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Full Length Research Paper

Prominent effect of Mefloquine on Murine *Schistosomiasis Mansoni* *Schistosomules* with no detectable Hepatocyte P53 protein

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The TP53 gene provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing (proliferating) too fast or in an uncontrolled way. The p53 protein is located in the nucleus of cells throughout the body, where it attaches (binds) directly to DNA. When the DNA in a cell becomes damaged by agents such as toxic chemicals, radiation, or ultraviolet (UV) rays from sunlight, this protein plays a critical role in determining whether the DNA will be repaired or the damaged cell will self-destruct (undergo apoptosis). If the DNA can be repaired, p53 activates other genes to fix the damage. If the DNA cannot be repaired, this protein prevents the cell from dividing and signals it to undergo apoptosis. By stopping cells with mutated or damaged DNA from dividing, p53 helps prevent the development of tumors. Because p53 is essential for regulating cell division and preventing tumor formation, it has been nicknamed the "guardian of the genome." This work is a trial to elucidate the effect of the antimalarial Mefloquine on the immature stages of *Schistosoma mansoni* schistosomules in experimental schistosomiasis *mansoni*. It is also a way to prove that, in spite of this previous effect, the level of hepatocytes p 53 protein is unchanged. Ninety (90) swiss albino mice were used in the experiment. Animals were divided into nine groups according to the time of drug administration. Group 1: infected control. Group 2: Mefloquine was given 24 hours prior to infection. Group 3: Mefloquine was given one week post infection. Group 4: Mefloquine was given two weeks post infection. Group 5: Mefloquine was given three weeks post infection. Group 6: Mefloquine was given four weeks post infection. Group 7: Mefloquine was given five weeks post infection. Group 8: Mefloquine was given six weeks post infection. Group 9: Mefloquine was given seven weeks post infection. Each group included three sub-groups corresponding to the three ascending doses used (200, 300 and 400 mg/kg). All animals were sacrificed nine weeks post infection. This study showed that mefloquine doesn't induce any hepatic dysplastic or malignant changes. This was indicated by the negative nuclear p53 in the hepatocytes with the highest dose of 400 mg/kg b wt. Therefore, Mefloquine can be safely used as anti-schistosomal, in addition to its known anti-malarial effect. The higher dose of 400 mg/kg proved to be the most effective in the experiment. Again, this trial disclosed the anti-morbidity role of the drug, by marked improvement in liver pathology, with concurrent drop in both granuloma diameter and numbers. The improvement was more salient in the higher than in the lower doses.

Keywords: Mefloquine, schistosomiasis, *Schistosoma mansoni*, P53, protein

INTRODUCTION

Schistosomiasis is a chronic water-borne helminthic disease endemic in many tropical and subtropical countries. The current global estimate indicates that 258 million people, mostly children, are affected, with 90% of them living in Africa. It was reported that in 2014, 61.6

million patients have been treated for schistosomiasis. The estimated annual loss is nevertheless very high, with more than 200 thousand deaths in sub-Saharan Africa (WHO, 2016). Preventive chemotherapy of schistosomiasis, is based on regularly targeted treatment with praziquantel (PZQ), which is effective against all *Schistosoma* species. This drug needs to be regularly applied in mass treatment programs to achieve

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sustainable control over schistosomiasis, as it lacks activity against the young developing stages of the parasite (Doenhoff *et al.*, 2008). On the other hand, as a result of the intensive and widespread use of PZQ, reduced rates of cure and treatment failure have been reported (Gryseels *et al.*, 2001). Moreover, reduced susceptibility of *S. mansoni* isolates to PZQ has been reported in many areas. The appearance of resistant strains is a first step towards widespread drug resistance; therefore, it is urgent to seek potential alternative anti-schistosomal agents (Wang *et al.*, 2012). *Schistosoma* has extreme metabolic activities, especially female worms for the production of eggs that is beyond the control of the host hence, a potentially profitable starting point for new drugs discovery to fight parasites is to examine available compounds already developed with anti-parasitic properties (Eissa *et al.* 2017).

