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Hepatic Amyloidosis Secondary to Schiatosomal Infection: Therapeutic Effect of Colchicines Alone or in Combination with Praziquantel

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ABSTRACT *Amyloid deposits is one of the most complication were found secondary to schistosomal infection in both human and experimental animals. We aim to study the effect of common used amyloidosis drug "colchicines" for the treatment of secondary amyloidosis of schistosomal infection. Hamsters were infected with 200 S. haematobium cercariae and treated with Colchicines (Col) or/ and Prazequentel (PZQ), early and late of infection. Biochemical and histopathological investigations were measured to assess the effect of Col or/ and PZQ treatment. Significant reduction in hepatic Amyloid deposits and improvement in serum albumin and cholesterol were observed when combined treatments were given. This was nearly complete with early treatments and only partial when treatment was given late. We conclude that, colchicine is effective for the prevention of and cure hepatic amyloidosis secondary to schistosomal infection.*

Introduction:
Schistosomiasis is a parasitic disease caused by infection with helminthes Schistosoma species. The arrival of eggs in the liver during Schistosoma infection initiates a protective granulomatous response. However, as the infection progress, this response results in chronic liver complications including amyloidosis and fibrosis (Andrade, 2004) which are central events in progressive liver disease. This leads to cirrhosis, and associated morbidity and mortality caused by decompensation. Amyloidosis is a heterogenous group of fibrillary protein deposition disorders involving the extracellular matrix of various organ systems. The liver is a major site of amyloid deposition. Hepatic involvement in amyloidosis can occur in both primary and secondary forms of the disease. Although liver is often involved, clinically apparent disease is rare (Yellapu, et al., 2010). Liver amyloidosis is the results of an imbalance between synthesis and degradation of extracellular matrix proteins of the liver . Colchicine, a naturally occurring alkaloid, inhibited collagen synthesis and fibroblast proliferation when added to culture of fibroblasts isolated from Schistosoma mansoni infected liver and may therefore be useful in modulating schistosomal hepatic fibrosis (Mansour et al. 1988). Montasser et al. (1994) also suggested that Colchicine has therapeutic effect against schistosomal liver fibrosis. When colchicines were given concomitantly with Praziquantel (Badawy et al. 1996) although it normalized the elevated serum procollagen III propeptide of infected mice it could not significantly change the histological picture of schistosomal murine liver fibrosis. In this article we aim to study the effect of Colchicine alone or in combination with PZQ on liver amyloidosis deposits and functions.

Materials and Methods:
Experimental Design:
Male hamsters were used in this study as Hamed et al., (2007) recommend to use male hamsters when use this experimental animal in schistosomal infection research. Seventy hamsters were infected with ±200 S. haematobium, they were divided into three groups G2: infected not treated (positive control, PC); G3: infected with early treatment at 9 week post infection; G4: infected with late treatment at 15 week post infection. Another uninfected group was considering as negative control, NC (G1). Group 3 and 4 each contain 30 hamsters, were subdivided into three subgroup each of 10 hamsters differ according to the type of treatment (G3a, 4a) were treated with Colchicine only COL. Group (G3b, 4b) were treated with COL + PZQ. Group (G3c, 4c) were treated with PZQ only. Five hamsters from each group were sacrificed at 18 week of infection and the other 5 hamsters at 24 weeks.

Drug Dosage:
Drug dosage was given according Sobh et al., (1995). Both drugs are given orally, Colchicine in a dose of 60 µg/ kg daily until the time of sacrifice and PZQ as 100 mg/ kg once which was repeated weekly for 3 consecutive weeks.

Biochemical and Histopathoilogical investigations:
At sacrifice, animals were anesthetized, liver and spleen tissues were obtained and examined histopathologically. Liver and spleen specimens were fixed in 10% neutral buffered formalin and processed as paraffin sections. These were stained with hematoxylin and eosin, Masson's trichrome, periodic acid- Schiff and Congo red stains. Sections were examined by a pathologist unaware of the animal group. At sacrifice, mesenteric veins were explored for adult worms. Weekly estimates of serum total protein, albumin and cholesterol were measured to all hamsters involved in this study, A/ G ratio were calculated.

Statistical Analysis:
Biochemical changes in different treated group were compared with NC and PC using Student's t test. Pathological changes were given numbers (0- 3) according to the degree of amyloid deposits. A nonparametric method was used to analyze these changes using SPSS package. P value of < 0.05 was considered as significant.

