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Screening of Anti-Infectives against *Leishmania donovani*

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Abstract

Aim: To evaluate *in vitro* the effectiveness of several anti-infective agents alone and in combination against *Leishmania donovani*. Method: A convenient stratified sampling method was used to obtain selected anti-infective agents. For individual drug samples, Half Maximal Inhibitory Concentrations (IC_{50}) were obtained using the broth dilution method. The $IC_{50's}$ of the drugs which were active against *L. donovani* were used as reference values to prepare drug combinations for the modified microdilution checkerboard method. Results: Five (5) out of the fifty-six (56) drugs used showed activity (inhibition of cell growth) against *L. donovani* cells. They include Quinine sulphate (IC_{50} = 0.089 µg/ml), gentamicin (IC_{50} = 8.1 µg/ml), amodiaquine (IC_{50} = 138 µg/ml) and the two standard drugs: Amphotericin B (IC_{50} = 6.3 µg/ml) and Pentamidine (IC_{50} = 25 µg/ml). The remaining fifty-one (51) drugs did not show any inhibition within the range of concentrations used (1.25 - 160 µg/ml). The drug combinations of Pentamidine/Amodiaquine, Pentamidine/ Quinine sulphate, Pentamidine/Gentamicin, Amphotericin B/Quinine Sulphate, Amphotericin B/ Gentamicin, Amodiaquine/Quinine sulphate and Amodiaquine/Gentamicin showed synergistic effects against *L. donovani* whereas the Amphotericin B/Amodiaquine combination was antagonistic. Notable in the results obtained was the high effectiveness of quinine sulphate in inhibiting the growth of *L. donovani*. Quinine sulphate, though not indicated for leishmania treatment, was more effective than the two standard drugs and has a potential of playing a significant role in the treatment of leishmaniasis. Conclusion: This study has revealed five (5) anti-infective agents that by themselves or in combinations show activity against *L. donovani*. Some of the drug combinations which showed synergism should further be investigated. These results have to be confirmed by *in vivo* studies to define their roles in leishmaniasis treatment.

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Keywords

Anti-Infectives, Half Maximal Inhibitory Concentration, *Leishmania donovani*

1. Introduction

Leishmaniasis is a major vector borne disease caused by the obligate intramacrophage protozoa of the genus Leishmania [1]. The disease is transmitted by the bite of infected female Phlebotomine sand flies. The disease affects many mammals including humans. Leishmaniasis is considered a neglected tropical disease [2] and consists of four main clinical syndromes depending on the parasite species and the cellular immune system and function of the patient. Cutaneous leishmaniasis produces skin lesions mainly on the face, arms and legs. The diffuse cutaneous type of leishmaniasis is difficult to treat because of disseminated lesions that resemble leprosy and do not heal spontaneously. This type is especially related to a defective immune system and it is often characterized by relapses even after treatment. Mucocutaneous leishmaniasis, also called "espundia" in South America causes disfiguring lesions to the face and destroys the mucous membranes of the nose, mouth and throat. *Leishmania braziliensis* is responsible for most cases of mucocutaneous leishmaniasis. Visceral leishmaniasis (VL) also known as "kala azar", is the most severe form of leishmaniasis, and is usually fatal if left untreated. It is characterized by irregular fever, weight loss, swelling of the liver and spleen and anemia. The incubation period can be months or years and, unlike the cutaneous forms of leishmaniasis, the internal organs are involved. Visceral leishmaniasis is caused by the *Leishmania donovani* complex: *L. donovani* found mostly in East Africa and the Indian subcontinent and *Leishmania infantum* in Europe, North Africa and Latin America [3]. Humans are considered to be accidental hosts of these parasites [4] [5]. Malnutrition in *Leishmania donovani* infected subjects is able to alter the immune response and consequently increases the risk of clinical leishmaniasis [6] [7].

Historically, the treatment of leishmaniasis has been based on the use of pentavalent antimonial drugs. Recently, an increased incidence of emergence of antimony resistant parasite strains has demanded a shift in focus from antimony to other anti-leishmanial agents including Miltefosine, Amphotericin B, Pentamidine, Paromomycin and Sitamaquine among others [8].

Amphotericin B and Pentamidine are the second line alternative drugs [9]. Treatment also depends on the region where the disease was acquired, the type of infection and the species of *Leishmania.* Liposomal Amphotericin B is recommended in India, South America and the Mediterranean regions. In Africa, a combination of Paromomycin along with pentavalent antimonial is given. Other medications, such as Pentamidine and Amphotericin B, have been used as alternative drugs. However, most of these drugs are not orally active, requiring long-term parenteral administration, and display serious side effects.

