

Impact of Intake a Sidr (*Zizyphus Spina-Christi* L.) Extract on Enterobiasis Vermicularis Infection for Children

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Enterobiasis vermicularis (Ev), considered the most common helminthic infection in the world. Approximately 4% to 28% of children infected with Ev. Sidr (*Zizyphus Spina-Christi*) leaf has many therapeutic effects. The aim of the study promising anti-Ev agents by using Sidr extracts leaves. Choosing the Ev infected from 150 male children (7-10 yrs.), 5 children served as the normal control group and 25 children whose stool were infected with Ev. The data indicated the children treated with Sidr extracts at two levels (10 or 20 mg /day) where the (Pus cells & RBC's and Ova counts) in the stool decreased significantly from the first to the second week. After the continuation treat with Sidr, the Ev and Ova completely disappeared in the third week in dose 10 mg and second weeks of the dose 20 mg of Sidr. Furthermore, the children treated having improved significantly (P = 0.001) in (HB, RBC's, and WBC 's) parameters.

Keywords: Children; Enterobiasis Vermicularis; *Zizyphus Spina Christi*.

Enterobiasis vermicularis (Ev) called a human pinworm. A human parasitic malady brought by the pinworm (Adult females: 8 to 13 mm, grown-up male: 2 to 5 mm). The widest recognized indications were a triangle in the butt-centric zone; this could make a difficult rest¹. Consequently, the time frame from swallowing eggs to the presence of new eggs around the anus is about 1-2 months. Moreover, the illness spread between individuals by pinworm eggs². Enterobiasis vermicularis considers the most common helminthic infection in the world.^{3&4}. about 4% to 28% of children infected by Ev possesses the cecum and have at times been accounted for in the appendix⁵ Accordingly, the nearness of Ev in the appendix can cause an infected because of obstructive phenomena⁶

Fleming *et al.*,⁷ and Zourae, *et al.*,⁸ revealed variable Ev rates about 4%, 7%, and

9.8% among pediatric appendectomies, from various pieces of the world -Ireland, Iran, and USA, respectively separating eggs at first happen around the anus and can get by for as long as three weeks in the circumference. They might be swallowed the eggs from the pollution of the hands or diet. Those in danger are the individuals who go to class, live in a health services organization, or deal with infected individuals⁹. Yang, *et al.*,¹⁰ reported that persistent itching can cause disturbances in sleep and may be insomnia, later registered the female genitourinary infections have watery diarrhea in some patients. At times, abdominal pain and other serious complications, like appendicitis can occur due to worms blocking the lumen in the appendix or lead to inflammation around the appendix sometimes tiny thread-like worms may be visible to the naked eye in the terminal area. Consequently, the medical

treatment of Ev variety, e.g. Mebendazole, Pyrantel pamoate or Albendazole, are used, two weeks separated¹¹.

These medical occurrences, many side effects, including headache, nausea, dizziness, metallic taste, and failures in treatment are frequently reported Sik *et al.*,¹². Additionally, the development of parasites resistant to common therapies features the requirement for new elective restorative approaches and the significance of natural treatment as a novel enemy of parasitic specialists. Numerous restorative plants indicated a guarantee in the treatment of Ev, with low side effects and high impact illustrated by Eleni *et al.*,¹³.

Zizyphus Spina-Christi L. locally known as Sidr is a multipurpose tree species belonging to the botanical family Rhamnaceae. It is an important cultivated tree and one of the few truly native trees species in the Arabic area that is still growing to now¹⁴. It contains flavonoids, alkaloids and, saponins are the main phytochemicals. The major components of the leaves' volatile oil are geranyl acetone, methyl hexadecanoate, methyl octadecanoate, farnesyl acetone, hexadecanal, and ethyl octadecanoate. Sidr is one of the significant organic product crops in dry parts of tropical Asia and Africa¹⁴. The organic product of sidr is profoundly nutritious and plentiful in vitamin C. The dry organic product (i.e., per 100 g) contains 314 calories, 0.9% fat, 4.8% protein, 80.6% complete sugar, 140 mg Ca, 3 mg Fe, 0.13 mg riboflavin, 0.04 mg thiamin, 3.7 mg niacin, and 30 mg ascorbic acid¹⁵. The botanical extract of Sidr leaves contains four saponin glycosides: christanin A, christanin B, C and D. It was shown to contain butyric acid and kinetic acid, cyclopeptides, as well as flavonoids lipids, protein, free sugar and mucilage¹⁶. The aim of our study: The impact of Sidr (Zizyphus Spina-Christi L.) leaf against the Ev in a sample of children as a natural treatment and compared with the effect of metronidazole drug.

