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Research Article

Evaluation of Anthelmintic Efficacy of Equal Combination of Doxycycline and Mepacrine

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Abstract

The drawback of limited options in the fight against helminth infections caused by roundworms, hookworms and threadworms has remained a major concern in healthcare delivery especially in resource-poor nations. And the success of drug repurposing both in anthelmintic and other treatment landscape has made the choice of this technique in anthelmintic chemotherapy research compelling. A couple of reports have been documented on the strong anthelmintic activities of doxycycline and mepacrine both in combinations with other drugs and as single molecules. We aimed at investigating the efficacy of doxycycline and mepacrine against three selected geo-helminths using Fecal Egg Count Reduction (FECR) as a metric. Ethical approval was obtained from Abia State University Teaching Hospital Ethics Committee and a randomized controlled trial was conducted on a total of thirty two (32) volunteers diagnosed egg positive for the investigated helminths and randomly allocated to (1) mepacrine (2) doxycycline (3) doxycycline+mepacrine equal combination and (4) albendazole treatment

groups in respective doses of 100 mg twice daily over three days for mepacrine and doxycycline; 100 mg each of doxycycline +mepacrine STAT and 400 mg albendazole STAT as positive control. The fecal egg count reduction rates for *ascaris*, hookworm and *strongyloide* were determined using the modified Mc master method and the average FECR of 76.0±14.4;79.7±10.0;81.0±5.9 and 90.0±5.8%were obtained for mepacrine, doxycycline, doxycycline+mepacrine and albendazole respectively. The study identified doxycycline and mepacrine as potential alternative anthelmintic agents especially as combination therapy. Larger scale clinical trials are recommended.

Keywords: Egg Reduction Rate, Doxycycline, Mepacrine, Anthelmintic, Combination.

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1.0 Introduction

The attrition of drug pipelines for most Neglected Tropical Diseases (NTDs) and in particular anthelmintic agents have raised an urgent need for their resuscitation and exploration of alternative strategies for research (Geary et al., 2015).

Soil-transmitted helminth infections have become a major scourge, particularly among school-age children in resource-poor nations where frightening statistics of about 1.0 billion are reported to be at risk with a paucity of research into this disease domain in addition to the increasing incidences of parasite resistance development (Moser et al., 2017).

The declining efficacy of the flagship anthelmintic chemotherapeutic agent, the benzimidazoles (such as albendazole), further exacerbates the problem and calls for prioritization of research for radical approaches to deliver novel and effective treatments.(Levecke et al, 2014)

Two approaches that have received considerable attention recently are employment of drug combinations and repurposing of approved drugs. They

present the advantage of exploiting possible synergism from interaction of the combining entities and drastic time and cost conservation because of availability of preclinical and clinical data. As well, given that mass drug administration of a single dose of the benzimidazoles has been the common practice with recent observations of drop in their efficacy, clinicians have had to resort to multi-dosing or combination of the available anthelmintic agents. However, the drawback of compliance in multiple dosing makes the latter strategy more practicable (Falagas et al, 2015).Drug combinations though beneficial demand their chemical interactions must be studied in biological experiments and probably animal models to demonstrate the rationale and safety of the interactions prior to application in humans.

Our earlier investigation of the anthelmintic activities of doxycycline and mepacrine by *in silico* and *in vitro* procedures in worm models produced strong synergism capable of improving the treatment of helminth infections if validated *in vivo* (Agube and Uzochukwu, 2018).

Keiser et al (2012) have studied *in vitro* and *in vivo* effects of combinations of various standard anthelmintics such as albendazole, mebendazole, ivermectin and oxantel while other researchers have conducted drug combination trials on patients infected with hookworms and *T.trichiura* which results have all shown promise of more efficacious anthelmintic agents (Keiser et al., 2012; Speich et al., 2015; Steinmann et al., 2016; Palmeirim et al., 2018; Sutero et al., 2020). These favorable outcomes of anthelmintic drug combination studies, the urgent need to mitigate the fast-developing drug resistance by the common intestinal worms and the inadequacy found with single drug therapy threw up the need to undertake this *in vivo* study. A couple of researchers have reported anthelmintic activities of

doxycycline and mepacrine which has prompted their use clinically. Notable among these is the report of Katiyar and Singh (2011) in the elimination of *Wolbachia* with six weeks single dose treatment using doxycycline while Gayen et al (2013) confirmed an almost 100% suppression of circulating microfilaria when doxycycline is administered concurrently with albendazole. Similar outcomes were reported by Turner et al (2010) using a combination of doxycycline and ivermectin. It is noteworthy that mepacrine has long been employed in the treatment of tapeworm infections (Schapiro, 1983).

This study investigated the efficacy of an equal combination of doxycycline and mepacrine as an anthelmintic chemotherapeutic agent.