Although the last century has been characterized by a drastic dropping in the mortality caused by infectious diseases, leishmaniasis still remains a dreadful menace to human health and therefore there is the need for efficient control, which requires the steady development of new, more powerful, less toxic and inexpensive drugs for alternative treatment. The search for efficacious and, at the same time, safer anti-leishmania compounds is a continual process that needs convenient, reproducible and scalable drug screening assays.

The goal of this project is to evaluate the *in vitro* activity of individual and combinations of anti-infective agents against *Leishmania donovani*. The objectives are to determine from susceptibility studies, the single anti-infective agents that show activity against *L. donovani*, and to obtain their fifty percent Inhibitory Concentrations ($IC_{50's}$). The information obtained will be used to prepare possible combinations of the anti-infective agents at various concentrations and the susceptibility of *L. donovani* to these drug combinations determined. Finally the combination indices will be calculated and used and to characterize the activity of the drug combinations as synergistic, additive or antagonistic at the various concentrations.

2. Materials and Methods

2.1. Test Organism and Reagents

The test organism, *Leishmania donovani* (WHO strain DD8) was a gift from Dr. Neelo Singh of the Leishmania Research Society, India. Culture media, M199, Alamar blue and all other reagents used for experiments were purchased from VWR, USA.

2.2. Drug Samples and Standards

Drug standards were obtained as gifts from the Centers for Disease Control, Atlanta, GA, USA. All other antimicrobials were purchased as tablets, capsules, or injectables from various pharmacies in Ghana and the United States of America. A total of fifty-six (56) drugs, including the reference standards were used (**Table 1**). The drug choices were made from the most available antibiotic classes. This was done to ensure that various mechanisms of drug action were tested against the parasite. The various drugs were prepared initially at 4 mg/ml as a stock solution in Dimethyl sulfoxide (DMSO). The final drug solutions were prepared in M199.

Table 1. Table showing individual drugs and their corresponding $IC_{50's}$.

ANTI-INFECTIVE AGENTS	IC_{50} (mg/ml)	IC_{50} (µg/ml)
AMPHOTERICIN B (REFERENCE)	0.0063	6.3
PENTAMIDINE (REFERENCE)	0.025	25
QUININE SULFATE	0.000089	0.089
AMODIAQUINE	0.138	138
GENTAMICIN	0.0081	8.1
HYDROXYCHLOROQUINE	NA	NA
ARTEMETHER	NA	NA
LUMEFANTRINE	NA	NA
PRIMAQUINE	NA	NA
CEFAZOLIN	NA	NA
CEFOTETAN	NA	NA
CEFEPIME	NA	NA
AMPICILLIN	NA	NA
NAFCILLIN	NA	NA
PENICILLIN G SODIUM	NA	NA
FLUCLOXACILLIN	NA	NA
AMPICILLIN/SULBACTAM	NA	NA
POLYMIXIN B	NA	NA
TETRACYCLINE	NA	NA
SULFAMETHOXAZOLE/TRIMETHOPRIM	NA	NA
SULFADOXINE/PYRIMETHAMINE	NA	NA
CHLOROQUINE	NA	NA
CEFOXITIN	NA	NA
TIGECYCLINE	NA	NA
CEFTAZIDIME	NA	NA
RIFAMPICIN	NA	NA
CEFUROXIME	NA	NA
MEROPENEM	NA	NA
DOREPENEM	NA	NA
IMIPENEM	NA	NA
PIPERACILLIN/TAZOBACTAM	NA	NA
AMIKACIN	NA	NA
TOBRAMYCIN	NA	NA
CEFACLOR	NA	NA
CIPROFLOXACIN	NA	NA
LEVOFLOXACIN	NA	NA
DOXYCYCLINE	NA	NA
DICLOXACILLIN	NA	NA
TETRACYCLINE	NA	NA

Continued

AZITHROMYCIN	**NA**	**NA**
CLARITHROMYCIN	**NA**	**NA**
ERYTHROMYCIN	**NA**	**NA**
CLINDAMYCIN	**NA**	**NA**
CHLORAMPHENICOL	**NA**	**NA**
AMOXICILLIN	**NA**	**NA**
AMOXICILLIN/CLAVULANATE	**NA**	**NA**
CEFTRIAXONE	**NA**	**NA**
FLUCLOXACILLIN	**NA**	**NA**
PENICILLIN G SODIUM	**NA**	**NA**
NITROFURANTOIN	**NA**	**NA**
AZTREONAM	**NA**	**NA**
OSELTAMIVIR	**NA**	**NA**
VANCOMYCIN	**NA**	**NA**
ISONIAZID	**NA**	**NA**
ETHAMBUTOL	**NA**	**NA**
PYRAZINAMIDE	**NA**	**NA**

Table 1: Half Maximal Inhibitory Concentration (IC_{50}) of various anti-infectives obtained by the broth dilution method as compared with the reference anti-leishmania drugs Amphotericin B, and Pentamidine. NA = no activity.