MATERIALS AND METHODS

Chemicals and Plant Extracts

Dried Zizyphus Spina-Christi leaves (Sidr) were purchased from the agriculture organization market in Giza-Egypt. Sidr leaves were extracted according to the method described

by Adzu *et al.*,¹⁷. Preparation of water extract on the heat by collecting 10 g and/or 20 g of dry leaves powder of Sidr and put in a glass flask 500ml capacity contains 200 ml distilled hot water 100° C. Consequently, three layers of the coffee filter were nominated to separate the large plankton. Therefore, the final filtration was performed using a centrifuge for 15 minutes to separate the small plankton and obtain a clear solution. Then get a basic solution and complete the size to 100 ml of Sidr extract.

Experimental Protocol and Subjects

Planning the mothers' meeting to invite him to participate in the study. Accordingly, all mothers attending the meeting approved to examine the stools of her children. Nevertheless, 40% of them did not approve the bleeding examine. As well as, written informed consent was obtained from all the mothers and fathers who, her children will participate in this study. One hundred fifty of the children presented their stool samples. After examining it found about 25 subjected infected with Ev and/or Ova. Therefore, the total samples chosen were 30 children; males (7-10 yrs.) were carried out of three primary schools in Cairo, Egypt. The children were arranged into four groups. These presented in Fig. (1):

Five children served as normal control (-ve) G1. As well as, 25 children who as follows:

G 2: Children infected with Ev + treatment with Sidr (10 mg/200 ml distill water.) /21 days (n = 10) according to Adzu *et al.*,¹⁷.

G 3: Children infected with Ev + treatment with Sidr (20 mg/200 ml distill water.) /14 days (n = 10).

G 4: Children infected with Ev + treatment with Mebendazole (MBZ) (100 mg/7 days) for 14 days (n = 5).

Stool samples collected to determine the infection (pus cells and RBC's) by Ev before and after treatment, according to Wilson *et al.*,¹⁸. Red blood cells (RBC's) count and white blood cells (WBC's) were indicated by Linberg *et al.*,¹⁹. Hemoglobin (Hb) had decided by Hardison and Ross,²⁰.

Statistical Analysis

The results are reported as the mean \pm SD of values obtained from multiple observations. Differences between means were considered statistically significant at $P < 0.01$ variance²¹.

All statistical analyses were computed by SPSS version¹⁰.

Ethical Approval

The protocol was approved by the Ethics Committee for human studies of the Ain shams the University of Cairo, Egypt, and by the private education management in Cairo.

RESULTS

Our data in Figure (2) have indicated that assessment of the stool's analysis of children in four groups before treatment, the results showed that no pus cells and red blood cells (RBC's) in the stool for group 1 as negative control. Concerning to the groups of children have infected with Ev, it was observed that pus cells were elevated significantly between groups G 2, G 3 and G 4 at ($p < 0.001$) compared with control negative with a mean value of (4.0 ± 0.2 , 0.6 ± 4.4 and 0.4 ± 4.2), respectively. Accordingly, the existence of RBC's in the stool of children before treated was presented in Fig. (2). The data showed that there was a significant difference at ($p < 0.001$) between infected groups compared with the group of healthy children. Moreover, the results of a table (1) illustrated

the effect of the Sidr extract on stool analysis (Pus cells & RBC's) of children infected with Ev after treated for three weeks. Therefore, it was observed that infected children (G 2) have treated with Sidr at level one (10mg/day) the (pus cells & RBC's) in stool were decreased significantly at ($p < 0.001$) gradually from the first to second weeks furthermore, disappeared in the third week compared between them and other groups.

Furthermore, the data in a table (1) indicated that the group of infected children in (G3) that having the treatment with Sidr at level two (20mg/day) in the first week and follow up three weeks, the results of (pus cells & RBC's) in stools were decreased significantly at ($p < 0.001$) and disappeared in the second and third weeks. The results of treating the infected children in (G 4) with MBZ drug represent in table 1 the data observed that (pus cells & RBC's) in the stool were disappeared after the second week.