More studies on mefloquine induced toxicity are needed as a basis for possible wide use for schistosomiasis in humans. The drug is generally well tolerated by adults and children. However undesirable events in the gastrointestinal (Keiser *et al.*, 2010) and central nervous systems (Van Essen *et al.*, 2010) have been reported in few studies.

In *S. mansoni*–infected mice, intestinal schistosomiasis is characterized by a temporal and special recruitment of mast cell subsets, with mucosal mast cells predominantly present in the ileal mucosa during the acute phase (De Jonge *et al.*, 2002). Tissue mast cell number showed that mefloquine induced mastocytosis in infected treated mice while praziquantel reduced mast cell density, (Van Nassauw, 2008) . In addition, mefloquine induced a severe mastocytosis in the intestinal wall, pointing to intestinal inflammation and resulting in gastrointestinal disturbances (Van Nassauw, 2008).

The efficacy of Praziquantel against adult worms of all schistosome species that infect humans has led to its very widespread use (Fenwick and Webster 2006). At the first decade of the new century, progress has indeed been made, where several new antischistosomal compounds have been studied. They included the 9-(S)-[3-hydroxy-2-(phosphono-methoxy)propyl] (Botros *et al.* 2003), 2-(alkylamino)-1-phenyl-1-ethanethiosulfuric acids (Moreira *et al.* 2007), synthetic 1,2,4-trioxolanes, secondary ozonides or OZs (Xiao *et al.* 2007; Keiser and Utzinger 2007), an inhibitor of thioredoxin glutathione reductase, i.e., 4-phenyl-1.2.5.-oxadiazole-3-carbonitrile-2- oxide (Sayed *et al.* 2008), the cysteine protease inhibitor K11777 (Abdulla *et al.* 2007), and miltefosine (hexadecyl-phosphocholine, HePC), an antileishmanial drug (Eissa *et al.* 2011).

Mutations in the p53 gene are the most common genetic defect in human tumors (Esrig *et al.* 1993). The p53 gene is located on chromosome 17 p 13, encodes for a 53-kD protein, and is known to play a vital role in the regulation of the cell cycle (Lane 1992) .When DNA damage occurs, the level of p53 protein increases, causing cell- cycle

arrest, this allows for the repair of DNA and prevents propagation of the DNA defect. Mutations in the p53 gene result in the production of protein. Consequently, this abnormal protein accumulates in the cell nucleus and can be detected by immunohistochemical staining. Several studies have demonstrated that nuclear accumulation of p53 protein determined by immunohistochemical staining correlates with gene mutations detected by DNA-sequence analysis (Hollstein *et al.* 1991, Dalbagni *et al.* 1995 and Vet *et al.* 1995). Inactivation of p53, a key tumor suppressor protein, is an important factor for determining transition to neoplastic changes (Netto. 2011).

Keiser *et al.* (2009), reported that the antimalarial drug mefloquine-an arylaminoalcohol compound possesses antischistosomal properties.

This study aimed at elucidating the role of the anti-malarial Mefloquine against schistosomiasis in infected mice. Three different doses have been administered to animals: 200 ,300 and 400 mg/kg body weight, Animal groups were divided according to the time of treatment, starting 24 hours before ,then one week post- infection and at weekly intervals up to seventh week post infection. This timing would be targeting all stages of the parasite. Histopathological study includes examination of liver sections stained with either Hx and Eosin, Masson trichrome or Toluidine blue. Detection of p53 protein by immunohistochemistry was also undertaken to evaluate any possible effect on hepatocytes.

Material and methods

The parasite: *Schistosoma mansoni* cercariae were obtained from *Schistosoma* Biological Supply Program (SBSP) at Theodor Bilharz Research Institute (TBRI).

Experimental animals: C57BL/6 mice (6-8 weeks old) were obtained from Schistosoma Biological Supply Program (SBSP) at TBRI, and kept under standard housing conditions.