2.0 Materials and Methods

2.1 Ethical Approval of Clinical Studies

2.1.1 Ethical Statement

Ethical clearance was obtained from the Abia State University Teaching Hospital Aba in response to an application made to the Ethics Committee of the institution. Ethical Clearance Reference (Ref: ABSUTH MAC/117 vol.15).

2.1.2 Study Site

The study was carried out in Springs Hospital No.,15 Scotland Crescent Aba, a private secondary care centre in South Eastern Nigeria.

2.2 Trial Design/Field Work

2.2.1 Informed consent

Informed consent of the adult participants was obtained through the signing of the informed consent form provided and for the minors, the parents or guardians provided consent on their behalf both verbally and in writing.

2.2.2 Eligibility Criteria

The following criteria determined eligibility for inclusion:

i. Must be within the age range of 8 – 50 years. samples.

ii. Stool must be egg positive.

iii. Must not be pregnant or breastfeeding at the time of recruitment (for females).

iv. Must not be having diarrhea

Eligibility was carried out in two ways:

1. Verbal confirmation of age, pregnancy test, and readiness to provide pre and post-intervention stool samples.
2. Analysis of pre-intervention stool sample for determination of infection.

v. **2.2.3 Confidentiality**

This is a single blind study and participants were anonymized by assignment of specific codes from CT1 to CT4 representing the four intervention groups. For purposes of blinding, the drugs were packed into small dispensing envelopes before treatment and labeled with the unique IDs. The following brands, Zentel→, Doxycap→ and Eden Mepacrine→ being products of GlaxoSmithKline, Hovid and Eden respectively were used. An AUDIT OFFICER was appointed who was the custodian of all data and ensured recording materials, signatures and dates of any caregiver or other trialists were not compromised.

2.2.4 Sample Size

The sample size for the study was thirty- two (32).The sampling method employed was the method as described by Daniel, W.W. (1999)

2.2.5 Randomization / Allocation

Randomization was done by the Covariate Adaptive method since two prognostic factors, gender and infection intensity were involved in the study. As well, this technique is most suited for small to moderate sample sizes (Suresh, 2012).

The online randomization software www.graphpad/quickcalcs/index.cfm was used to generate a randomization plan for treatment assignment to patients. Thirty-two patients were enrolled and the gender stratification was twenty (20) females to twelve (12) males.

The randomization code created was used to assign to each ID a number representing the four treatment arms thus:

i. Mepacrine only (100 mg) CT1

ii. Doxycycline only (100 mg) CT2

iii. Albendazole only (200 mg) CT3

iv. Doxy + mepa combination (100+100 mg) CT4

The implementation of the 50-50 combination of doxycycline and mepacrine (that is concurrent administration of a capsule and a tablet respectively in this study despite *in vitro* combination study revealing the optimal activity at the 60-40 ratio was due to the unavailability of the pure chemical samples of the two drugs to carry out physicochemical and stability studies, hence the 50-50 was implemented since the compounded drugs are readily available and the administration is uncomplicated.