The comparison between the blood analyses of children Pre and after the treatment with Sidr extract is present in the table (2). Consequently, the results indicated that a decline significantly at ($p < 0.001$) of Hb levels, RBC's and WBC's, count of children infected with Ev pre-

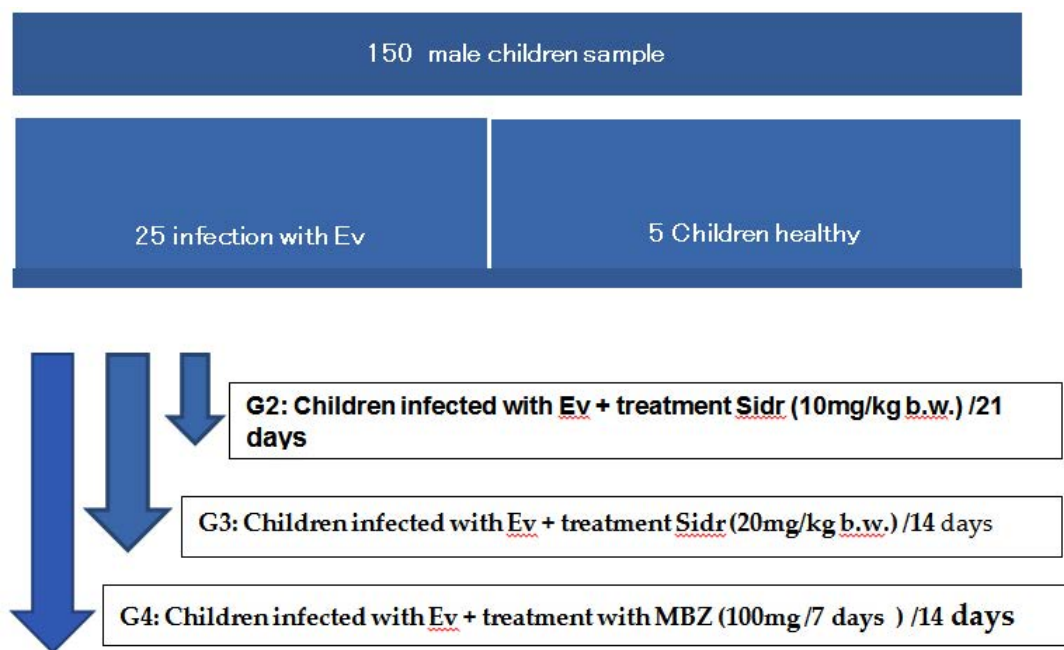


Fig. 1. Showed the protocol of chosen samples and distribution groups and treatment

treated (G2 G3 and G4) compared with the healthy children in group one. However, the parameters of blood (Hb, RBC's, and WBC's) were improved significantly after treatment. Accordingly, the best results of Hb, RBC's and WBC's in the group (3) treated with Sidr (at level 2 of 20 mg) with the mean value of (15.0± 0.3 g/dL, 5.0±0.1 M/ μ L and 9.0 ±0.8 K/mm), respectively compared with the same group before the treated and other groups. Besides all blood variables were risen and improved significantly in treating groups post-treatment compared with the same groups before treated.

The impact of *Zizyphus Spina-Christi* L. extracts on (Ev) – Ova infected children post-treatment for three weeks was presented in Table (3). Accordingly, the results illustrated that the Ova count disappeared significantly at ($p < 0.001$) completely in the third week for three groups (G 2: G 4). As well as, the data illustrated that the children treated with Sidr at (20 mg /or MBZ 15 mg) the Ova significantly at ($p < 0.001$) disappeared totally in the second week compared with before treated.

DISCUSSION

Enterobius vermicularis (pinworm) is one of the most well-known human parasitic helminths, and It is widely prevalent among kids.

Some behaviors and natural components in the environment may encourage pinworm disease. Our results indicated that from 150 children stools analyses were founded 25 (16.6%) of children infected with pinworms. These agree with Fan, *et al.*,²² reported that the overall prevalence of pinworms infection was 22.4% in samples. Girls (20.31%) had a lower prevalence than boys (24.5%) age range bigger than 5 years. Since individual cleanliness and exposure are significant transmission factors as poor personal or group hygiene, for example, kindergartners age, schools, orphanages, and family groupings 23 and 24. Ahamed *et al.*,²⁵ identified Ev in a sample of children aged from (6 – 12) years in a rural area at Beheira, Egypt were found the 11.8% of specimens infected with Ova in stool. Our data represented that the children treated with Sidr extract at two levels (10 or 20 mg/day) were the (pus cells & RBC's and Ova counts) in stool decreased significantly at ($p < 0.001$) gradually from the first to the second week. After the continuous treat with Sidr extracts, the Ev and Ova completely disappeared in the third week in the dose, 10 mg of Sidr and the second week of the dose 20 mg of Sidr. Our illustrated explain that the efficacy of Sidr against Ev and Ova in children as similar results of the MBZ drug in group 4. Many of the studies reported the potential effect of *Zizyphus* against diarrhea²⁶, antinociceptive¹⁷, anticarcinogenic²⁷,

