Efficacy of a Single dose versus a Multiple Dose Regimen of Mebendazole against Hookworm Infections among School Children: A Randomized Single-Blinded Trial

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KEYWORDS
Hookworm, Single Dose, Multiple Dose, Mebendazole, Efficacy
Abstract

Background: Despite the existence of population-based control program using single dose albendazole or mebendazole as a preventive chemotherapy, Hookworm disease transmissions remains high. It causes a negative impact on the growth and school performance of children. In connection to this preventive chemotherapy, different studies produced conflicting results. This study evaluated the efficacy of single (500mg) versus multiple doses (100mg twice a day during three consecutive days) of mebendazole against Hookworm infections among school aged children.

Methods: This randomized single-blinded clinical trial took place among school-aged children (6-14 years old) in Burie and Debre Elias towns, Northwest Ethiopia. Using simple randomization, eligible Hookworm positive children were allocated (1:1) to either a single or multiple doses treatment arm. Stool samples were collected and processed using McMaster method at baseline and follow-up period (14-21 days after treatment). Main outcome measures: The cure rate against Hookworm and egg reduction rate for determining the changes in infection intensity were the main outcome measures after 14-21 days following dosing. An independent t-test was used to compare group means, and logistic regression was used to calculate odds ratio (OR). P-value < 0.05 at 95% CI was considered statistically significant.

Result: 109 children were participated in both treatment arms. Cure rate against Hookworm was significantly higher in the multiple dose (96.1%) than in the single dose (30.8%) with (OR=55.125; 95% CI: 11.92-254.9; P < 0.001). The egg reduction rate in the multiple dose treatment arm (99.5%) was also significantly higher than in the single dose arm (68.9%) with difference (t (101) =5.38; 95% CI 230.95-505.36; P < 0.001). Conclusion: The single dose regimen of mebendazole for the treatment of Hookworm infection showed poor efficacy, while the multiple dose revealed satisfactory efficacy. Moreover, infection intensity reduction was not achieved following single dosing. Therefore, we strongly recommend replacing the single dose mebendazole regimen with multiple dose regimen during deworming program in hookworm endemic areas.

Trial registration: This trial is registered in www.pactr.org, # PACTR201911466695052

Background

Globally, Hookworm’s disease burden remains high, and an estimated around 500 million people are
infected(1). In 2017, the global burden of Hookworm infections was estimated at 845,000 disable-adjusted life years (DALYs) (2). The burden mainly associated with hypo-albuminemia, iron deficiency anemia, and malnutrition, which cause more subtle chronic health problems like physical and intellectual growth retardation in children, and adverse pregnancy outcomes (3,4). Moreover, it cause annual productivity loses with estimated range from $7.5 billion to $138.9 billion(1). The highest burden and intensity of infections occur in sub-Saharan Africa followed by Asia, Latin America and the Caribbean (5). The majority of Hookworm infections occur in the sub-Saharan Africa, with an estimated infections of 40–50 million school-aged children and 7 million pregnant women (6). In general, over 267 million preschool-aged children and over 568 million school-aged children live in areas where the parasite is intensively transmitted and are in need of treatment and preventive interventions (7).

Currently, control efforts for Hookworm infections to are implemented through periodic mass drug administration (8,9). Single dose mebendazole (500mg) is one of the recommended chemotherapy and is in the World Health Organization’s (WHO) Essential Medicine List for treating and controlling helminthes infections (10). This controlling measure is implemented without prior information on the infection status of individuals. These days, studies have revealed conflicting reports on the impact of preventive chemotherapy (PC) (4)

In addition to this, multiple studies have shown different efficacies of single dose mebendazole against Hookworm infections. Moreover, the cure rate (CR) and egg reduction rate (ERR) are not satisfactory (11). For instance, a multi-centered study conducted on the efficacy of single dose mebendazole in terms of ERR from six endemic countries among school children revealed 65.4% - 95% with overall ERR of 80.6% (12). Other studies also experienced inconsistent and unacceptable efficacy status of single dose mebendazole in terms of CR ranging from 17.6% - 58.5% (10,13–15). Generally, this regimen gave unsatisfactory results for clearing of Hookworm infections in different regions of the world. However, it is relatively cheap and widely available (16).

