Histopathological Studies on the Effects of Ethanolic Leaf Extract of *Senna italica* Treatment of Rats Infected with *Hymenolepis diminuta*

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors MA and MS performed all the experiments. Authors TA, AS and MA supervised the work. Authors MA, MS and MBB helped with the manuscript writing and statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Helminths infections are among the major ailments affecting humans and livestock in many tropical and sub-tropical regions of the world which necessitates the current need for the use of traditional plants in the treatment of parasitic diseases. The aim of this study was to determine the pathological effects of *Senna italica* leaves extract on *Hymenolepis diminuta* infection. Plant leaf were collected and processed using cold maceration method. Phytochemical composition of the plant extract was determined. Rats were grouped into five groups of five rats each for the experiment. Albendazole was used for treatment at 7.5 mg/kg as positive control, while different concentrations of 75, 150, 300 and 0.0 mg/kg of the plant extracts were used for treatment of rats in the other groups. Daily worm-egg counts from all the rats for all the groups were recorded for up to 7 days post infection.
Histopathology of tissues from representative rats from all groups was conducted to determine any visible change due to the treatments. The findings have shown a significant (p<0.05) reduction in mean egg/gram (EPG) in positive control after three days. Rats in group 2 (75 mg/kg) showed a fluctuating faecal worm-egg count throughout the period of the study, with no significant difference observed. Rats in group 3 (150 mg/kg) showed a slightly decreased mean worm-egg count with no significant difference observed at P<0.05. Rats treated with 300 mg/kg of the extract have shown significant decreased of worm-egg count within three days of the treatment (P<0.05) which was observed to be similar to the treatment with Albendazole. Histopathological studies of intestine, liver and lung tissues of the infected and treated rats have shown that the plant extract has no significant histopathological effects. Leaves of *Senna italica* have therapeutic effect against *Hymenolepis diminuta* infection with no observed pathology in an experimental animal model.

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

1. **INTRODUCTION**

Current estimates suggest that over half of the world population is infected with intestinal helminthes, and that most of these infected people live in remote rural areas in the developing countries Horton, [1]. These diseases which are currently referred to as neglected diseases, cause enormous hazards to the health of people, particularly children, by contributing to malnutrition, anaemia and retarded growth Hotez et al. [2]. The treatment for helminthiasis is made with anthelmintic drug which has been reported to be less effective due to drug resistance by parasites Siddiqui and Berk, [3]. Drug are also costly, and in some cases associated with gastrointestinal side effect, and a higher relapse rate. It is said to be avoided during pregnancy, lactation as well as among younger animals due to adverse effects they cause WHO [4].

Plants have great potential uses, especially as traditional medicine and as a component of modern pharmaceutical drugs. A large population of the world depends on traditional medicine because of the scarcity and high costs of orthodox medicine Hudaib et al. [5]; Tagboto and Townson [6]. Many plants contain a variety of pharmaceutical products, which have been found very important for application in the field of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drugs for the treatment and prevention of several diseases Newman et al., [7]; Gilani and Rahman [8]. This has called the attention of many researchers in extracting natural product from plants for the treatment of many diseases which are cheap and affordable for the poor people. *Senna italica* has been used for many years as traditional medicine for the treatment of helminth parasite infection in our locality (Northwestern, Nigeria) as recommended by many herbalists.

Pharmaceutical companies have expanded a large chunk of time and money in developing natural products extracted from plants to produce effective remedies that are affordable to the poor populace Dahiru et al. [9]. Medicinal plants are widely and successfully used all over the world for treatment of various ailments Hoareau and Dasilva, [10]. They play important role in human health worldwide. According to world health organization, almost 80% of the population relies heavily on natural substances as medicine WHO [11]. This study reports the histopathological effects of the *Senna italica* plant leave extract in the treatment of *Hymenolepis diminuta* infection in rats.

2. **MATERIALS AND METHODS**

2.1 **Collection and Identification of Plant Material**

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

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 Jaqueline et al. [12], for further analysis.