2.3. Individual Drug Screening and Alamar Blue Assay

Working drug solutions of concentrations between 1.25 and 160 µg/ml were prepared from each stock solution of 4 mg/ml. Aliquots (100 µl) of each drug concentration were pipetted in triplicates into 96-well plates. To each of the aliquot was added 50 µl of M199 media followed by the inoculation of the wells, under aseptic conditions. 50 µl inoculum-equivalent to a McFarland turbidity standard absorbance of 0.02 and containing approximately 1.88×10^6 cells/ml of *L. donovani* was used. Negative control wells containing 200 µl of the media were included in the same plate. Each plate had positive control wells, which contained 50 µl of inoculum and 150 µl of media. The plates were covered and incubated at 26˚C for 24 hours, after which 10 µl alamarBlue® was added to each well and incubation continued for another 24 hours. The plates were read (λex =540nm; λem = 590 nm) after the 48-hour incubation period using a fluorescent microplate reader. Percentage inhibitions were calculated for each concentration, and a graph of percentage inhibition against log of drug concentrations was plotted to obtain the IC_{50} for each anti-infective agent.

2.4. Modified Micro-Dilution Checkerboard Method

Using $IC_{50's}$ obtained for individual drug susceptibility tests as reference values, combinations of anti-infective agents at various concentrations were prepared and used to determine the susceptibility of *L. donovani*. Briefly, 50 µl of the first anti-infective of the combination was put, in increasing order of the seven (7) concentrations, into wells along the ordinate, while 50 µl of the second drug was put into wells along the abscissa (**Figure 1**). 50 µl of M199 media was put into each well of the micro-dilution plates and 50 µl each of the two drugs was put in the corresponding wells to produce drug combination volume of 100 µl in varying concentrations. Each well was inoculated with 50µl inoculum-equal to a McFarland turbidity standard absorbance of 0.02, containing approximately 1.88×10^6 cells/ml of *L. donovani*. Positive control wells without any drug content and negative control wells without any inoculum were included on the plates. The plates were covered and incubated erect at 26˚C for 24 hours, after which alamarBlue® was added to each well and incubation continued for another 24 hours. Fluorescence spectrophotometry was used to obtain the number of viable cells in each well after the 48-hour incubation period and the percentage inhibitions for each well were calculated. The combination index for each well was also calculated from the concentrations of the drugs used in the well and IC_{50} of the individual drugs. The additive effect of a combination of antimicrobials is one in which the effect of the combination equals that of the sum of the effects of the individual drugs. The synergistic effect of a combination of antimicrobials is present if the effect of the combination is greater than the additive effect of the individual drugs whereas