Recruitment flow chart

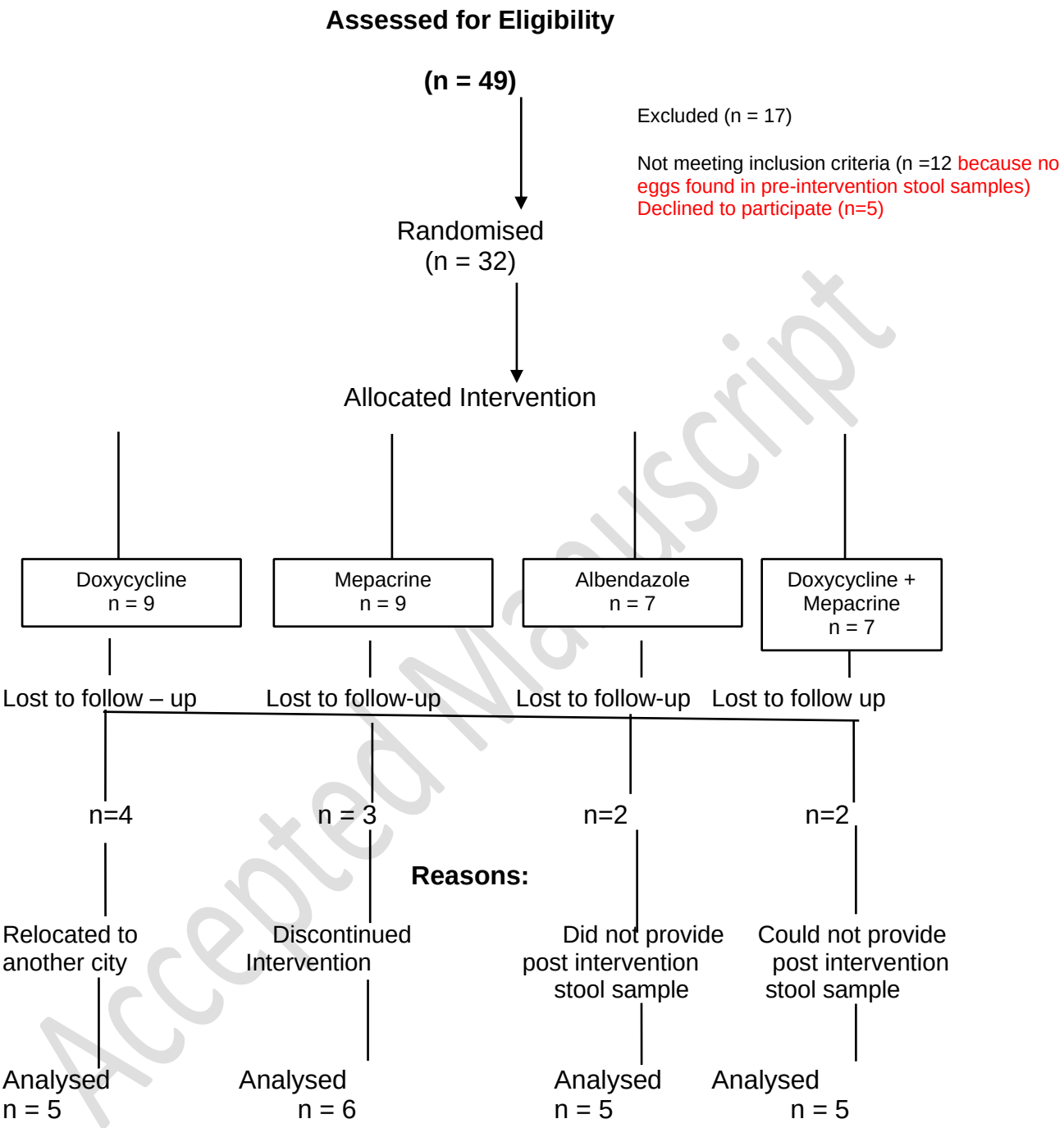

Fig. 1: The flow chart of progress through the phases of the stratified randomized trial of four groups that comprised enrolment, intervention, allocation, follow-up and data analysis.

Table 1. Participants Lost – To Follow Up And Reasons

Intervention	Relocated	Discontinued	No post-intervention stool sample
Doxycycline	3		1
Mepacrine		2	1
Doxy+mepa			2
Albendazole			2

2.3 Efficacy Evaluation of Doxycycline, Mepacrine, and their equal combination Stool (Feces) Screening

Stool samples of patients were screened qualitatively by wet mount to determine the presence of the eggs of the helminths of interest as described by Cheesbrough (1982) while the modified Mc Master Fecal Egg count was applied in quantitative screening to estimate the eggs found in stool samples (Vercruysse et al., 2011). This was done in duplicates and the mean values were recorded with egg count values up to and beyond nine thousand (9,000) being accepted as clinically significant.

2.3.1 The Mc Master Counting Technique

The McMaster counting technique was based on the modified McMaster described by the United Kingdom Ministry of Agriculture, Fisheries and Food (1986). Two (2) grams of fresh stool samples were suspended in 28 ml of saturated salt solution ($MgSO_4$) of density 1.2. The suspension was poured three times through a wire mesh to remove large debris. Then 0.15 ml aliquots were

added to each of the two (2) chambers of a McMaster slide. Both chambers were examined under a light microscope using a 100X magnification and FEC for each helminth species was obtained by multiplying the total number of eggs by a factor of 50 to convert to eggs per gram.

2.4 Data Analysis

The efficacy of the treatment for each of the three soil-transmitted helminths (STH) was evaluated quantitatively based on a reduction in fecal egg counts (FEC). The outcome of the FECR, ERR (egg reduction rate) was calculated using the formula below.

The data were statistically analyzed using the Kruskal-Wallis test at a confidence limit of 95%.

FECR (%)

$$= 100 \times \frac{EPG\ (pre{-}intervention\ FEC) - EPG\ (post{-}intervention\ FEC)}{EPG\ (pre{-}intervention\ FEC)}$$

Where EPG = egg per gram of feces.

Volunteers were drawn from among the patients whose stool egg counts fell within the range specified. Thereafter they were made to sign the CONSENT FORM to validate their acceptance to participate in the study.

2.5 Design of Protocol

Applying the inclusion criteria, thirty-two (32) patients were found eligible for participation.

2.5.1 Intervention

Doxycycline (100 mg) and Mepacrine (100 mg) were administered to candidates in the respective groups at a dosage regimen of one tablet and capsule twice daily for 3 days with clean water.