Drug preparation and adjustment of the dose: Mefloquine tablets (Mephaquin®) tablets were obtained (Mepha Ltd, Aesch-Basel, Switzerland, lot 0850074) and were administered orally as a fresh suspension in 7% (v/v) Tween-80 and 3% (v/v) ethanol. Mefloquine was administered in ascending doses till the high single oral dose of 400 mg/kg (Keiser *et al.* 2009).

Parasitological parameters

Worm burden: Perfusion of adult worms from the liver and portomesenteric system was performed 8 and 16 weeks after infection according to (Ebeid *et al.* 2005).

Tissue egg load: The number of eggs per gram tissue (liver and intestine) was studied according to the procedure by (Botros& Bennett 2007).

Egg developmental stages (Oogram): The percentages of immature, mature and dead eggs from

the small intestinal wall of infected mice were computed from a total of hundred eggs per intestinal segment. Immature eggs were characterized by partially developed embryos with clear transparent parts within the eggs shell. The mature ones contained fully developed miracidium. Dead eggs exhibited dark, retraction and irregular outline of dead embryos. Three segments per animal were examined [Kamel et al 2006].

Histopathology and granuloma measurement: Livers were harvested from mice, fixed in 10% buffered formalin and processed to paraffin blocks. Sections (4 µm thick) were cut every 250 µm to avoid measuring the same granuloma. Five liver sections were prepared from each animal and stained with the haematoxylin and eosin and Masson trichrome stains. Measurements of the granulomas were conducted on non-contiguous granulomas, each containing a single egg (with intact or degenerated miracidia), using an ocular micrometer. The mean diameter of each granuloma was calculated by measuring 2 diameters of the lesion at right angles to each other [Ebeid et al 2005]. Granuloma structural configurations, including cellular components and associated hepatic histopathological changes, were recorded.

Toluidene blue stains mast cells red-purple background blue (Sridharan and Shankar 2012).

P53 protein detection: Immunohistochemical stain was stained in both, infected control group (I) and the groups receiving the highest dose of mefloquine (400 mg/kg) six weeks post infection to evaluate any possible impact of the drug on inducing undesirable hepatocyte side effects.

Immunohistochemical staining: Immunohistochemical staining performed on 4-µm, formalin- fixed, paraffin –embedded sections using p53 antibody at 1: 50 dilution (DAKO, Carpinteria, CA) Antigen retrieval performed in all cases by steam heating the slides in a 1-mmol/L solution of EDTA (pH 8.0) for minutes .After blocking of endogenous biotin, staining performed using an automated immunostainer (DAKO) followed by detection by using a streptavidin –biotin detection system (DAKO) Positive and negative control sections used for each assay (Vet et al 1995).

Experimental design: *Mice infection:* *S. mansoni* cercariae were inoculated subcutaneously using (60 ± 10) freshly shed cercariae per mouse [Kamel et al 2011].

Animals were divided according to the time of drug administration:

- Group 1:** Infected control.
- Group 2:** Mefloquine was given 24 hour before infection.
- Group 3:** Mefloquine was given one week post infection.
- Group 4:** Mefloquine was given two weeks post infection.
- Group 5:** Mefloquine was given three weeks post infection.

Group 6: Mefloquine was given four weeks post infection.

Group 7: Mefloquine was given five weeks post infection.

Group 8: Mefloquine was given six weeks post infection.

Group 9: Mefloquine was given seven weeks post infection.

Each group included three sub groups corresponding to the three ascending doses used (200, 300 and 400 mg/kg). All animals were sacrificed nine weeks post infection.

Statistical Analysis

- Data were coded and entered using the statistical packages SPSS version 7.5.
- Comparisons between groups were done using chi-square test for qualitative variables and analysis of variants (T-test) and multiple comparison post Hoc test for quantitative variables.
- P-values lower than 0.05 were considered as statistically significant.