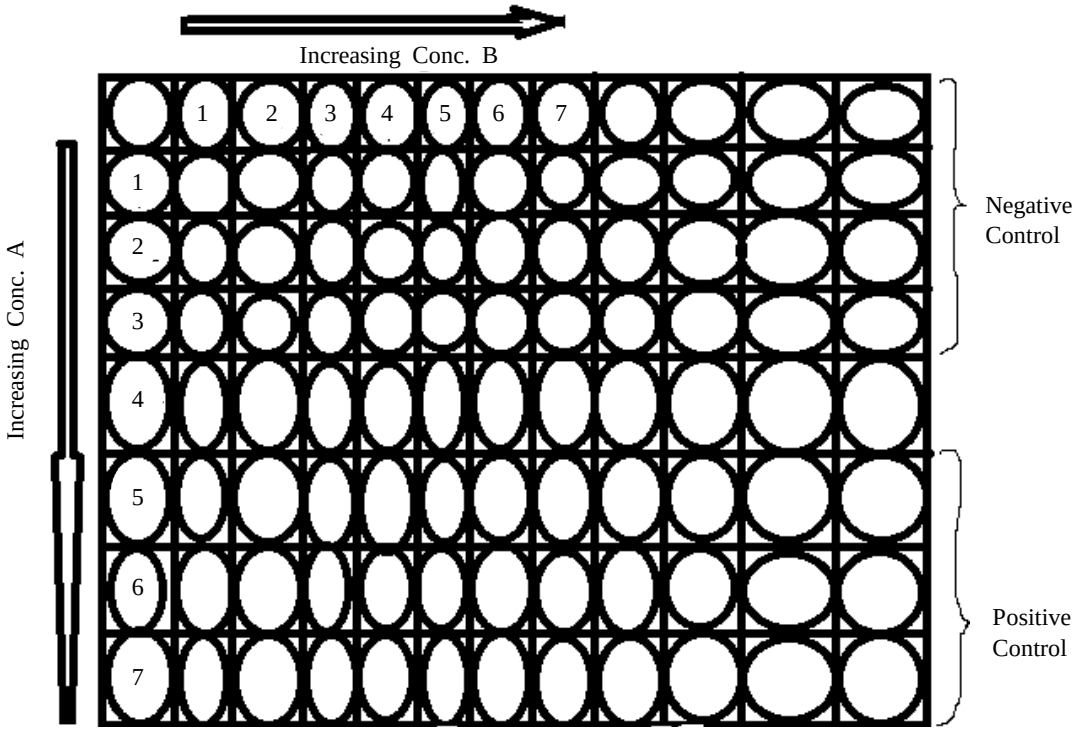

Figure 1. 96 well plate containing two drugs in different proportions**.** This Figure shows testing of drug combination using the modified micro-dilution checkerboard method.

antagonism is present if there is a reduced effect of a combination of antimicrobials observed in comparison with the effect of the most effective individual substance [10].

For Pentamidine/Quinine combination, the combination index (CI) was calculated as:

CI = (Conc. of Pentamidine in the well)/(IC_{50} of Pentamidine) + (Conc. of Quinine in the well)/(IC_{50} of Quinine) + {(Conc. of Pentamidine × Conc. Of Quinine)/(IC_{50} of Pentamidine × IC_{50} of Quinine)}.

This was repeated for each combination and used to characterize the activity of the drug combinations as synergistic, additive or antagonistic at the various concentrations.

3. Results and Discussion

3.1. Results

Five (5) out of the fifty-six (56) drugs used showed activity (inhibition of cell growth) against *L. donovani* cells. This includes the two standard drugs; Amphotericin B and Pentamidine. The remaining fifty-one (51) drugs did not show any inhibition within the range of concentrations used (1.25 - 160 µg/ml) (**Table 1**).

$IC_{50's}$ obtained were 0.089, 6.3, 8.1, 25, and 138 µg/ml for Quinine Sulphate, Amphotericin B, Gentamicin, Pentamidine, and Amodiaquine respectively. **Figure 2** and **Figure 3** show sample plots for Pentamidine from which IC_{50} values were obtained. Similar plots were made for all the other drugs. Comparing Amodiaquine, Gentamicin and Quinine Sulphate with the standard drug therapies (Pentamidine and Amphotericin B), Quinine Sulphate had a lower IC_{50} than both standard drug therapies whereas the IC_{50} of Amodiaquine was higher than that for Amphotericin B and Pentamidine. The IC_{50} value for Gentamicin was lower than that for Pentamidine but higher than Amphotericin B (**Table 1**).

Other than testing the susceptibility of micro-organisms to individual anti-microbial agents, their susceptibility to combined anti-infectives too can be tested for. Multi-drug susceptibility tests do not only give results for susceptibility, but also qualifies the activity of the combined anti-infective agents [11].

The minimum inhibitory concentration (MIC) is used to measure the potency or effectiveness of an antimicrobial agent, and not necessarily the IC_{50} [12]. This means the anti-infective agent does not necessarily have to inhibit 50% of the microbial cell population before it is considered effective. Once the agent shows some form

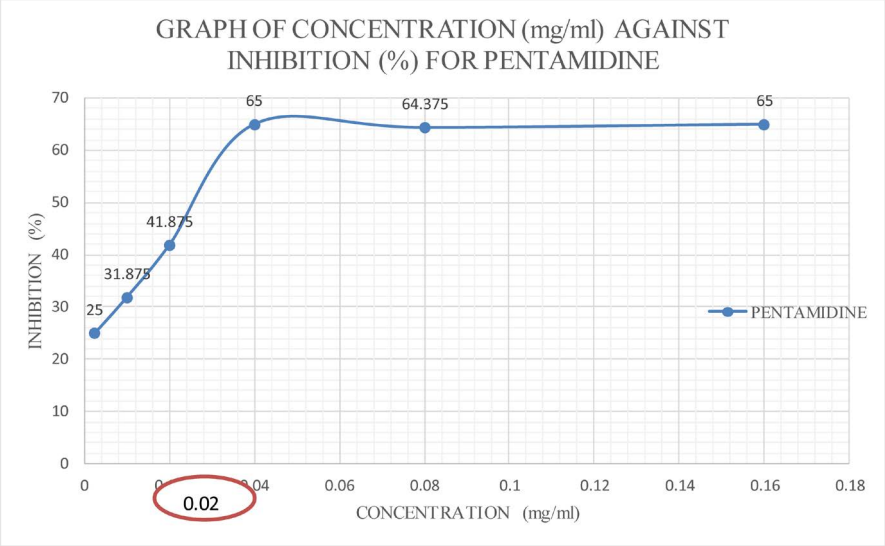

Figure 2. Plot of drug concentration versus percent inhibition. figure showing a hyperbolic curve of concentration (mg/ml) against inhibition (%) for pentamidine. 50% extrapolation on the inhibition (%) scale corresponded to 0.025 mg/ml when concentration (mg/ml) is plotted against inhibition (%) for pentamidine.