While the combination of doxycycline and mepacrine was given as concurrent administration of 2.2 mg/kg body weight as a tablet and a capsule respectively as STAT dose which formed a 50:50 combination to contrast the longer duration administration of the monotherapy and check for the synergistic effect observed in vitro with the combination.

Albendazole as a positive control was administered as a single dose of 400 mg tablets.

After fourteen (14) days of intervention, post-intervention stool samples were analysed, and egg count was implemented in duplicate, and recorded as mean fecal egg count with standard deviation.

2.5.2 Precautions / Safety / Ethics Maintenance

In the course of the study, all volunteers' confidentiality was maintained and data integrity was ensured by AUDIT OFFICER. All exclusion and inclusion criteria were applied as well as contraindications and special precautions were observed.

Drugs used were purchased from two registered pharmacies, Chrisdon Pharmacy Ltd. Aba and Boots Company United Kingdom. All details about the drugs such

as batch number, expiry date, NAFDAC number, and country of origin were documented.

Table 2: Profile of drugs used for clinical studies

Drug	Strength	Dosage form	Batch No	Expiry Date	NAFDAC Code Number	Origin / Source
Mepacrine	100 mg	Tablet	T-268047	09/2018		Eden Pharm. Leicester, England.
Doxycycline	100 mg	Capsule	BG08559	07/2019	04-1259	Hovid,Malaysia.
Albendazole	200 mg	Caplet	341471	11/2019	04-2400	Smithkline Beecham, France.

2.6 Statistical Analysis

The data obtained were expressed as mean ±SD. Data analysis was done using Kruskal Wallis and ANOVA tests. The differences between the treatment groups were determined by multiple comparisons of mean ranks for all groups.

In all cases, a probability error of less than 0.05 was selected as the criterion for statistical significance. A post hoc test was also undertaken.

3.0 Results

3.1 Results of Baseline Characteristics of Candidates

The baseline characteristics showed a demography of twenty (20) against twelve (12), female to male ratio. The infection intensity was mostly of heavy mode except for *Ascaris lumbricoides* where the moderate mode predominated. The allocation was fairly distributed among the four groups receiving respectively: doxycycline-mepacrine combination, doxycycline, and mepacrine only and albendazole only. This result is summarized in Table 3.

Table 3: Baseline Characteristics of Candidates in the trial

Characteristics	Overall	Doxy +Mepa	Only Doxy	Only Mepa	Only Albendazole
No of females / males	32 20/12	7 5/2	9 6/3	9 5/4	7 4/3
(Infected with *A. lumbricoides*)					
No. Infected (%)	10 (31.25)	2 (28.57)	4 (44.44)	2 (22.2)	2 (28.57)
(Infection Intensity) **No % Infected**					
Moderate (5000 – 9,999 EPG)	7	1 (14.28)	3 (42.85)	3 (42.85)	
Heavy (10,000 ≥ EPG)	3	1 (14.28)			2 (28.6)
Infected with Hookworm	8 (25)	2 (28.57)	1 (11.1)	1 (11.1)	4 (57.1)
Infection Intensity					
Moderate	2	2 (28.57)			
Heavy	6		2 (22.2)	1 (11.1)	3 (33.3)
(**Infected with *S. stercolaris)***					
No infected (%)	14 (43.75)	1 (14.28)	7 (77.7)	6 (66.67)	
(Infection Intensity (%)					
Moderate	2	1 (11.1)		1 (11.1)	
Heavy	12	3 (42.85)	4 (44.4)	2 (28.57)	3 (42.85)

3.2 Results of Efficacy Determination of Doxycycline, Mepacrine and their equal Combination across the three Investigated Helminths

The efficacy determination of doxycycline, mepacrine and the equal combination showed high egg reduction rates (ERR) against the investigated helminths. The percentage ERR, all fell above the World Health Organization (WHO) recommended threshold levels for anthelmintic chemotherapeutic agents.

The result is presented in Fig 2. The combination of doxycycline and mepacrine yielded 81% which compared to that of albendazole, the standard treatment (90%) and these results were not significantly different at $p<0.05$ (Kruskal-Wallis test). Figure 3 is the Box and whisker diagram showing the spread of percentage fecal egg reduction across treatment groups – the minimum and maximum points, median points, 1st and 3rd quartiles.