RESULTS

There was a high significant reduction in total worm burden in all treated groups when the dose 400 mg/kg B.W. of mefloquine was given (groups 4, 5, 6, 7, 8). Complete disappearance of worms when treatment was given .3 weeks post infection with the dose of 400 mg/kg (Gr.5). Starting treatment 24 hours before infection (Gr.2) or seven weeks post infection (Gr.9), resulted in insignificant reduction in worm burden with the doses 200 mg/kg and 300 mg/kg B.W. of mefloquine.

Both hepatic and intestinal tissue egg loads showed remarkable reduction as compared to infected control, in all groups treated with rising doses of mefloquine .Administration of the drug 3 weeks post infection (Gr.5) resulted in disappearance of tissue eggs in liver and intestine when mefloquine was given in the dose 400mg/kg B.W.

As regards the oogram pattern, no developmental egg stages were seen in groups 5, given mefloquine (400mg/kg), 3 weeks post infection. the dose 300 gm/kg of mefloquine increased the percentage of dead ova to reach 92.2 when treatment was given 6 weeks post infection (Gr.8).At the same time of treatment the dose 400 mg/kg B.W. resulted in 100% dead ova with complete disappearance of all egg stages.

The mean number of granulomas in ten successive power fields decreased as compared to infected control group. Disappearance of granulomas was noticed in the group given mefloquine (400 mg/kg B.W.) three weeks post treatment, treatment 5 weeks with 300mg/kg or 400

Table 1 Total worm burden \pm SD in *Schistosoma mansoni* infected mice given Mefloquine(200mg/kg, 300mg/kg and 400mg/kg respectively) at different time intervals pre and post infection.

Animal group	Dose of mefloquine		
	200 mg/kg	300 mg/kg	400 mg/kg
1) Infected control. Without treatment.	23.66 \pm 4.38		
2) 24 hour before infection.	25.67 \pm 1.15	18.33 \pm 3.05*	14.88 \pm 2.47***
3) One week after infection.	21.66 \pm 4.93	20.66 \pm 5.86	11.33 \pm 2.08***
4) Two weeks after infection.	11 \pm 3***	9.5 \pm 0.7***	4.33 \pm 1.15***
5) Three weeks after infection.	8 \pm 1.7***	2.33 \pm 1.5***	0***
6) Four weeks after infection.	12.33 \pm 0.57	7 \pm 1.4***	3 \pm 1***
7) Five weeks after infection.	6 \pm 1***	2.33 \pm 1.52***	0.33 \pm 0.57
8) Six weeks after infection.	14 \pm 1***	6 \pm 1.41***	3.3 \pm 3.65***
9) seven weeks after infection.	18.66 \pm 2.94*	13.4 \pm 3.44***	12.5 \pm 4.65***

*Statistically significant difference at P<0.05 low sig.
 **Statistically significant difference at P<0.01 moderate sig
 *** Statistically significant difference at P<0.001 highly sig

Table 2 Hepatic and Intestinal tissue egg loads in *Schistosoma mansoni* infected mice given Mefloquine (200mgm/kg, 300mg/kg and 400mg/kg respectively) at different time intervals pre and post infection.