A multiple dose (100 mg twice a day over three consecutive days) of mebendazole is among the recommended and widely used anthelminthic regimen for treating Hookworm and other soil-
transmitted helminths (STH) infection throughout the world (17,18). Only limited numbers of studies were conducted to evaluate the multiple dose efficacies against Hookworm infections. Some studies revealed various efficacy status of multiple dose with the CR ranging from 26% to 97.9% and ERR from 85% to 100% (15,19,20). This inconsistent efficacy status of the drug warrant further studies. The possibility to increase and assure the efficacy of the drug is given special consideration on the treatment regimen. Previously, one study was conducted in Tanzania about the standard single dose of mebendazole to multiple dose (100 mg twice a day for three days) and it showed that the multiple regimen is much better (19). However, it is the only randomized clinical trial conducted so far. To create more evidence, we conducted another clinical trial with these treatment arms, the first of its kind in Ethiopia.

**Methods**

**Study design**

This randomized, single-blinded clinical trial was conducted at Burie and Debre Elias towns’ primary schools, Northwest Ethiopia from March to May, 2019 and included school-aged children aged 6 to 14 years. The study was approved by the Ethical and Review Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Ethiopia. This trial is retrospectively registered in www.pactr.org, number PACTR201911466695052 on November 26, 2019.

Prior to participant enrolment, all parents/ legal guardians’ of the children were informed about the objective, purpose, study procedures, and the potential risk and benefits of participating in the study. Parents/ legal guardians who agreed their child to be included in the study were asked to sign a written informed consent. Verbal assent was also sought from each participant. Moreover, for those parents/legal guardians who were unable to read and write, they were asked to give thumbprint after having been read the full informed consent form by a data collector.

**Intervention, trial medication, and outcome measures**

This randomized trial was carried out in two treatment arms: (i) single dose (500mg) and (ii) a multiple dose mebendazole (100 mg twice a day for three consecutive days).
mebendazole (WORMIN tab) was commercially obtained from private pharmacy in the local market, while the single dose mebendazole (Vermox®) was provided by the local coordinator office of the deworming program. The CR against Hookworm and ERR for determining the changes in infection intensity were the main outcome measures after 14-21 days following dosing.

**Eligibility criteria and Sample size**

Eligible for inclusion were all Hookworm-positive children with a signed informed consent and who did not have additional health problems (based on medical history, physical examination, vital signs) other than Hookworm were randomized and allocated to one treatment arm. The following exclusion criteria were also applied: children who received any form of anthelminthic treatment within the past 30 days, had diarrhea at the time of the first sampling, had a hemoglobin level <8g/dl, experienced severe concurrent medical condition, had any known history of allergic reaction to mebendazole, and infected with other parasitic infection.

The desired sample size was determined by using WHO guideline(21) with the following assumptions: the local prevalence of Hookworm infections was assumed to be 50% due to lack of recent finding and a total of 100 Hookworm infected individual (50 in each of the two treatment arms) would be needed to detect differences in the CR following different treatment arms for the cure of Hookworm infections with 80% power using a 2-sided statistical test with alpha-level of 0.05. Moreover, by considering the potential loss to follow-up; 20% as a non-response rate was added. Finally, 300 school aged children was screened for Hookworm infections microscopically to get the minimum required sample size.

**Data collection and Laboratory procedures**

Study participants had responded to a short questionnaire investigating demographic and other health-related issues using the WHO drug efficacy assessment from. A specific identification number was given to each participant. Then, each participant received a sterile stool container labeled with his/her unique identification number and was asked to provide approximately 10mg of fresh stool. All children were well informed to avoid any contamination of the sample. Following this, samples were immediately transported to the nearby health center laboratory in Debre Elias and Burie hospital laboratory.
The McMaster concentration technique, which is the standard reference method for evaluating drug efficacy in Veterinary Parasitology and has recently been evaluated for human helminthes, was used for this study(21,22). Laboratory quality control was performed through re-reading 10% of the slides of each laboratory technician by an expert microscopist. Only one stool sample was collected from each participant in both baseline and follow-up survey. To ensure the objectivity and avoid the risk of prejudgment on the treatment response, the laboratory technicians were blinded to the dose allocation, the hypothesis and objective of the study.