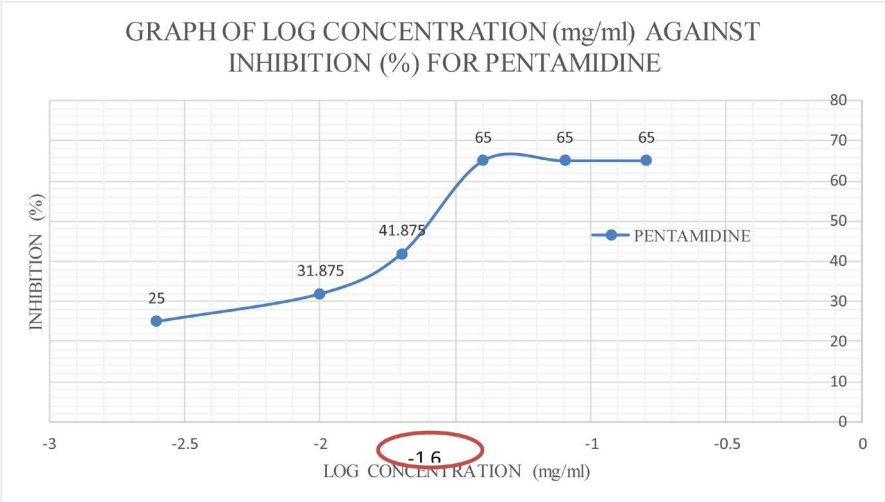

Figure 3. Plot of log drug concentration versus percent inhibition. Figure showing a sigmoidal curve of log concentration (mg/ml) against inhibition (%) for pentamidine. The reduced dispersion of the data set, on extr-apolation, produced a 50% inhibition corresponded to −1.60 mg/ml on the log concentration axis of the log concentration (mg/ml) against inhibition (%) graph. This therefore gave an IC_{50} of 0.025 mg/ml (25 µg/ml).

of inhibition of microbial growth, the agent can be said to be effective against the micro-organism. It could be inferred from the results that most of the combined anti-infectives were effective against *L. donovani* at the various concentrations used. Comparing the number of wells that showed activity against *L. donovani* to the number of wells that showed no inhibitory effect, it could be inferred that Amodiaquine/Quinine was 61.22% effective, Pentamidine/Quinine was 89.80% effective, Pentamidine/Amodiaquine was 83.67% effective and Gentamicin/Amodiaquine was 100% effective (**Table 2**).

The results from the microdilution method also indicated that only the combination Amphotericin B/Gentamicin showed a 100% inhibitory activity against *L. donovani*. The drug combination Quinine Sulphate/Amphotericin B gave a 98% inhibitory activity against *L. donovani*. The combination of Amphotericin B/Amodiaquine

Table 2. Table of drug combinations and their respective percent inhibitions.

Drug Combination	% Inhibition	% Synergy	% Additivity	% Antagonism	Interpretation
Pent/Quin	89.80	89.80	0	0	Synergy
Pent/Amod	83.67	36.73	14.29	32.65	Synergy
Amod/Quin	61.22	46.94	0	14.28	Synergy
Gent/Amod	100	48.98	10.20	40.82	Synergy
AmphoB/Quin	98	83.67	12.25	2.04	Synergy
AmphoB/Amod	69.94	25	2.04	42.9	Antagonism
AmphoB/Gent	100	81.6	10.2	8.16	Synergy
Gent/Quin	85.7	85.7	0	0	Synergy

Table 2: Interpretation of activity of drug combinations on *L. donovani* promastigotes. Most drug combinations showed a synergistic effect except for Amphoterin B and Amodiaquine, a combination which proved antagonistic.

also gave a 69.4% inhibitory activity against *L. donovani* while that of Gentamicin/Quinine Sulphate showed an inhibitory activity of 85.7% against *L. donovani*. Although the combination of Amphotericin B/Gentamicin showed an activity of 100% against *L. donovani*, the two drugs showed a synergistic potential of 81.6% when used in their respective concentrations against *L. donovani*. Also 10.2% of the total number of microtiter wells showed additivity whiles 8.16% of the wells showed antagonistic effect. Moreover, all of the wells with this combination showed percentage inhibitions greater than 50% (IC_{50}) (data not shown) with the lowest percentage inhibition in this plate being 58.62% and the highest percentage inhibition being 74.31% (**Table 2**).