2.3 **Preparation of Ethanolic Extract**

The ethanolic extract was obtained using the cold maceration method as described by Shehu.
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 Jeruto et al. [14]; Vijaya et al. [15] Semenya et al. [16].

2.5 Source of Experimental Animals

Hymenolepis diminuta infected (positive for worm egg) Wistar rats were obtained from Biological Garden, Department of Biological Science, Usmanu Danfodiyo University Sokoto, Nigeria.

2.6 Experimental Design

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

- **Group A:** Infected rats and treated with standard drugs; Albendazole at 7.5 mg/kg once (positive control).
- **Group B:** Infected and treated with 75 mg/kg of the Senna italica extract, the rats were given food and water *ad libitum*.
- **Group C:** Infected and treated with 150 mg/kg of the Senna italica extract, the rats were given food and water *ad libitum*.
- **Group D:** Infected and treated with 300 mg/kg of the Senna italica extract, the rats were given food and water *ad libitum*.
- **Group E:** 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 Taylor et al. [17] for proper identification.

2.8 Preliminary Acute Toxicity Test

The extract was administered orally in the doses of 400, 800, 1,600 and 3,200 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 Erhirhie et al. [18].

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 Chandrawathani et al. [19] was performed before and after treatment of *Hymenolepis diminuta* infected rats with different concentration of the plant extract.

2.10 Sacrifice of Experimental Animals

At the end of the treatments in all the groups, and in order to confirm the effectiveness of the treatments through elimination of adult stages of the tapeworm, one rat from each group (1-5) was euthanized using chloroform. They were dissected and small intestines were detached, split longitudinally and their contents were removed. The luminal surfaces were washed in warm (34°C) normal saline. Adult worms were then harvested from the surfaces of the lumen and identified using Stereomicroscope. Tissues were also collected from the small intestine, liver and lung of each rat in all the groups and were harvested for histopathological examination.

2.11 Histopathological Analysis of Tissues

Tissues (approximately 1.0 cm in length) from lung, liver and small intestines were fixed in 10% formalin, dehydrated, cleared in absolute alcohol/xylene and embedded in paraffin according to standard protocol Matsuda et al. [20]. Histopathological sections were cut in 4 µm thickness, and stained with haematoxylin-eosin (HE) prior to examination. The slides were microscopically examined at different magnifications to assess histological and pathological alterations in the organs.

2.12 Statistical Analysis

Data of worm-egg count was expressed as means (Mean ± 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 ($P<0.05$) were determined using the Dunnett Post Hoc test for multiple comparison.

3. RESULTS

3.1 Phytochemical Composition of *Senna italica*

Some phytochemical components of the plant were quantitatively determined. Among which, Steroids and Tannins were found in large amount and others were in moderate amount (Table 1).

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 Anthelmintic Efficacy of the *Senna italica* Extract

In order to compare the worm-egg per gram (EPG) of the faecal samples from rats of the different groups, those treated with Albendazole (GROUP A) at 7.5 mg/kg has shown significant decrease similar to those treated with 300 mg/kg (GROUP D) 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 A and D compared to the negative control (GROUP E). In order to compare egg per gram (EPG) of the faecal samples from rats of different groups; Negative control (Group E), rats treated with albendazole at 7.5 mg/kg Group A (positive control) and the rats treated with ethanolic extract of *Senna italica* at 75, 150 and 300 mg/kg in Group B, C and D 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 is presented (Fig. 1).

3.4 Post Mortem Findings

Three of the rats from group 2, 3 and 5 (negative control), had large numbers of adult tapeworms found in the lumen of their intestines, (16, 11 and 21 respectively) as shown in Plate 1(a,b,c). Histopathological examination of the tissues from the intestines (Plate a), liver (Plate b) and lung (Plate c) were conducted. For each of the tissues, comparison was then made with those of normal histology in standard texts and micrographs for any differences.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>3.3±0.252</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>3.1±0.252</td>
</tr>
<tr>
<td>Glycosides</td>
<td>0.7±0.238</td>
</tr>
<tr>
<td>Saponins</td>
<td>4.0±0.163</td>
</tr>
<tr>
<td>Steroids</td>
<td>79.1±0.309</td>
</tr>
<tr>
<td>Tannins</td>
<td>96.7±0.163</td>
</tr>
</tbody>
</table>