Upon completion of all the baseline parasitological and participant information survey, all children who were found to be positive for Hookworm infection were subjected to a physical and clinical examination by senior health officer. Height was measured with a standard meter (to the nearest 0.1 cm), and weight with an electronic balance (to the nearest 0.1 kg). Haemoglobin levels were measured in capillary blood using the finger-prick method (HemoCue®301).

**Randomization**

Finally, eligible Hookworm positive children were randomly assigned either to the single (500mg) or multiple dose regimen of mebendazole (100 mg twice a day for three consecutive days) arms of the study with 1:1 ratio. Randomized of the children were not based on their age, sex or by any means of personal parameter before randomization. Using simple randomization lottery techniques, 55 eligible children were categorized into the single and 54 into multiple dose mebendazole arms.

**Drug administration**

A slice of biscuit was given to each eligible child before drug administration. Participants who were randomized in single dose arm were asked to take the drug in front of their parents and a public health officer at school. After administering the drug, children were monitored for 3 to 4 hours for the occurrence of any vomiting and other adverse events following treatment. In the case of children who were randomized into multiple dose arm; parents/guardians were asked to take home the remaining tablets in a sealed envelope and were instructed on how to administer the drugs. They were instructed to give the drug twice a day (every morning and evening for three days), avoid skipping/doubling any dose, follow strictly their child up to the end of treatment and reminded not to
drink alcohol. Participants/parents/guardians were requested to report any medical discomfort following treatment to the investigators or the nearby health extension worker.

**Follow-up data collection**

Each treated children were revisited after 14-21 days of drug administration and asked to provide one stool sample for the second time. At this time point, children were also asked about the occurrence of vomiting and diarrhea following drug administration. A participant who vomited within 4 hours after drug administration or a participant with diarrhea was excluded for the final analysis. The same laboratory procedures took place at the follow-up. Children who remained infected with Hookworm and other STH were treated with albendazole (400mg) at the end of the study.

**Statistical analysis**

Data was entered to Epi-data software to check data completeness and clearance, and then transferred to SPSS version-23 for statistical analysis. All analyses were performed on a per-protocol basis. Only children with complete data sets were included in the analysis to determine the treatment efficacy. The baseline characteristics of the study participants are summarized using frequencies, mean and standard deviation. Infection intensity with Hookworm were grouped in to light, moderate and heavy infections, according to WHO guideline (23). Cure rate and ERR were used to assess the efficacy of the drug based on the following mathematical calculation. Cure rate was assumed to be the proportion of individual hosts positive for parasites who become parasitologically negative after treatment (24). Whereas egg reduction rate was the arithmetic mean egg count at baseline of the treatment group minus mean egg count at the end of treatment period divided by the mean egg count at the baseline and express it in percent (25).

\[
\text{ERR} = 100\% \times \left(1 - \frac{\text{arithmetic mean (post-intervention FEC)}}{\text{arithmetic mean (pre-intervention FEC)}}\right)
\]

Confidence intervals for ERR were calculated using bootstrap re-sampling method with 5000 iterations. An independent t-test was used to compare group means, whereas CRs were compared by calculated Odds Ratio (OR) using logistic regression. For all statistical analyses a *P-value* of 0.05 was
considered as the limit for statistical significance.

Result
A total of 300 school-aged children were enrolled in the baseline screening. Of these, 120 (40%) children; (64 females and 56 males) were found to be Hookworm-positive. Eleven Hookworm infected children were excluded because they were absent from school on the clinical and physical examination day. Furthermore, from the remaining 109 randomized infected children, one child from the single dose of mebendazole arm was not willing to receive the allocated treatment. Thus, 108 eligible children, 54 in each treatment arm had completed baseline data and received allocated treatment. After follow-up, a total of 103 children had complete data records, 52 in single dose and 51 in multiple dose mebendazole arm were included for the final efficacy analysis (Figure 1).