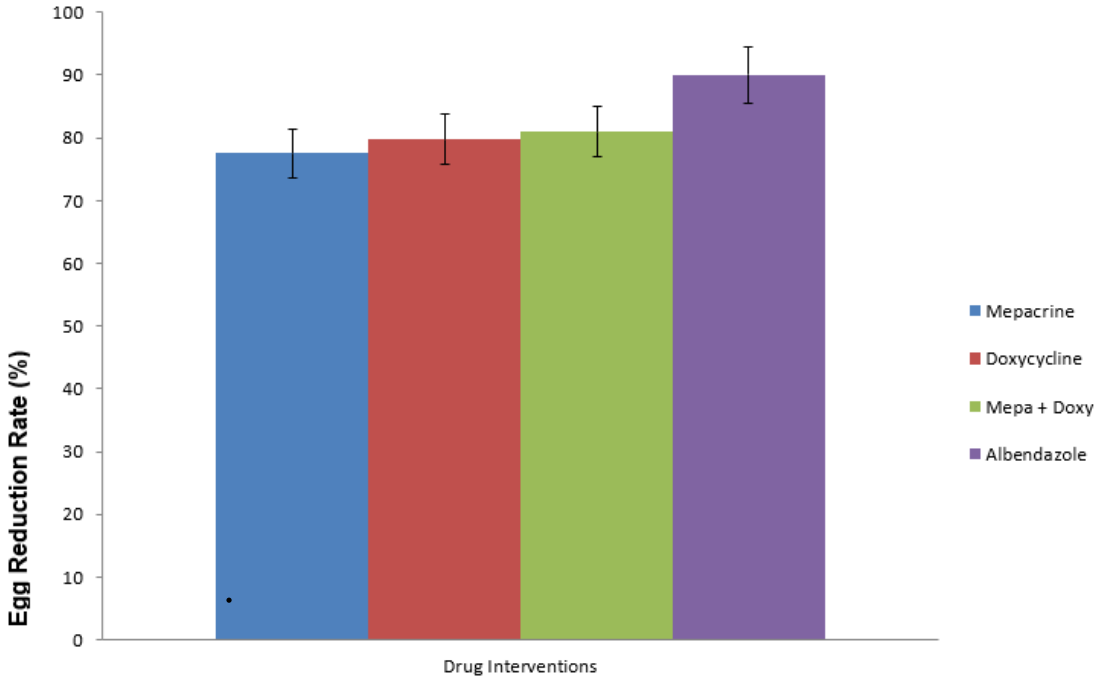

Fig. 2: Fecal Egg Count Reduction Rate of the various Interventions (Mean ± SD) Doxycycline (1 tablet BD 3 days); Mepacrine (1 tablet BD 3 days); Doxycycline +Mepacrine Combination (Single dose); Albendazole (Single dose).

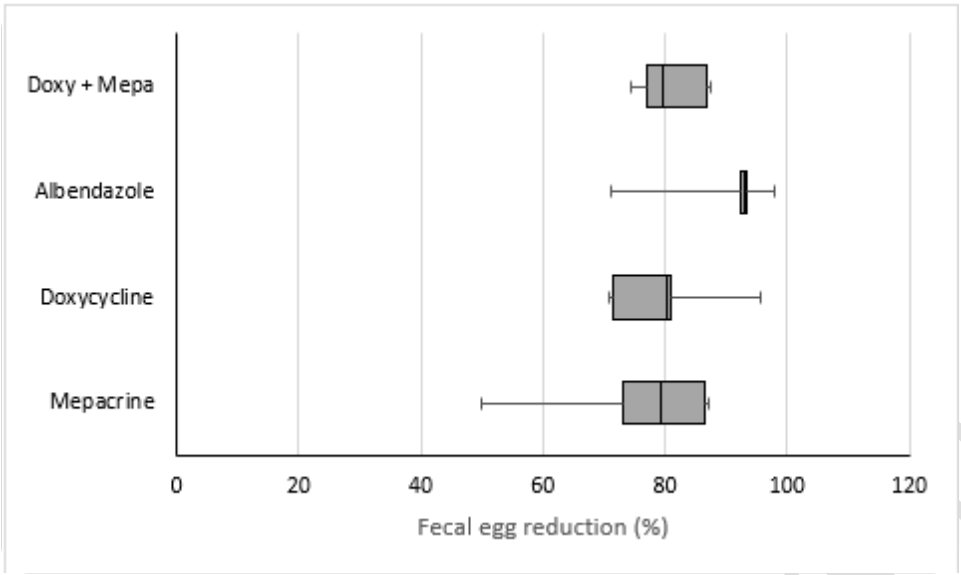

Figure 3: Box and whisker diagram showing the spread of percentage fecal egg reduction across treatment groups

Results of Efficacy Determination of Doxycycline, Mepacrine and their equal Combination against the individual Helminths

As all the drugs under investigation produced good efficacy levels a further investigation was undertaken to determine their respective efficacy levels against each of the three helminths being studied. The equal combination of doxycycline and mepacrine produced ERR of 77, 82.2 and 86.7% against hookworm, *ascaris* and *strongyloide* respectively.