Plate 1. Numbers of adult tapeworms fund in the lumen of the intestines of rats from the different groups (group 2 = 16, group 3 = 11 and group 5 = 21)
3.5 Histopathology Results

From the results of the histopathology of the intestine of rats in group 1, a normal orientation of Tunic (Serosa, muscularis, sub-mucosa) with clearly normal intestinal gland and Tunica mucosa having normal orientation of villi and crypt of Lieberkühn were observed (Plate 2a). From the liver tissue of rats in the same group, there was a normal distribution of hepatocytes, normal orientation of sinusoid with normal clear central vein (Plate 2b). It was observed that the lung tissue also shows normal alveoli with clear alveoli sac and alveoli wall and normal type I and type II pneumocytes (Plate 2c).

Results from tissues of the rats in group 2, the intestine has shown normal orientation of Tunic (Serosa, muscularis, sub-mucosa) with clearly normal intestinal gland, Tunica mucosa but having highly degenerated orientation of villi and crypt of Lieberkühn (Plate 3a). The liver from rats has shown normal distribution of hepatocytes, slight widening of sinusoidal space but with normal clear central vein (Plate 3b). Tissue of the Lung has shown normal alveoli with clear alveoli sac, alveoli wall and pulmonary bronchus, with normal type I and type II pneumocytes (Plate 3c).

Results from the intestines of rats in Group 3, shows normal orientation of Tunic (Serosa, muscularis, sub-mucosa), with clearly normal intestinal gland, and Tunica mucosa having moderately degenerated orientation of villi and crypt of Lieberkühn (Plate 4a). Tissue of the liver has also shown normally distributed hepatocytes, moderate widening of sinusoidal space with a normally clear central vein (Plate 4b). Results have shown normal alveoli with clear alveoli sac, alveoli wall, with normal type I and type II pneumocytes (Plate 4c).

Results from the tissues of rats in Group 4 has shown normal orientation of Tunic (Serosa, muscularis, sub-mucosa) with clearly normal intestinal gland and Tunica mucosa having normal orientation of villi and crypt of Lieberkühn (Plate 5a). It was also observed that tissues of the liver showing normally distributed hepatocytes, severe widening of sinusoidal space with a normal clear central vein (Plate 5b). The results of the tissues from the lung have shown normal alveoli with clear alveoli sac and alveoli wall, with normal type I and type II pneumocytes (Plate 5c).

Fig. 1. 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

A= Albendazole at 7.5 mg/kg (positive control); B= Senna italica extract at 75 mg/kg; C= Senna italica extract at 150 mg/kg; D= Senna italica extract at 300 mg/kg; E= infected but not treated (Negative control)
Plate 2. Photomicrographs of rat organs with different treatments

A= Photomicrograph of Rat intestine treated with albendazole at 7.5mg/kg (Group 1) showing normal orientation of Tunica Serosa (Red arrow), Tunica muscularis (C), Tunica submucosa with clearly normal intestinal gland (B), and Tunica mucosa (A) having normal orientation of villi (Green arrow) and crypt of Lieberkühn (Yellow arrow), H&E; X250.

B= Photomicrograph of Rat Liver treated with albendazole at 7.5mg/kg (Group 1) showing normally distributed hepatocyte (Red arrow), normal of orientation of sinusoid (Green arrow) with normal clear central vein (CV). H and E; X250.

C= Photomicrograph of Rat Lung treated with albendazole at 7.5mg/kg (Group 1) showing normal alveoli (A) with clear alveoli sac and alveoli wall, normal type I pneumocytes (Red arrow) and type II pneumocytes (Green arrow), H&E; X200.

Plate 3. Photomicrographs of rat organs with different treatments

A= Photomicrograph of Rat intestine treated with Senno italica extract at 75mg/kg (Group 2) showing normal orientation of Tunica Serosa (Red arrow), Tunica muscularis (C), Tunica submucosa with clearly normal intestinal gland (B), and Tunica mucosa (A) having highly degenerated orientation of villi (Green arrow) and crypt of Lieberkühn (Yellow arrow), H&E; X250.