Animal group	Dose of mefloquine					
	200 mg/kg		300 mg/kg		400 mg/kg	
	Hepatic tissue egg count.	Intestinal tissue egg count.	Hepatic tissue egg count.	Intestinal tissue egg count.	Hepatic tissue egg count.	Intestinal tissue egg count.
1) Infected control. Without treatment.	29461.56 \pm 4685			24663.67 \pm 8830.463		
2) 24 hour before infection.	5402.66 \pm 1275.58 ***	12357.33 \pm 6183.07 ***	4826.67 \pm 1193.83 ***	7104.67 \pm 2526.62 ***	10953 \pm 12832 ***	7236 \pm 7834.93 ***
3) One week after infection.	29221 \pm 5456.37	8635 \pm 5308.14 ***	5543.67 \pm 586.97 ***	3291 \pm 1121.24	1700 \pm 788.59 ***	2240 \pm 848.43 ***
4) Two weeks after infection.	10021.67 \pm 3681.44***	11816 \pm 16101***	8126 \pm 377595***	5034.5 \pm 2342.64***	6362 \pm 5516.55***	2249.33 \pm 589.38***
5) Three weeks after infection.	1354 \pm 848***	992.33 \pm 1433.24**	787 \pm 826.61***	595 \pm 546.37***	0***	0***
6) Four weeks after infection.	11306.67 \pm 8635.81***	5558.67 \pm 3233.22***	4344.5 \pm 4827.42***	1465.5 \pm 1887.27***	1425 \pm 259.43***	536.67 \pm 71.62***
7) Five weeks after infection.	5389 \pm 2215.42**	1645.67 \pm 2257.47**	2637.67 \pm 1226.74 ***	1363.33 \pm 1529.79***	1863.67 \pm 2094***	1032.67 \pm 1383.15**
8) Six weeks after infection.	4249 \pm 1389.27**	1804.33 \pm 604.49**	4944.5 \pm 962.3***	3258.5 \pm 614.48***	1098.32 \pm 636.98***	94.4 \pm 169.66***
9) Seven weeks after infection.	5665.5 \pm 1164.19 ***	3172.83 \pm 491.07**	4581.6 \pm 1045.98**	1845.6 \pm 474.42***	3363 \pm 1563.89***	1040.25 \pm 350.5***

*** Statistically significant difference at P<0.001 highly sig

Table 3 Oogram pattern in *Schistosoma mansoni* infected mice treated with Mefloquine (200mgm/kg,300mgm/kg and 400 mgm/kg respectively) at different time intervals pre and post infection.

Animal group	Dose of mefloquine								
	200 mg/kg			300 mg/kg			400 mg/kg		
	Immature Stage \pm SD	Mature Stage \pm SD	Dead Stage \pm SD	Immature Stage \pm SD	Mature Stage \pm SD	Dead Stage \pm SD	Immature Stage \pm SD	Mature Stage \pm SD	Dead Stage \pm SD
control. Without 1) Infected treatment.	49.67 \pm 4.39			43.22 \pm 2.59			7.11 \pm 1.45		
2) 24 hour before infection.	53 \pm 2.65	40 \pm 2	7 \pm 1 ***	52.3 \pm 2.52	40.7 \pm 4.04	7 \pm 1.73 ***	47.25 \pm 2.92	45 \pm 2.73	7.25 \pm 1.58
3) One week after infection.	52 \pm 2	42.67 \pm 2.52	5.33 \pm 0.58 ***	50 \pm 3	42.67 \pm 2.52	7.33 \pm 0.57 ***	50 \pm 2	42 \pm 2.65	8 \pm 1
4) Two weeks after infection.	48.67 \pm 2.08	43.67 \pm 1.53	7.67 \pm 1.15 ***	47.5 \pm 0.71	43 \pm 1.41	9.5 \pm 0.71 ***	46 \pm 1	44 \pm 1	10 \pm 1 ***
5) Three weeks after infection.	30.33 \pm 4.51 ***	38.33 \pm 2.88 ***	28 \pm 2.65 ***	25 \pm 5 ***	36.7 \pm 2.89 ***	45 \pm 13.23 ***	0 ***	0 ***	0 ***
6) Four weeks after infection.	36.67 \pm 2.08 ***	42.33 \pm 2.52	21 \pm 1	35.5 \pm 0.71	39 \pm 1.41 ***	25.5 \pm 0.71 ***	30 \pm 2.65 ***	38 \pm 3 ***	32 \pm 2 ***
7) Five weeks after infection.	23.33 \pm 3.51 ***	36.67 \pm 2.89 ***	40 \pm 6.24 **	23.67 \pm 5.69 ***	31 \pm 5.29 ***	45.33 \pm 2.52 ***	4 \pm 6.92 ***	8.33 \pm 14.43 ***	87.67 \pm 21.36 ***
8) Six weeks after infection.	22.33 \pm 6.43 ***	32.67 \pm 6.43 ***	44.7 \pm 11.50	2 \pm 3.56 ***	6.8 \pm 8.92***	92.2 \pm 11.88	0***	0***	100***
9) Seven weeks after infection	46.17 \pm 2.32	41.67 \pm 2.33 **	12.17 \pm 2.48 ***	35.8 \pm 5.12 ***	39.8 \pm 2.86	24.4 \pm 4.39 ***	27.5 \pm 6.45 ***	30 \pm 9.13 ***	42.5 \pm 11.9 ***