Baseline survey
At baseline, the eligible children allocated in the two treatment groups were comparable in terms of several characteristics. Participant’s characteristics expressed in the form of mean ± standard deviation (SD), age in single dose group was 10.78 ± 2.1 years; while in the multiple dose it was 10.48 ± 1.34 years. The mean weights of the participants for single and multiple doses were 31.45 ± 7.96 and 29.69 ± 6.2 kg, respectively. Moreover, most participants were diagnosed with light infection (see table 1).

Table 1: Baseline characteristics of randomized children, at Burie and Debre Elias towns, North West Ethiopia, January - June 2019
Efficacy of multiple and single dose regimen of mebendazole for treating Hookworm infections

In this analysis, the follow-up fecal egg intensity of Hookworm infection in terms of arithmetic mean were 378.04 EPG and 5.88 EPG in single and multiple doses regimen of mebendazole, respectively. Cure rates of single and multiple dose regimen of mebendazole for treating Hookworm infection were 30.8% (19.2 - 44.2%) and 96.1% (90.2-100%), respectively. In addition, the ERRs were 68.9% in the single dose arm and 99.5% in the multiple dose arm. Overall, 36 (69.2%) school-aged children who were treated with single dose and two (3.9%) treated with multiple dose mebendazole remained Hookworm-egg positive at the follow-up period (see table 2).

Table 2: Cure and egg reduction rates of single and multiple dose mebendazole against Hookworm infections among school aged children from Burie and Debre Elias towns, January - June 2019
<table>
<thead>
<tr>
<th></th>
<th>Single dose</th>
<th>M</th>
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<tbody>
<tr>
<td>No. of Infections before treatment (%)</td>
<td>52 (100%)</td>
<td>5</td>
</tr>
<tr>
<td>No. of cured after treatment</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>CR (95% CI)</td>
<td>30.8 (19.2-44.2)</td>
<td>9</td>
</tr>
<tr>
<td>EPG arithmetic mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment (95% CI)</td>
<td>1216 (845.8-1586.86)</td>
<td>1</td>
</tr>
<tr>
<td>After treatment (95% CI)</td>
<td>378.04 (237.1-510.99)</td>
<td>5</td>
</tr>
<tr>
<td>ERR (95% CI)</td>
<td>68.9% (48.07-73.14)</td>
<td>9</td>
</tr>
<tr>
<td>Infection intensity after treatment</td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Light (1-1999 EPG)</td>
<td>35 (67.1%)</td>
<td>3</td>
</tr>
<tr>
<td>Moderate (2000-3999)</td>
<td>1 (1.9%)</td>
<td>-</td>
</tr>
<tr>
<td>Heavy (&gt;=4000)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Comparative efficacy status of the multiple versus the single dose regimen of mebendazole for the treatment Hookworm infections**

There was a substantial difference for both CR and ERR between multiple and single dose regimen of mebendazole for treating Hookworm infections [(CR: 96.1% versus. 30.8%; OR=55.125; 95% CI: 11.92-254.9; p < 0.001), (ERR= 99.5% versus 68.9%; 95% CI 230.95-505.36; p <0.001)].

**Discussion**

Ethiopia is one of the hotspot areas for Hookworm and other STHs in the world. School children are disproportionately affected by the parasite(6, 26). Mass drug administration for selected risk groups such as children using single dose of albendazole or mebendazole is the mainstay for the control of soil transmitted helminthiasis in Ethiopia(27). However, there are recent reports which showed a reduction of single dose mebendazole efficacy in some endemic areas (13,19,28,29). Moreover, an increased use of the single dose of mebendazole in many endemic areas may lead to the possible
developments of drug resistance and it should be put into special attention on drug regimen. Thus, this and other similar reasons call for continuous monitoring of its therapeutic efficacy.