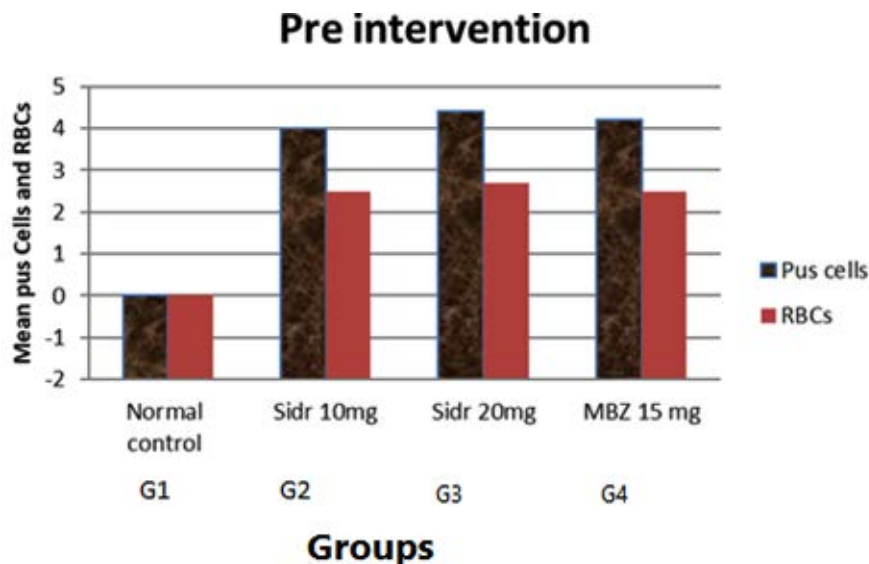


Fig. 2. Assessment the Stool Analysis (Mean pus cells and RBC's) for children pre intervention for groups

Table 1. Effect of *Zizyphus Spina-Christi L.* extracts on Stool analysis of children infected with Ev after treatment for three weeks

Groups	Treatment doses (mg/ day)	Mean Pus Cells \pm SD (First week)	Mean RBC's (hpf) \pm SD (First week)	Mean Pus Cells \pm SD (second week)	Mean RBC's (hpf) \pm SD (second week)	Mean Pus Cells \pm SD (third week)	Mean RBC's (hpf) \pm SD (third week)
Normal control	Non-infected	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Treated groups	Group 1	1.2** \pm 3.0	0.8** \pm 2.0	1.01 \pm 0.3**	1.4 \pm 0.5**	0.00 \pm 0.00**	0.00 \pm 0.00**
	Group 2	0.9** \pm 2.0	0.5** \pm 1.0	0.00 \pm 0.00**	0.00 \pm 0.00**	0.00 \pm 0.00**	0.00 \pm 0.00**
	Group 3	1.1** \pm 2.7	0.7** \pm 1.5	0.00 \pm 0.00**	0.00 \pm 0.00**	0.00 \pm 0.00**	0.00 \pm 0.00**
Group 4							

P values represent the relationship between non-infected and treated groups for children.

**Highly significant at $P \leq 0.001$

Ev= *Enterobiasis vermicularis*

Table 2. The comparison between the blood analysis of children pre and post- treatment with *Zizyphus Spina-Christi L.* extracts

Groups	Treatment doses (mgD day)	Pre- treatment		Post-treatment	
		Mean HB g/dL	Mean RBC'sM/ μ L	Mean HBg/dL	Mean RBC'sK / mm
Normal control	Healthy	13.0 \pm 0.1	4.3 \pm 0.05	13.1 \pm 1.0	4.2 \pm 0.2
Treated groups	Group 1	11.0 ** \pm 0.7	3.7** \pm 0.23	14.0 \pm 2.0**	4.0 \pm 0.3**
	Group 2	10.2** \pm 0.5	3.4** \pm 0.18	15.0** \pm 0.3	5.0** \pm 0.1
	Group 3	10.5** \pm 0.5	3.5** \pm 0.16	14.1** \pm 1.0	5.0** \pm 0.2
Group 4					

P values represent the relationship between group1 and other groups for children.