**Statistically significant difference at P<0.01 moderate sig
 *** Statistically significant difference at P<0.001 highly sig

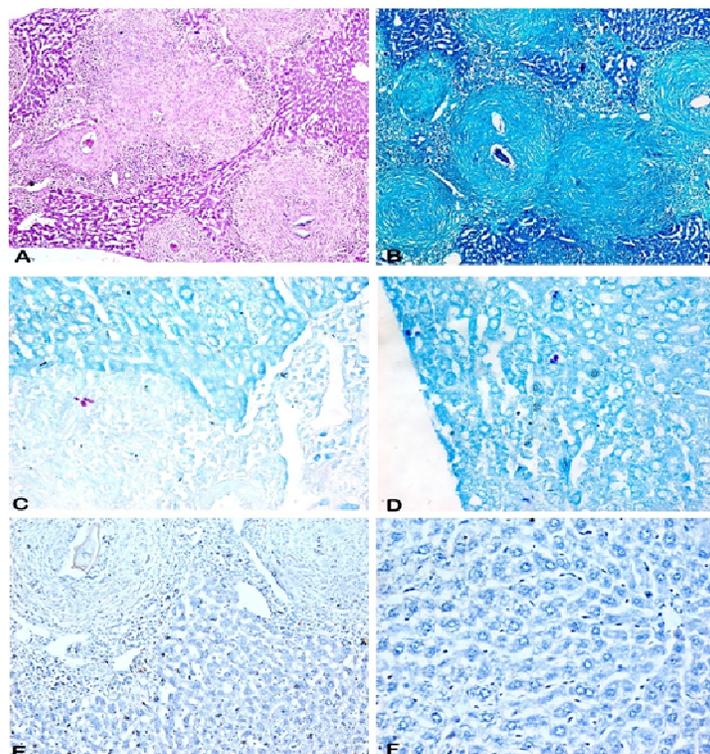
Table 4 Hepatic granuloma number and diameter in *S. mansoni* infected mice given Mefloquine (200mg/kg, 300mg/kg and 400mg/kg respectively) at different time intervals pre and post-infection

Animal group	Dose of mefloquine					
	200 mg/kg		300 mg/kg		400 mg/kg	
	number of granuloma in 10 successive power fields (10x10)	mean granuloma diameter in μm	number of granuloma in 10 successive power fields (10x10)	granuloma diameter in μm	number of granuloma in 10 successive power fields (10x10)	granuloma diameter in μm
1 control.)Infected Without treatment.	13.67 \pm 1.53			334.33 \pm 7.02		
2) 24 hour before infection.	8.77 \pm 0.24**	149.14 \pm 1.87***	7.11 \pm 1.20***	148.74 \pm 1.19**	6.72 \pm 0.61***	146.51 \pm 1.32***
3) One week after infection	8 \pm 1**	150.33 \pm 3.21	6.33 \pm 1.53**	148 \pm 1**	6 \pm 0.82**	140.71 \pm 3.19***
4) Two weeks after infection.	3.69 \pm 2.08***	87.58 \pm 9.72***	3.18 \pm 0.08***	75.51 \pm 1.96***	2.59 \pm 0.51***	65.83 \pm 4.16***
5) Three weeks after infection.	6.67 \pm 1.15**	141 \pm 8.72***	5.67 \pm 1.15**	139 \pm 3.61**		
6) Four weeks after infection.	4 \pm 1***	116.67 \pm 7.02***	2.25 \pm 2.63***	36.5 \pm 36.5***	2 \pm 2.12***	41 \pm 37.64***
7) Five weeks after infection.	7.33 \pm 0.58**	188 \pm 33.15**				
8) Six weeks after infection.	8.33 \pm 0.58**	248 \pm 3*	7 \pm 1.41**	241.5 \pm 8.92*	5.67 \pm 1.15**	193.67 \pm 8.39**
9) Seven weeks after infection	11.5 \pm 1.52***	242.66 \pm 7.74*	10.4 \pm 2.30*	233.4 \pm 5.94*	7.25 \pm 1.71**	221.5 \pm 1.29**