It is important to note that the ERR produced by mepacrine alone (87%) was superior to that of the equal combination against hookworm but was at par with albendazole; however mepacrine recorded the least ERR against *A.lumbricoides* at 61% (Table 4).

TABLE 4: Comparison of Percentage Fecal Egg Reduction Rate For The Three Drugs And Control Against Each Of The Helminths Investigated (±SD)

Helminth	Fecal Egg Reduction Rate (%) For Each of the Drugs			
	Doxycycline	Mepacrine	Doxy + Mepa	Albendazole
Ascaris	75.90± 7.14	61.30±16.00	82.20± 7.50	92.70±0.35
Hookworm	71.40± 0.00	87.10± 0.00	77.00± 3.68	87.00±14.40
Strongyloide	87.90± 10.67	82.00± 6.90	86.70± 0.00	--------

Values are Means ± standard deviation (SD) which were derived from Kruskal wallis test. Statistical analysis was not done to compare the effects of the treatments on individual helminth fecal egg reduction due to small sample size. There was no data for the Albendazole effect on strongyloide due to none submission of post-intervention stool by participants allocated to that group.

Results of the Fecal Egg Clearance of the Trial Drugs against the Standard Reference Treatment on Mixed Helminths

The comparison of the fecal egg clearance of the trial drugs against the standard treatment using the One-way ANOVA statistical test revealed no significant difference in the p-values as shown respectively for doxycycline 0.191; mepacrine 0.535; and 0.399 doxycycline and mepacrine equal combination (Table 5).

TABLE 5: Comparison of Fecal Egg Clearance of Mixed Helminths for the Trial Drugs against the Reference Standard

Treatment	Mean Fecal Egg Clearance (FEC)(x10^3)	P-value	Fecal Egg Clearance (%)	P-value
Doxycycline	7.89± 0.47*	0.191	79.84± 9.98*	0.506
Mepacrine	8.96±1.13*	0.535	75.98±14.25*	0.203
Doxy + Mepa	8.56± 0.72*	0.399	81.02± 5.85*	0.608
Albendazole	10.74±1.11		89.56±10.61	

* = P > 0.05 compared to Albendazole(statistically no significant difference for p = 0.05; 95% confidence interval). N = 6 for mepacrine and 5 for other groups.

4.0 Discussion

This study was initiated to validate the computational and *in vitro* observations of strong anthelmintic activities of an equal combination of two approved drugs, doxycycline, and mepacrine as treatments against *A.lumbricoides*, *S. stercolaris*, *N. americanus,* and *A. duodenale* (Agube et al., 2020).

This, together with our earlier published work as much as we are aware, is the first attempt at repurposing approved drugs as anthelmintics that embraced *computational*, *in vitro,* and clinical investigations.

This non-inferiority, proof-of-concept, randomized clinical trial was implemented with an equal combination of doxycycline and mepacrine as well as the individual drugs as monotherapy with albendazole serving as the positive control.

There was observed good correlation between the reported *in vitro* activities and clinical efficacy using egg reduction rate (ERR) as a metric and both the equal combination and the individual drug molecules produced ERR values that surpassed the World Health Organization (WHO) recommended anthelmintic chemotherapy efficacy threshold of ≥ 70% for *A. lumbricoides* and ≥ 50% for hookworm species in endemic areas (Fig.2) (WHO, 2017). However, ERR due to albendazole treatment was higher than that of the rest of the treatments though the difference in the means was not statistically significant. Hookworm clinical performance is given prominence because together with Trichirus trichiura, they exhibit the least sensitivity to anthelmintic agents amongst the soil-transmitted helminths. The post-hoc multiple comparisons of the fecal egg clearance rates of the trial drugs with the standard reference treatment, albendazole against the three investigated helminths revealed non-inferiority as the p-values (0.60, 0.20 & 0.50) obtained from One-way ANOVA

statistical analysis did not show any significant difference (Table 5). The efficacy of the trial drugs can be considered good given that albendazole remains the gold standard in anthelmintic chemotherapy with only the other benzimidazole compound such as mebendazole approximating its efficacy threshold. However, there was an observation of a decline in the efficacy of albendazole which seemed to align with the speculation of anthelmintic resistance being muted among clinicians, though the claims of resistance remain contentious as several conflicting reports had revealed wide bands of efficacy with ERR values as high as 77% and low as 22% reported by Sacko et al (1999) and Reynoldson et al (1997) respectively for albendazole monotherapy against hookworm. Hence the 89.56% fecal egg clearance rate observed in this study as compared to the standard threshold for albendazole (95-99%) needed to be properly situated because the economic and social consequences of confirmation of resistance would be huge, particularly in regions with high prevalence such as Nigeria given the issues of re-infection after weeks of treatment. For this reason, we surveyed factors that confound drug efficacy besides parasite resistance especially those that interfere with drug pharmacology as immediate consideration of resistance may be too hasty (Geary et al 2010). First among our considerations was the moderate to high infection intensity that predominated in this study (Table 2) which we speculated would have contributed to the reduced efficacy of the interventions since the high density of infection affects drug bioavailability which seemed a very probable cause of the observed decline. (Bennett & Guyatt 2000; Kotze & Kopp 2008). Next, we considered that all the participants in this study operated from their homes hence their diets were not controlled and could have impacted the results of this study since certain diets may lead to increased stomach emptying and loose stool resulting in less exposure time of the worms to the drugs (Sanchez et al 2006; Lacey 1990). But none of the participants