The present study showed that multiple dose of mebendazole is significantly more efficacious at clearing Hookworm infections than the single dose (96.1% versus 30.8) with (OR=55.125; 95% CI: 11.92-254.9; \( P<0.001 \)). This implies that almost all Hookworm-infected children were cured following multiple dose of mebendazole treatment (CR=96.1%, CI: 90.2 to 100). This is in line with another head to head comparative RCT study, which revealed superiority efficacy status of multiple dose over single dose mebendazole (CR=13%, OR=389.1, 95% CI 95.2 to 2885.7%, \( p = 0.001 \))(19). On the other hand, the therapeutic efficacy of multiple dose in the current study is considerably higher than those previously reported in Iran(CR = 35% & ERR= 40.83%) (30) and Brazil (CR = 58.5%)(31). This inconsistency in efficacy results could be related to the use of different diagnostic techniques, the sample size variation, the age of study participants, the parasite genetic diversity, and study site. For instance, a study conducted in Iran applied the Stoll diagnostic technique; whereas the study conducted in Brazil had used the duplicated Kato-Katz and Hoffmann’s spontaneous sedimentation techniques. Thus, variation in the sensitivity of the diagnostic techniques might be the possible source for the discrepancies.

So far, several researches have been conducted to assess the efficacy of single dose of mebendazole on Hookworm infections (19,20,28,29,32–34). Overall, their CRs ranged from 7.6% to 70.3% and ERRs ranged from 52% to 76.3%. The efficacy of single dose in our finding is; CR=30.8; CI: 19.2 to 44.2 & ERR=68.9; CI: 48.07 to73.14. This is in line with studies conducted in China (CR= 29%)(28), Vietnam (CR= 38% & ERR= 52%) (20), and Tanzania (CR=24.4 & ERR= 59.5%)(33). However, it does not agree with other studies conducted in Zanzibar (CR= 7.6%) (29), Tanzania (CR= 13%)(19), Lao PDR (CR= 17.6% & ERR= 76.3%)(34), and Cameroon (CR= 70.3%)(32). The above mentioned reasons could also have resulted in the discrepancies.

Lower efficacy of single dose than the multiple doses of mebendazole might be associated with the extensive and frequent use of single dose in deworming program; since frequent use of the single dose usually raises concern about drug resistance or tolerance. Although administration of single dose
of mebendazole for mass treatment is convenient in terms of practical implementation, our finding revealed that its capacity to succeed the primary objective of preventive chemotherapy on intensity reduction is questionable. In other words, 43 (82.7%), of the infected study participants were under light infection category at the base line, and 35 (67.1%) remained in this category after treatment (Table 2).

On the other hand, managing multiple dose of mebendazole as mass chemotherapy is complex and the cost of administration is high. However, the result of the present study has indicated that it is encouraging to use it as prevention and control measure in hookworm endemic areas.

Although comparing the two commonly used dose of mebendazole in head to head manner is considered as strength of the study, the infection intensity of the parasite was determined by the examination of a single stool sample of each study participant. And also, the multiple dose mebendazole was given by care giver of each participant. That might have affected the prevalence and the accuracy of the egg count of Hookworm infections. Thus, the findings should be interpreted with that limitation in mind.

In conclusion, the multiple dose regimen of mebendazole showed satisfactory efficacy with significantly higher CR and ERR than the single dose regimen, against Hookworm infection. These results advocate a need to revise treatment guidelines of the current deworming programs in predominantly hookworm endemic areas. Moreover, we recommend conducting further studies using large sample size and more sensitive diagnostic procedures. It is also better to conduct further investigation on species identification with respect to efficacy status of the drug.

**Abbreviations**

AM: Arithmetic Mean; BZ: Benzimidazole; CI: Confidence Interval; CR: Cure Rate; DALYs: Disable Adjusted Life Years; EPG: Egg per Gram; ERR: Egg Reduction Rate; OR: Odds Ratio; PC: Preventive Chemotherapy; STH: Soil-Transmitted Helminths; WHO: World Health Organization

**Declarations**

**Authors’ contributions**

TE conceptualized the study, contributed to the format of the data collection instruments, analysis,
and write up of the first draft manuscript. MA contributed to the study protocol, development of the data collection tools field data collection and analysis. AjZ was involved in the proposal development and data analysis. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of Data and Materials**

The data generated or analyzed during this study is included in this manuscript. Other data will be available from the corresponding author upon request.