*Statistically significant difference at P<0.05 low sig.

**Statistically significant difference at P<0.01 moderate sig

*** Statistically significant difference at P<0.001 highly sig



mg/kg of mefloquine also resulted in disappearance of granulomas, the mean granuloma diameter (μm) also decreased significantly in all groups treated post infection when using either of the 3 doses.

Figure 1: A- Control infected liver shows bilharzial granulomas, H&E x100. B- Control infected liver shows few eosinophils and mast cells (purple in color) around a bilharzial granuloma, Toluidine blue x400. C- Mefloquine

treated liver shows back to normal architecture with no residual granulomas or fibrosis in contrast to A, H&Ex40. D- Mefloquine treated liver shows back to normal architecture with no residual granulomas or fibrosis in contrast to A, Masson Trichromex40. E- Mefloquine treated liver shows negative p53 in hepatocytes no granulomas P53 immunohistochemistryx200. E- Mefloquine treated liver shows no eosinophils or mast cells, Toluidine blux400.

DISCUSSION

It's worth investigating if mefloquine treatment could be of value in patients who have mixed malaria and *Schistosoma* infections. Such co-infection is not rare in endemic countries. Prevalence of *S. mansoni* – *P. falciparum* co-infections was 22.6% among schoolchildren in rural northwest Tanzania (Mazigo et al., 2011).

Therefore, a closer collaboration between malaria and schistosomiasis communities might facilitate the discovery and development of novel antischistosomal drugs (Keiser and Utzinger 2012)

In this study, mefloquine (400 mgm/Kg b wt) given three weeks post infection, revealed complete worm eradication. While treatment seven weeks post infection, resulted in mild drop in worm load. Again, there was a significant difference in worm load between the three studied doses and their corresponding control groups. Finally, a high significant drop in worm burden was evident in the group treated six weeks post infection with the highest dose.

Again, in this work, both hepatic and intestinal tissue egg loads, showed evident reduction, compared to infected control, in all groups given rising doses of mefloquine. In addition, administration of the drug (400mgm/Kgm b wt) three weeks post infection, resulted in absence of both hepatic and intestinal tissue egg loads.

Vanishing of developmental egg stages was seen in the oogram pattern when mefloquine (400mg/kg) was given 3 weeks post infection. The percentage of dead ova reached 100% when the same dose was given 6 weeks post infection. Parasitological data revealed a remarkable activity against immature stages of *S. mansoni*. To sum it up, the higher dose of 400mg/kg mefloquine, proved to be the most effective in all experimental groups. Previously, Keiser et al. (2009) found a worm burden reduction of 45% and 72% in doses of 100 and 200 mg/kg mefloquine respectively. Similarly, Xiao et al. (2009b) tested a single mefloquine dose of 200 mg/kg. Their results showed total worm burden reductions of 56.3% on juvenile and 89.1% on adult schistosomes. When they increased the mefloquine dose to 400 mg/kg, worm reduction rates were 81.1-100% on juvenile and adult worms respectively. Xiao et al. (2009a)

assessed the effect of mefloquine 400 mg/kg single dose – on the morphology of adult *Schistosoma japonicum* under a light microscope. They found severely dilated guts and the entire worm body was swollen. Moreover, reproductive glands showed signs of degeneration, which resulted in disturbance of ova formation and cessation of oviposition.