B= Photomicrograph of Rat Liver treated with Senno italica extract at 75mg/kg (Group 2) showing normally distributed hepatocyte (Green arrow), slight widening of sinusoidal space (Red arrow) with normal clear central vein (CV), H&E; X250.

C= Photomicrograph of Rat Lung treated with Senno italica extract at 75mg/kg (Group 2) showing normal alveoli (A) with clear alveoli sac and alveoli wall, pulmonary bronchus (PB), normal type I pneumocytes (Red arrow) and type II pneumocytes (Green arrow), H&E; X200.

Plate 4. Photomicrographs of rat organs with different treatments

A= Photomicrograph of Rat intestine treated with Senno italica extract at 150mg/kg (Group 3) showing normal orientation of Tunica Serosa (Red arrow), Tunica muscularis (C), Tunica submucosa with clearly normal intestinal gland (B), and Tunica mucosa (A) having moderately degenerated orientation of villi (Green arrow) and crypt of Lieberkühn (Yellow arrow), H&E; X250.

B= Photomicrograph of Rat Liver treated with Senno italica extract at 150mg/kg (Group 3) showing normally distributed hepatocyte (Red arrow), moderate widening of sinusoidal space (Green arrow) with normal clear central vein (CV), H&E; X250.

C= Photomicrograph of Rat Lung treated with Senno italica extract at 150mg/kg (Group 3) showing normal alveoli (A) with clear alveoli sac and alveoli wall, normal type I pneumocytes (Red arrow) and type II pneumocytes (Green arrow), H&E; X200.
The results from the tissues of rats in Group 5, it was observed that there is a normal orientation of the Tunics (Serosa, muscularis, sub-mucosa) with clearly normal intestinal gland and Tunica mucosa having a completely degenerated orientation of villi and crypt of Lieberkühn (Plate 6a). Tissues of the liver have also shown a normal distribution of hepatocytes, normal orientation of sinusoid with a normal and clear central vein (Plate 6b). The tissues of the lung have shown normal alveoli with clear alveoli sac and alveoli wall, with normal type I and type II pneumocytes (Plate 6c).

4. DISCUSSION

As several researchers have tested and reported the anthelmintic effect of medicinal plants against several other helminth parasites Merlin and Benjamin [21]; Cock et al. [22], this is also evident with Senna italica extract against Hymenolepis diminuta. 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 300mg/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 Samia and Sherif, [23]; Bizhani, [24]. Similarly, Arun and Vareishang, [25], 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 our environment, Senna italica represents a potential replacement of albendazole which is a synthetic drug that has been associated with gastrointestinal and reproductive side effects.

Histopathological analysis of tissues revealed that animals treated with 75 mg/kg and 150 mg/kg of the plant extract showed partial degeneration of villi and crypt lieberkhum in the intestine. This could be attributed to higher frequency of the parasites which were not cured completely by the concentration given in those groups, as earlier reported by Godfrey et al. [26] in their study on the antihelminthic effects of Anacardium occidentale in Wistar rats. Similarly, Tedong et al. [27], reported the degeneration of lieberkhum while studying acute and sub chronic toxicity of Senna occidentale Linn leaves hexane extract in mice infected with intestinal cestodes. Furthermore, the sinusoidal space was also observed to increase with increase in the concentration of the plant administered although increase in widening of sinusoidal space does not show any toxic effect to the animals as reported by Madara et al. [28].

5. CONCLUSION

This study suggested that Senna italica leaf extract has anthelmintic effect against Hymenolepis diminuta. Toxicity and histopathology results has also shown that the extract has a wide safety margin and a low toxicity level as observed in the experimental subjects (rats).

6. RECOMMENDATIONS

The extract can be recommended for researchers keeping laboratory colonies of rats for experimental purposes for its anthelmintic efficacy in order to improve the health status of
the experimental animals (rats). The extract can also be further validated for its efficacy before it can be approved for use as anthelmintic agent.

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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