affirmed such during the study. Finally, we considered the quality of drugs used as an intervention and did not find any lacuna as only the brands of the originators or notable manufacturers were used and purchased from reputable sources such as Boots, United Kingdom. On the strength of the survey, we were convinced that the decline in efficacy observed is either due to infection intensity or resistance development. Amid the challenge of the notion of emerging development of resistance, most clinicians still insist there remain cogent concerns of such development given the very high exposure of the standard treatment through periodic mass deworming campaigns, confirmed reports of parasite resistance to anthelmintic agents in livestock and lack of close alternatives making the exploration of combination therapy an attractive option (Geets & Gryseels 2000). Increasing the frequency and duration of monotherapy has been viewed as a welcome strategy and this has received extensive studies since the ineffectiveness of the standard single monotherapy could point to such dose as suboptimal (WHO,2017;2021). Already some studies have suggested a possibility of single-dose monotherapy of albendazole and mebendazole as insufficient since repeat doses of these drugs had shown very marked improved efficacy. Steinmann et al (2011) reported 97% and 84% ERR with a three (3) and six (6) dose regimen of albendazole against ascaris and hookworm respectively.

Notwithstanding the current setbacks in anthelmintic chemotherapy, we do not subscribe to the abandonment of albendazole as the gold standard anthelmintic agent yet, though the concern remains that the continual resort to albendazole monotherapy as the gold standard is threatened by the clinical drawback of potential total ineffectiveness of single molecule treatments capable of progressing to full-scale resistance hence our effort at the development of a close alternative.

There was an observed clinical effect of 81% with confidence intervals of 78.9-83. for the equal combination of doxycycline and mepacrine at a 95% level of significance that was found not significantly different from the control, albendazole. This was considered a bright patch in the horizon and reinforced the clamour for the combination therapy strategy against the backdrop of the paucity of new chemical entities (Albonico et al, 2004). There was no synergistic effect observed in contrast to the in vitro strong synergism reported in the earlier work and the reasoning is that it may be due to the non-implementation of the in vitro 60/40 ratio combination at which point the synergism occurred. Albeit the administration of doxycycline and mepacrine as monotherapy in our study yielded ERR values of 79.8% and 75.9% respectively the fact that helminthiasis often occurs as mixed infections which usually require broad-spectrum anthelmintic agents to treat, demands research efforts in the direction of combination therapy be made a priority because optimisation of drug efficacy can be delivered through the combined action of the interacting molecular entities through possible novel mechanisms of action (Goldberg et al., 2012).

The observed marginal clinical superiority of the combination over the single molecular entities was speculated to have derived from some additive effect though this effect was not further investigated (Table 5). This could be of significance if validated given that the single-dose monotherapies were administered twice daily over a three-day period against a STAT dose of the combination. It is expected that a much greater clinical effect would result from either increased dosing or the implementation of the 60/40 ratio, the point of synergism in vitro.

The additive effect may have been from the activity of mepacrine in the combination given that alkaloids act on the central nervous system of worms causing paralysis, a mechanism of action absent in the benzimidazoles which may enable a bypass of the

speculated resistance most current soil-transmitted helminths are assumed to have shown against the standard anthelmintic agents (Roy et al., 2010).

The effectiveness of anthelmintic combination therapy is supported by several studies involving the administration of different benzimidazole compounds as combinations that delivered clinical successes over monotherapies (Keiser et al 2012; Moser et al 2019). Also, studies that successfully implemented combinations of some other currently available anthelmintic agents with the benzimidazoles with much-improved outcomes have been reported (Soukhathammavong et al. (2012); Tegene et al. (2020); Keiser et al 2012; and Clarke et al 2019). These reports give us confidence in our advocacy for combination treatments against soil-transmitted helminths. Besides the benefits of multiple dosing and extended duration of the existing treatments is the twin issue of cost and compliance. The WHO epidemiological data stratified the prevalence of most soil-transmitted helminth infections to regions of the world ravaged by poverty which includes sub-Saharan Africa and South-East Asia and hence most households can hardly afford repeat doses of the standard anthelmintic agents and this is a major drawback considering that very few research is focused on neglected tropical diseases (Lobo et al, 2011).