In this study, the mean number of granulomas in ten successive power fields, decreased as compared to infected control group. Disappearance of granulomas was noticed in the group given mefloquine (400 mg/kg B.W.) three weeks post treatment. The mean granuloma diameter (μm) also decreased significantly when the drug was given two weeks post infection when using each of the three ascending doses.

In a study by Abdel –Fattah and Ahmed (2011), mefloquine monotherapy was found to be superior to PZQ both in juvenile and mature worm infection. The drug resulted in worm eradication, and when combined with PZQ, full or half dose regimens, much improved the course of infection (Abdel –Fattah and Ahmed, 2011). In vivo studies showed that the efficacy of mefloquine, is independent from the host immune response (Keiser et al. 2010). Mefloquine possesses a rapid onset of action, and causes extensive and severe morphological, histopathological, and ultra-structural damage to adult and juvenile schistosomes. The worm tegument, musculature, gut, and vitelline glands of female worms are more vulnerable to the drug action (Zhang et al. 2009; Xiao and Zhang 2009). Again, mefloquine showed a significant chemoprophylactic and a therapeutic effect against juvenile and adult *S. mansoni* in infected mice compared to artemether (juvenile only) and praziquantel. Scanning Electron Microscopy, revealed that mefloquine induced tegumental damage in adult schistosomes. This model could need to replicate in human individuals as it may be a promising treatment regimen preventing treatment failures and a strategy for schistosomiasis *mansoni* control programs. (Ingram et al. 2012). El Sayed et al (2012), did a scanning electron microscopy (SEM) and found that mice treated with mefloquine 400 mg/kg eight hours post infection, showed focal swelling of the worm body, fusion of tegumental ridges, and enlargement of sensory structures. El Badry et al (2014), stated that administration of single oral dose of MFQ-Ag-NPs (Silver nanoparticles) composite three and six weeks post-infection, gave more statistically significant results than PZQ-AgNPs composite as regards the anti-schistosomal effect. This may introduce Ag-NPs as a novel antischistosomal compound in addition to the available drugs praziquantel (PZQ) or mefloquine (MFQ).

Examination of liver after mefloquine treatment (400mg/kg), either 3 weeks post infection or 6 weeks post infection showed back to normal architecture with no granuloma or fibrosis as compared to infected control. In the same treated groups, the liver showed no eosinophil's or mast cells.

On the other hand, there was no association between tissue mast cell density and clearance of infection in treated mice (Sobhy et al.2014). Again, there was no association between tissue mast cells count and both the worm burden and tissue egg count in infected treated mice. The increased aggregation of mast cells was associated with infection; treatment with MFQ resulted in an insignificant increase in mast cells compared to infected control group (Sobhy et al . 2014).

The present study aimed at investigating any possible impact of mefloquine on P53 gene, the immunohistochemical staining of liver of treated mice revealed negative P53 in hepatocytes.

As regards the potential harmful side effects induced by mefloquine, this study showed that mefloquine doesn't induce hepatic dysplastic or malignant changes indicated by the negative nuclear p53 in the hepatocytes with the highest dose of 400 mg/kg gm.

In conclusion, mefloquine can be safely used as an anti-schistosomal agent in addition to its known anti-malarial effect. The dose of 400 mg/kg, revealed the most promising anti-schistosomal action. This was shown by the marked improvement in liver pathology, with significant decrease in both granuloma diameter and number. The improvement was dose dependent. Again, the timing of treatment post-infection had a salient impact on most experimental data. Since praziquantel is active against adult worms, and mefloquine is active against both adult and juvenile *S. mansoni* stages, combination therapy with both drugs could be a beneficial resort for transmission control of schistosomiasis worldwide (Abdel-Fattah and Ahmed 2011).

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