**Consent for publication**

Not applicable

**Ethics approval and consent to participate**

Before starting the data collection process, Ethical clearance was obtained from Ethical and Review Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences University of Gondar, Ethiopia. Then, permission and support letters were obtained from Zonal Health bureau and District Health Office. Finally, after obtaining written informed consent from each participant parent/guardian, data were collected anonymously.

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**Authors’ detail**
References


Tables

Table 1. Baseline characteristics of hookworm infected eligible school aged children, at Burie and Debre Elias towns, North West Ethiopia, January - June 2019.

<table>
<thead>
<tr>
<th></th>
<th>Single dose (N=52)</th>
<th>Triple dose (N=51)</th>
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<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (32.7%)</td>
<td>28 (54.9%)</td>
</tr>
<tr>
<td>Female</td>
<td>35 (67.3%)</td>
<td>23 (45.1%)</td>
</tr>
<tr>
<td><strong>Mean (SD) age, year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10.78 (2.1)</td>
<td>10.48 (1.34)</td>
</tr>
<tr>
<td>Female</td>
<td>10.56 (2.3)</td>
<td>10.48 (1.34)</td>
</tr>
<tr>
<td><strong>Mean (SD) weigh, KG</strong></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>31.45 (7.96)</td>
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<tr>
<td>Female</td>
<td>31.15 (8.1)</td>
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<td><strong>Mean (SD) height, m</strong></td>
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<tr>
<td>Male</td>
<td>1.35 (0.13)</td>
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<td>Female</td>
<td>1.35 (0.13)</td>
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<tr>
<td><strong>Mean (SD) haemoglobin, g/dl</strong></td>
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<tr>
<td>Male</td>
<td>13.8 (1.09)</td>
<td>14.02 (0.93)</td>
</tr>
<tr>
<td>Female</td>
<td>13.6 (1.07)</td>
<td>14.02 (0.93)</td>
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<td><strong>Baseline EPG</strong></td>
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<tr>
<td>Arithmetic mean (95% CI)</td>
<td>1216.35 (845.84-1586.86)</td>
<td>1134.3 (864.4-1404.96)</td>
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<td><strong>Infection intensity</strong></td>
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<tr>
<td>Light (1-1999 EPG)</td>
<td>43 (82.7%)</td>
<td>45 (88.2%)</td>
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<tr>
<td>Moderate (2000-3999)</td>
<td>7 (13.5%)</td>
<td>5 (9.8%)</td>
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<td>Heavy (&gt;=4000)</td>
<td>2 (3.8%)</td>
<td>1 (2%)</td>
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SD = standard deviation

Table 2. Cure and Egg Reduction Rate of single and triple dose of mebendazole against hookworm infection among school aged children from Burie and Debre Elias towns, January - June 2019
<table>
<thead>
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<th>Single dose</th>
<th>Triple dose</th>
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<td>No. of hookworm-infected children before treatment (%)</td>
<td>52 (100%)</td>
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<tr>
<td>No. of children cured after treatment</td>
<td>16</td>
<td></td>
<td>4!</td>
</tr>
<tr>
<td>No. of children remained infected (%)</td>
<td>36 (69.2%)</td>
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<td>2</td>
</tr>
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<td>CR (95% CI)</td>
<td>30.8 (19.2-44.2)</td>
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<td>EPG arithmetic mean</td>
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<td>Before treatment (95% CI)</td>
<td>1216 (845.8-1586.86)</td>
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<td>After treatment (95% CI)</td>
<td>378.04 (237.1-510.99)</td>
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<tr>
<td>ERR (95% CI)</td>
<td>68.9% (48.07-73.14)</td>
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<td>9!</td>
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</table>
Figure 1

Flow chart for identification procedures of eligible children. This diagrammatical representation showed the allocation and selection procedure of eligible children for this study.