It was in the light of this that we considered drug repurposing as a low-hanging fruit that is capable of mitigating drug development costs in those regions. Along with drug combinations, drug repurposing has gained traction recently as one of the new approaches to quicken drug discovery at much-reduced cost because of the merits of exploitation of novel mechanisms of action and safety since repurposing leverages approved drugs with reasonable clinical experience. (Tyagi et al., 2018; Weeks et al., 2018; Gouveia et al., 2019). This informed our exploration of this strategy in this study

and fruitful attempts at repurposing antimalarials, antiprotozoal, and anticancer agents as anthelmintic chemotherapeutic agents have been reported (Panic et al (2014).

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Among the limiting factors in this study are infection intensity and the low number of participants since moderate to high infection intensity confounds the efficacy of drugs by perturbation of certain pharmacokinetic parameters which we speculated to have affected the efficacy observed in this study. However, the use of the heavy-intensity mode of infection in analysing ERR has been considered the most suitable measure of efficacy since the main objective of soil-transmitted helminth control is a reduction in the number of people harboring heavy parasite load (Vecryusee, 2011,). The limiting effect on drug efficacy of high-intensity infection has been variously reported (Benneth and Guyatt (2000); Levecke et al 2014).

The small number of participants in our study reduced the statistical power and clinical effects of the study which was further impacted by an appreciable number that either discontinued intervention, relocated, or failed to provide post-intervention stool samplses (Table 1), albeit this is a proof-of-concept trial that permits the use of a small number of participants. The negative impact of a small number of subjects in a study was corroborated by the very poor efficacy outcomes reported by Reynoldson, (1997) in a trial that involved just 15 participants underscoring the need for much larger study participants.

Finally, the inability to formulate and implement the optimal fixed dose (60/40) obtained *in vitro*, in the clinical trial is a major step capable of improving the observed efficacy of this combination. This and the conduct of larger-scale multicenter trials are expected to significantly scale up the efficacy of this combination treatment and point it as a

possible close alternative to benzimidazoles in the treatment of soil-transmitted helminth infections.

Conclusion

This study revealed a broad spectrum of activity of an equal combination of doxycycline and mepacrine against the common soil-transmitted helminths in humans and validated the in silico and in vitro observations.

Although the combination therapy showed lower efficacy when compared to the standard treatment, the differences were not statistically significant suggesting that the combination therapy can serve as an alternative treatment, especially in parasite-resistant areas to anthelmintic monotherapy.

We strongly recommend the formulation of the optimal fixed dose, the conduct of chemical interaction and stability studies, and larger-scale clinical studies.

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Appendix: Raw Fecal Egg Count Data

Candidate Code	Helminth Identified	Pre-Treatment Fecal Egg Count	Post – Treatment Fecal Egg Count	Fecal Egg Clearance (Mean)	Intervention	Post Intervention Interval	Percentage Egg Reduction Rate	Cumulative Mean Egg Reduction Rate (%
CTI A	Ascaris	11,000	3,000	8,000	Mepacrine	14 Days	72.7	
CTI B	Strongloide	13,000	2,000	11,000	"	"	84.6	
CTI C		14,000	1,800	12,200	"	"	87.1	
CTI D	Hookworm	10,500	2,700	7,800	"	"	74.2	76
CTI E	Strongyloide	9,000	4,500	4,500	"	"	50.0	±14.35
CTI F		11,800	1,500	10,300	"	"	87.3	
	Ascaris				"			
	Strongyloide							
CT2 A	Hookworm	10,500	3,000	7,500	Doxycycline	14 Days	71.4	
CT2 B		10,200	2,000	8,200	"	"	80.4	
CT2 C	Strongyloide	9,500	1,800	7,700	"	"	81.0	79.8
CT2 D	Ascaris	9,300	2,700	6,600	"	"	70.9	±5.85
CT2 E	Ascaris	9,900	450	9,450	"	"	95.5	
	Strongyloide				"			
CT3 A	Ascaris	12,000	900	11,100	Albendazole	14 Days	92.5	
CT3 B	Hookworm	15,000	1000	14,000	"	"	93.3	90
CT3 C		10,000	700	9,300	"	"	93.0	±5.75
CT3 D	Ascaris	12,000	200	11,800	"	"	98.0	

CT3 E	Hookworm Hookworm	10,500	3000	7,500	“ “	“	71.0	
CT4 A CT4 B CT4 C CT4 D CT4 E	Hookworm Strongyloide Ascaris Hookworm Ascaris	9,800 9,000 12,000 9,000 13,000	2000 1,200 1,500 2,300 3,000	7,800 7,800 10,500 6,700 10,000	Doxy + Mepa “ “ “ “	14 Days “ “ “ “	79.6 86.7 87.5 74.4 76.9	81 ±9.97