3.2. Discussion

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, *i.e*. an enzyme, cell, cell receptor or microorgamism) by half. For any two agents that inhibit the same biological process, the agent with the lower IC_{50} value is more effective at inhibiting that biological process. IC_{50} was measured for drugs that showed activity against *L. donovani* and then compared to that obtained from standard (second line) drugs used in the treatment of the disease, Pentamidine and Amphotericin B. The lower IC_{50} of Quinine Sulphate than either Amphotericin B or Pentamidine is an indication that it is more effective than either drug. Gentamicin, on the other hand is less effective than Amphotericin B, but more effective than Pentamidine as indicated by the $IC_{50's}$. Finally, the IC_{50} of Amodiaquine shows that it was the least effective drug. A higher concentration of Amodiaquine is needed to inhibit *L. donovani* cell growth to the same extent as the standard drugs.

Notably, each combination of anti-infective agent had effectiveness against *L. donovani*. However, the aim of drug combinations is to achieve susceptibility with synergy. Drug synergy, the combined boost of drug efficacy, is a highly pursued goal of combinational drug development [13]. Synergistic drug combinations have been shown to be highly efficacious and therapeutically more specific [14]. This is indicative of a good combination which can be developed for clinical use. Drug antagonism, in contrast, is often undesirable, but could be useful in selecting against drug resistant mutations [15]. The modified micro-dilution checkerboard method further tests the drug combinations and characterizes their susceptibility as synergistic, additive or antagonistic. An additive effect of a combination of anti-infectives is one in which the effect of the combination is equal to that of the sum of the effects of the individual components. Synergistic effect of a combination of antibiotics is present if the effect of the combination exceeds the additive effects of the individual components whereas antagonism is present if a reduced effect of a combination of antibiotics is observed in comparison with the effect of the most effective individual substance [10].

From the characterization by the modified micro-dilution checkerboard method, combinations of Amodiaquine/Quinine showed 46.94% synergy and 14.28% antagonism out of the total 61.22% effectiveness. Pentamidine/Quinine showed 89.80% synergy which was the same as the percentage of its effectiveness. Pentamidine/Amodiaquine showed 36.73% synergy, 14.29% additivity and 32.65% antagonism out of the 83.67% effectiveness. Gentamicin/Amodiaquine showed 48.98% synergy, 10.20% Additivity and 40.82% antagonism (**Table**

2). Results for other combinations involving Amphotericin B are also outlined in **Table 2**. According to characterization by the modified micro-dilution checkerboard method, the character that dominates is used to classify the overall activity of the drug combination. So it could be inferred that seven out of eight combinations were synergistic in activity, since the highest percentage of activity was synergistic.

For a drug combination to be considered good to be developed for clinical use, it is desired that it shows synergism as well as good percentage inhibition. From the results, Amodiaquine/Quinine had percentage inhibitions ranging from 31.77% to 69.13%. Most of the inhibitions were more than 50% with just three (3) wells showing less than 50% inhibition. Pentamidine/Quinine showed percentage inhibitions ranging from 22.71% to 61.55%, with thirteen (13) wells showing less than 50% inhibition. Pentamidine/Amodiaquine showed percentage inhibitions ranging from 35.60% to 67.27%, with just one (1) well showing less than 50% inhibition. Gentamicin/Amodiaquine showed percentage inhibitions ranging from 63.87% to 71.34%, with none of the wells showing less than 50% inhibition.

The mechanisms of action of two drugs have an effect on their overall effect in combination. Therefore for the use of the CI in characterizing the effect of two drug combinations, there is the assumption that the two agents being combined have different mechanisms of action and exhibit a dose-response relationship [16].

Amodiaquine is a 4-aminoquinoline similar in structure and activity to chloroquine. It has been used as both an antimalarial and an anti-inflammatory agent for more than 40 years. The mode of action of amodiaquine has not yet been determined. However, in general, 4-aminoquinoline derivatives appear to bind to nucleoproteins and inhibit DNA and RNA polymerase. High drug concentrations are found in the malaria parasite's digestive vacuoles [17]. Amodiaquine and synthesized derivatives of amodiaquine have been reported to have activity against three (3) species of *Leishmania*; *L. braziliensis*, *L. chagasi* and *L. amazonensis*. The activity was achieved even in concentrations of micromoles (μm) [18].