Results:
Complete parasite eradication was achieved in the groups treated with PZQ alone or in combination with COL (G3, b, c and G4a, c) as no adult worms were detected in the mesenteric circulation. In PC or G2 and COL treated group (G3a and G4a) the mean adult worms recovered was 10.98 ± 7.4; 9.5 ± 7.1 and 14.75 ± 8.34 adult worms, respectively.

Table 1 show the biochemical changes observed in hamsters

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sacrificed 18 weeks after infection in NC, PC and hamsters treated 9 weeks, G3 and 15 weeks, G4 post infection. A significant improvement in serum albumin and cholesterol in COL, PZQ and COL + PZQ subgroups was observed when compared to PC (P2). These observations were the same when a treatment was given 9 or 15 weeks of infection. When compare the same biochemical parameters at different time of treatment (P3) a better significant improvement in serum albumin and cholesterol was observed in groups treated at 9 weeks more than groups treated at 15 weeks of infection.

Table1: Prophylactic (9 wks) and Curative (15 wks) Effect of Colchicines and/ or Praziquantel on Biochemical Parameters Measured at 18 weeks post infection Between Different Groups.

Groups		Alb	TP	A/G	Chol
G1	M	2.75	6.18	0.801	124
NC	S.D.	0.1	0.25	0.03	20
G2	M	1.91	5.65	0.483	209
PC	S.D.	0.44	0.73	0.125	78
	P1	0.00002	0.22	0.00005	0.004
G3a	M	2.51	6.14	0.702	123
COL9	S.D.	0.26	0.69	0.135	32
	P1	0.01	0.86	0.03	0.93
	P2	0.002	0.41	0.002	0.005
G3b	M	2.88	6.16	0.886	111
COL+PZQ9	S.D.	0.34	0.26	0.144	22
	P1	0.25	0.88	0.08	0.2
	P2	0.0001	0.3	0.00001	0.003
G3c	M	3.18	6.27	1.03	138
PZQ9	S.D.	0.15	0.41	0.081	60
	P1	0.00002	0.55	0.00003	0.51
	P2	0.00001	0.18	0.00002	0.05
G4a	M	2.08	6.16	0.511	170
COL15	S.D.	0.38	0.59	0.101	29
	P1	0.00008	0.93	0.00002	0.001
	P2	0.4	0.38	0.62	0.2
	P3	0.01	0.94	0.004	0.005
G4b	M	2.41	6.07	0.662	125
COL+PZQ15	S.D.	0.23	0.24	0.112	33
	P1	0.0009	0.39	0.001	0.9
	P2	0.01	0.48	0.007	0.01
	P3	0.006	0.5	0.003	0.32
G4c	M	2.74	6.8	0.679	157
PZQ15	S.D.	0.29	0.37	0.124	34
	P1	0.92	0.0004	0.007	0.02
	P2	0.0001	0.002	0.003	0.07
	P3	0.001	0.01	0.00003	0.41

Table 2 show the biochemical changes observed in hamsters sacrificed 24 weeks after infection in NC, PC and hamsters treated 9 weeks, G3 and 15 weeks, G4 post infection. A significant improvement in serum albumin and cholesterol in PZQ and COL + PZQ subgroups was observed when compared to PC (P2) while COL treated group was the same like PC group. These observations were noticed when a treatment was given 9 weeks of infection but not when treatment was given 15 weeks of infection. When compare the same biochemical parameters at different time of treatment (P3) a better significant improvement in serum albumin and cholesterol was observed in groups treated at 9 weeks more than groups treated at 15 weeks of infection only in COL + PZQ subgroup.

Table2: Prophylactic (9 wks) and Curative (15 wks) Effect of Colchicines and/ or Praziquantel on Biochemical Parameters Measured at 24 weeks post infection Between Different Groups.

