal health, particularly in tropical and under-developed regions of the world [18,19]. Globally, 30% of the population are estimated to be infected with intestinal worms that are mainly attributed to poor sanitation and hygiene [20]. It is in this context that the World Health Organization in its Tropical Diseases Control Programme has advocated that the use of traditional medicines be promoted to combat the menace of parasitic diseases globally [21,22]. Although some studies suggested the use of traditional medicine against intestinal worms and antihelmintic effects of plant extracts on the parasites [23,24,25,26].

This study has assessed the efficacy of *Senna italica* leaf extract against *Hymenolepis diminuta* infection. At high concentrations of the extract, it was found to be effective in controlling rodent infection similar to the recorded therapeutic effect of the conventional Albendazole at 7.5 mg/kg body weight of the treated host animals. This study provided the basis for the use of *Senna italica* extract as therapeutic agent for helminth parasites.

Based on the present findings, the extract was more effective at 300 mg/kg as it had completely cured the entire worm load and parasite eggs in the feces of the treated rats. Indeed, the efficacy of the extract at 300 mg/kg was comparable to the standard reference drug (Albendazole at 7.5 mg/kg). On the other hand, a little reduction in the worm load and EPG was observed when lower doses of the extract were administered. A number of studies have also highlighted the prospects of traditional medicinal plants in anthelmintic therapy. For instance, other authors [27], demonstrated that *Z. rhetsa* leaf extract possesses significant anthelmintic efficacy with little or no toxicity to the experimental animals. Furthermore, a more or less similar trend was also observed in the efficacy of the extract against the adult stage of parasites. In view of its affordability and availability in the environment, *Senna italica* represents a potential replacement of albendazole which is a synthetic drug that has been associated with gastrointestinal and reproductive side effects.

The results of this study showed that the plant contained phytochemicals, some of which are known to be nutritionally beneficial while others

have some antinutritional activities. The highest mean value of Tannins was obtained in the plants, followed by steroids, saponins, alkaloids, flavonoids and least mean value was obtained in glycosides. The tannins of *Senna italica* are unlikely to pose toxicity problems when the plant is consumed [27]. Similarly, the phenolic compounds have been proven to be protective against free radicals, thereby participating in ameliorating the risk of cancer, coronary heart disease, stroke, atherosclerosis, osteoporosis, inflammation and other neurodegenerative diseases associated with stress [28]. The presence of steroids in reasonable concentration in *Senna italica* is an indication that the plant may be a good source of steroids which serve as important component of cell membrane, as signaling molecules and also as primary precursors of cytochrome P450 oxidase enzymes [29].

High content of saponin obtained in *Senna italica* indicate that the plant might be of benefit both nutritionally and commercially as reported in the work of [30], Asl and Hossein, [31] and [32] who reported that the Plant extract containing saponins are commonly used as foaming agents for beverages such as beer and soft drinks. Saponins are also thought to have commercial applications such as ore separation in industrial and mining operations. They are also useful products in photographs, emulsions, fire extinguishers foam, tooth paste and shampoos. Saponin rich herbs also contribute to the lowering of blood cholesterol and inhibition of cancer proliferation. Alkaloids also have pharmacological effects; they are used as medications and as recreational drugs. The above confers pharmacological advantage to *Senna italica* and may be the basis for its use as folk medicine.

Flavanoids were also found in *Senna italica*. They are known to possess antioxidant activity. This implies that consumers of plants may be protected against oxidative cell destruction by free radicals. On the other hand, flavonoids are often reported to be toxic and are able to initiate physiological changes in the body when consumed [33]. Flavanoids were found to be abundant in the plant; they are mainly water-soluble compounds which occur both in the free-state and as glycosides. Moderate mean value of glycosides was obtained in the plant. Glycosides play numerous important roles to living organisms. Many plants stored chemicals in the form of inactive glycoside; these can be activated

by enzyme hydrolysis which causes the sugar part to be broken off, making the chemical available for use. Many glycosides found in plants are used in medications in animals and humans [34].

5. CONCLUSION

This study suggested that *Senna italica* leaf extract has anthelmintic effect against *Hymenolepis diminuta*. The extract is more effective at a concentration of 300 mg/kg which is similar to that of albendazole at 7.5 mg/kg.

6. FUTURE PERSPECTIVE

The number of drugs available for treatment of parasitic diseases is still limited, this fact making search from other sources almost necessary for new, diverse, effective and safe agents. The possibility of identifying and evaluating the compounds is of paramount significance and desirable. Therefore, it is expected that the number of traditional compounds with anthelmintic activity will increase in the near future, taking the search of new therapeutic approaches to a higher level.

ETHICAL APPROVAL

The research protocol used in this experiment was approved by and conducted in accordance with the Ministry of Animal Health and Fisheries, Sokoto State, Nigeria. Ref No. MAH&FD/VET/166/11.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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