Quinine is a quinolone-containing antimalarial. These drugs are thought to act by interfering with the digestion of haemoglobin in the blood stages of the malaria life cycle. The drug diffuses down the pH gradient to accumulate in the acidic vacuole of the parasite. The high intravacuolar concentration of quinine is proposed to inhibit the polymerisation of haem. As a result, the haem which is released during haemoglobin breakdown builds up to poisonous levels, thereby killing the parasite with its own toxic waste [19].

Assuming that these two drugs employ these same mechanisms in *L. donovani*, the extreme difference in their mechanism of action could account for the greater synergy they exhibited.

Pentamidine is active against a variety of protozoal infections, including many trypanosomes. Although its mechanism of action has not yet been defined, evidence exists that the drug is concentrated in the organism by an energy-dependent high uptake system. The drug then binds to the parasite's DNA and interferes with its synthesis of RNA, DNA, phospholipids and proteins [20]. Pentamidine is a second-line drug for treatment of leishmaniasis. There is also a vast difference between the mechanism of action between Pentamidine and Quinine. Both drugs are also different in antibiotic class. Assuming these same mechanisms are employed in *L. donovani*, this could account for the 89.90% synergy with no antagonism for Pentamidine/Quinine.

Pentamidine/Amodiaquine had percentage synergism of 36.73% which was quite low. Again, assuming that the drugs employ these same mechanisms in *Leishmania*, it could be observed that both drugs could have some similarity in their mechanisms of action. Thus, both drugs tend to be accumulated in the parasite and eventually inhibit DNA synthesis. When two drugs have a similar effect in action but act by different mechanisms, they are expected to exhibit more of summation than synergism when combined [21]. The similar effect in action, but different mechanisms could account for the low percentage synergism.

Gentamicin/Amodiaquine could also have exhibited low percentage synergy, 48.98% for the same reason as Pentamidine/Amodiaquine. This is because Gentamicin is an aminoglycoside. It interferes with protein synthesis by binding to the 30S ribosomal subunits. Gentamicin has been reported to be effective in combination with Paromomycin topically for the treatment of cutaneous leishmaniasis [22]. Amodiaquine inhibits DNA and RNA polymerase. The two agents, by their mechanisms of action may eventually end up with the same effect, thus, inhibition of protein synthesis. Due to the similarity in effect of actions but different mechanisms of actions, they showed a low percentage synergy. The probable reasons for the results obtained could be extrapolated for the other drug combinations as well.

4. Conclusion

From the susceptibility screening test of individual anti-microbials, Quinine Sulphate, Amodiaquine and Genta-

micin showed considerable activity against *L. donovani* as well as to the standard drug therapies; Amphotericin B and Pentamidine. Seven of the eight drug combinations showed synergistic activity against *L. donovani*. This would have to be confirmed by further *in vitro* and *in vivo* studies and possibly affect treatment options for leishmaniasis.

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References

[1] Banuls, A.-L., Hide, M. and Prugnolle, F. (2007) Leishmania and the Leishmaniases: A Parasite Genetic Update and Advances in Taxonomy, Epidemiology and Pathogenicity in Humans. *Advances in Parasitology*, **64**, 1-458. http://dx.doi.org/10.1016/S0065-308X(06)64001-3

[2] Organization, W.H. (2000) WHO Report on Global Surveillance of Epidemic Prone Infectious Diseases, Communicable Diseases and Surveillance Response. WHO/CDS/CSR/ISR/2000, World Health Organization, Geneva.

[3] Lukeš, J., Mauricio, I.L., Schönian, G., Dujardin, J.-C., Soteriadou, K., Dedet, J.-P., *et al.* (2007) Evolutionary and Geographical History of the *Leishmania donovani* Complex with a Revision of Current Taxonomy. *Proceedings of the National Academy of Sciences*, **104**, 9375-9380. http://dx.doi.org/10.1073/pnas.0703678104

[4] Andrade, B.B., Oliveira, C.I. de, Brodskyn, C.I., Barral, A. and Barral-Netto, M. (2007) Role of Sand Fly Saliva in Human and Experimental Leishmaniasis: Current Insights. *Scandinavian Journal of Immunology*, **66**, 122-127. http://dx.doi.org/10.1111/j.1365-3083.2007.01964.x

[5] Titus, R.G. and Ribeiro, J.M. (1988) Salivary Gland Lysates from the Sand Fly *Lutzomyia longipalpis* Enhance Leishmania Infectivity. *Science*, **239**, 1306-1308. http://dx.doi.org/10.1126/science.3344436

[6] Anstead, G.M., Chandrasekar, B., Zhao, W., Yang, J., Perez, L.E. and Melby, P.C. (2001) Malnutrition Alters the Innate Immune Response and Increases Early Visceralization Following *Leishmania donovani* Infection. *Infection and Immunity*, **69**, 4709-4718. http://dx.doi.org/10.1128/IAI.69.8.4709-4718.2001