Groups		Alb	TP	A/G	Chol
G1	M	2.91	6.45	0.821	127
NC	S.D.	0.19	0.28	0.05	36
G2	M	1.66	6.28	0.46	187
PC	S.D.	0.39	0.58	0.05	37
	P1	0.00001	0.0001	0.00001	0.01
G3a	M	2.18	5.7	0.557	130
COL9	S.D.	0.52	0.73	0.133	30
	P1	0.001	0.01	0.003	0.85
	P2	0.11	0.36	0.29	0.03
G3b	M	3.7	6.45	0.96	120
COL+PZQ9	S.D.	0.12	0.31	0.028	25
	P1	0.02	1	0.005	0.75
	P2	0.0001	0.008	0.0001	0.01
G3c	M	2.85	6.07	0.882	118
PZQ9	S.D.	0.19	0.29	0.088	9
	P1	0.61	0.04	0.11	0.65
	P2	0.0009	0.04	0.0007	0.009
G4a	M	1.9	5.82	0.482	210
COL15	S.D.	0.11	0.8	0.053	47
	P1	0.00006	0.001	0.002	0.002
	P2	0.25	0.11	0.74	0.42
	P3	0.33	0.75	0.33	0.01
G4b	M	2.12	5.57	0.615	190
COL+PZQ15	S.D.	0.57	0.6	0.179	35
	P1	0.001	0.002	0.01	0.01
	P2	0.19	0.48	0.16	0.9
	P3	0.01	0.04	0.008	0.01
G4c	M	2.88	6.31	0.845	134
PZQ15	S.D.	0.33	0.32	0.152	20
	P1	0.81	0.31	0.63	0.58
	P2	0.0003	0.0006	0.0003	0.003
	P3	0.1	0.1	0.31	0.2

Table 3 show the semi quantitative evaluation of hepatic amyloid deposits detected at the end point of infection in PC and hamsters treated 9 weeks, G3 and 15 weeks, G4 post infection. No amyloid deposits were seen in ant of the nor-

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mal uninfected group. A partial reduction in hepatic amyloid deposits was observed when drugs given alone ether early G3 or late G4 of infection. Combined drug treatment shows a complete significant reduction of hepatic amyloid deposits only when the drugs were given early G3 but not late G4.

Table3: Significant of Colchicines and/ or Praziquantel treatment on hepatic Amyloid deposits detected at the end point of infection.

Group		Liver	Spleen
G2	M	1.66	1.44
PC	S.D.	0.5	0.88
G3a	M	1.55	1.1
COL9	S.D.	0.5	0.31
	P	0.67	0.27
G3b	M	0.55	0
COL+PZQ9	S.D.	0.72	0
	P	0.005	0.0009
G3c	M	1.25	1.25
PZQ9	S.D.	0.7	0.5
	P	0.17	0.65
G4a	M	2	1.66
COL15	S.D.	0	0.51
	P	0.11	0.56
G4b	M	1.37	1
COL+P15	S.D.	0.51	0.53
	P	0.26	0.25
G4c	M	1	0.6
PZQ15	S.D.	0.94	1.07
	P	0.07	0.08

Discussion:

The evaluation of Colchicine effect on hepatic amyloid deposits biochemical and histopathological study was the main aim of the present study. We compare this effect when the drug was given alone or in combination with anti-schistosomal drug. These treatments were tried early 9weeks of infection, where no true amyloid deposits were detected in different tissues (preventive) and late 15 weeks of infection, where true amyloid deposits were detected in different tissues. Complete histological and biochemical regression was achieved when combined treatment was given early, yet this was partial when the drugs (COL or PZQ) were given alone. When combined treatment was given after establishment of amyloidosis an incomplete but significant reduction in disease markers (amyloid deposits and serum albumin) was achieved. When Colchicine or Praziquantel were given alone after establishment of amyloidosis, no histological was observed, yet there was significant partial reduction in serum albumin and cholesterol.

Amyloidosis is not a single disease, but a series of diseases in which there is extracellular deposition of a protein, often leading to prominent end organ dysfunction. The mechanisms of Colchicine action in the therapy of amyloidosis are still unclear. Colchicine was suggested to have a therapeutic effect against schistosomal liver fibrosis (Montasser et al., 1994; Si et al., 1995; Jiang et al., 1996). With other combined treatment, augmented reduction of liver fibrogenesis was achieved (Al-Harbi et al., 2012). Its effect was greatly improved when its treatment was combined to the anti-schistosomal drug Praziquantel (Sobh et al., 1995; Badawy et al., 1996 &1999). These findings were concise with our findings.

We conclude that Colchicine has a significant effect on Schistosoma- induced hepatic amyloidosis, especially when it given early of infection and in combination with anti-schistosomal drug Praziquantel.

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