**Highly significant at $P \leq 0.001$

antibilharzial 28, analgesic 29. Besides, Gheith & El-Mahmoudy, 30 finding that *Zizyphus* plant extracts were significant inhibitory effects against most Gram-positive microbial strains, namely, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium perfringens*. A lot of studies investigated the common medical drugs using to treat the *Enterobius vermicularis* and having side effects.

Yang *et al.*,¹⁰ showed that children were injured to pinworm and therapy with mebendazole have associated with increased bacterial diversity, an increased abundance of Actinobacteria including the probiotic *Bifidobacterium*, and a decreased proportion of Fusobacteria. Further, the change in the microbial composition detected two weeks after administration of mebendazole on pinworm-infected children could be confounded by late-onset effects on the metabolism of commensal bacteria and subsequently influence a host's immune system. Our data showed the children infected with Ev pre-treatment having a significant decline at ($p < 0.001$) of Hb levels, RBC's and WBC's counts. However, *Enterobius vermicularis* caused many lesions our findings accepted by Ahamed, *et al.*,²⁶ who found a significant correlation between parasitic infection and headache, fatigue, loss of appetite, pallor, perianal itching, abdominal colic, diarrhea, loss of weight and salivation during sleeping. An addition, Bassam, 31 found the prevalence of anemia in a village, Gaza, Egypt among the children infected with Ev (30.8%) was greater than among those non- infected (19.9%),

and that pattern appeared in all age groups. On the other hand, the finding that the Hb concentration was affected by Enterobiasis this result agreed with the studies 32, 33 and 34 an addition, Hama,³⁵ who found there was a significant relation between Enterobiasis and hemoglobin deficiency. Also, Ahamed *et al.*,²⁵ reported a significant correlation in the white blood cell counts and the presence of Enterobiasis, (80%) of the affected children. Our Finding that the children, after treating with Sidr extracts having improved significantly in (Hb, RBC 's, and WBC 's) levels compared pre-treatment. However, these results compatible with the results of a stool analysis. So, disappeared the Pus Cells, RBC's and Ova completely disappeared in the third week in dose 10 mg and the second week of dose 20 mg of Sidr. However, the Sidr considers the rich plant with nutrients Rabia, *et al.*,³⁶ described that *Zizyphus Spina-Christi* L. the extract contains a high percentage of copper about 0.0116 to 0.066 mg/g. Furthermore, the Cobalt concentration varied from 0.006 to 0.03 mg\ g in plant samples. Besides, it contains a higher amount of iron (11.39 to 24,98 mg/g). Although, the fruit of Sidr contains a high level of copper (0.53 mg/100 g), iron (10 mg/100 g) and zinc (4.2 mg/100 g). Marcelino, *et al.*,³⁷ reported that the seed was distinguished by its high content of total dietary fiber (82.1 g/100 g), calcium (540 mg/100 g), copper (0.54 mg/100 g), chromium (1.1 mg/100 g) and zinc (4.5 mg/100 g).

Abeer and Reem, 14 showed the protective and therapeutic effects of the Sidr extract in male albino rats treated with pentylenetetrazol and

Table 3. The impact of *Zizyphus Spina-Christi* L. extracts on (Ev) – Ova infected children post-treatment for three weeks

Groups	Treatment doses (mg D day)	Pre intervention. Ova± SD	Post intervention.		
			Mean Ova± SD (First week)	Mean Ova ± SD (Second week)	Mean Ova ± SD (third week)
Normal control	Group 1	Non-infected	No there		
Treated groups	Group 2	Sidr 10mg	4.0±1.0**	3.0±1.0**	1.0 ± 1.0**
	Group 3	Sidr 20mg	4.0±2.0**	1.0±1.0**	0.0 ± 0.0**
	Group 4	MBZ 15mg	4.0±1.0**	1.0±2.0**	0.0 ± 0.0**

P values represent the relationship between no-infected and treated groups for children.

**Highly significant at $P \leq 0.001$

Ev= *Enterobiasis vermicularis*

confirmed that Sidr improves RBC 's, and WBC 's levels in rats. As well as, the results agree with El-Desouky *et al.*, 38 who's showed that 12.5 mg of aqueous extract of Sidr leaves for two weeks, increased significantly the RBC's and WBC's count and improve Hb in rats.

CONCLUSION

Finally, we can conclude that using the Zizyphus Spina-Christi L. extract considers the potential effect against Ev, Ova and the side effect of infection, children can be used for two weeks at dose 20 mg/kg or 10 mg/kg dose for three weeks.

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