[7] Harrison, L.H., Naidu, T.G., Drew, J.S., de Alencar, J.E. and Pearson, R.D. (1986) Reciprocal Relationships between Undernutrition and the Parasitic Disease Visceral Leishmaniasis. *Reviews of Infectious Diseases*, **8**, 447-453. http://dx.doi.org/10.1093/clinids/8.3.447

[8] Murray, H.W. (2001) Clinical and Experimental Advances in Treatment of Visceral Leishmaniasis. *Antimicrobial Agents and Chemotherapy*, **45**, 2185-2197. http://dx.doi.org/10.1128/AAC.45.8.2185-2197.2001

[9] Rojas, R., Valderrama, L., Valderrama, M., Varona, M.X., Ouellette, M. and Saravia, N.G. (2006) Resistance to Antimony and Treatment Failure in Human Leishmania (Viannia) Infection. *The Journal of Infectious Diseases*, **193**, 1375-1383. http://dx.doi.org/10.1086/503371

[10] Eucast (2000) Terminology Relating to Methods for The Determination of Susceptibility of Bacteria to Antimicrobial Agents. Eucast Definitive Document E. Def 1.2. *Clinical Microbiology and Infection*, **6**, 503-508.

[11] Milne, K. and Gould, I. (2012) Combination Antimicrobial Susceptibility Testing of Multidrug-Resistant Stenotrophomonas Maltophilia from Cystic Fibrosis Patients. *Antimicrobial Agents and Chemotherapy*, **56**, 4071-4077. http://dx.doi.org/10.1128/AAC.00072-12

[12] Levison, M.E. and Levison, J.H. (2009) Pharmacokinetics and Pharmacodynamics of Antibacterial Agents. *Infectious Disease Clinics of North America*, **23**, 791-815. http://dx.doi.org/10.1016/j.idc.2009.06.008

[13] Fitzgerald, J.B., Schoeberl, B., Nielsen, U.B. and Sorger, P.K. (2006) Systems Biology and Combination Therapy in the Quest for Clinical Efficacy. *Nature Chemical Biology*, **2**, 458-466. http://dx.doi.org/10.1038/nchembio817

[14] Lehár, J., Krueger, A.S., Avery, W., Heilbut, A.M., Johansen, L.M., Price, E.R., *et al.* (2009) Synergistic Drug Combinations Tend to Improve Therapeutically Relevant Selectivity. *Nature Biotechnology*, **27**, 659-666. http://dx.doi.org/10.1038/nbt.1549

[15] Chait, R., Craney, A. and Kishony, R. (2007) Antibiotic Interactions That Select against Resistance. *Nature*, **446**, 668-671. http://dx.doi.org/10.1038/nature05685

[16] Chou, T.-C. (2006) Theoretical Basis, Experimental Design and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies. *Pharmacological Reviews*, **58**, 621-681. http://dx.doi.org/10.1124/pr.58.3.10

[17] Marquez, V.E., Cranston, J.W., Ruddon, R.W., Kier, L.B. and Burckhalter, J.H. (1972) Mechanism of Action of Amodiaquine. Synthesis of Its Indoloquinoline Analog. *Journal of Medicinal Chemistry*, **15**, 36-39.

http://dx.doi.org/10.1021/jm00271a010

[18] Coimbra, E.S., Da Silva, A.D., Dias, R.M., Corrales, R.C., De, L.F., Bispo, M., Kaiser, C.R., *et al.* (2011) Amodiaquine Analogs. Synthesis and Anti-Leishmanial Activity. *Mediterranean Journal of Chemistry*, **1**, 106-113. http://dx.doi.org/10.13171/mjc.1.3.2011.26.09.22

[19] Foley, M. and Tilley, L. (1997) Quinoline Antimalarials: Mechanisms of Action and Resistance. *International Journal for Parasitology*, **27**, 231-240. http://dx.doi.org/10.1016/S0020-7519(96)00152-X

[20] Clark, M., Finkel, R., Rey, J. and Whalen, K. (2012) Lippincott's Illustrated Reviews: Pharmacology. 5th Edition, Lippincott Williams and Wilkins, New York, 451.

[21] Vij, K. (2014) Textbook of Forensic Medicine & Toxicology: Principles & Practice. Elsevier Health Sciences.

[22] Monge-Maillo, B. and López-Vélez, R. (2014) Topical Paromomycin and Gentamicin for New World Cutaneous Leishmaniasis in Panama. *The American Journal of Tropical Medicine and Hygiene*, **90**, 1191-1191. http://dx.doi.org/10.4269/ajtmh.14-0040a