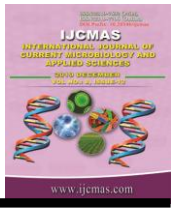
Int.J.Curr.Microbiol.App.Sci (2019) *8*(12): 1136-1151

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 12 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.812.143

Antifibrotic Alleviative Effect of Bone Marrow-Derived Mesenchymal Stem Cells on Experimentally induced *Schistosoma mansoni* Related Liver Fibrosis

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Keywords

BM-MSCs, *Schistosoma mansoni*, Praziquantel, Hyaluronic acid, α SMA

Article Info

Accepted:
10 November 2019
Available Online:
10 December 2019

A B S T R A C T

With increased national programs for eliminating schistosomiasis and viruses affecting the liver in Egypt, treating the remained pathology represented mainly by liver fibrosis will probably be a competitive goal in the coming few years. We aimed in our work to reinforce the antifibrotic effect of BM-MSCs in liver fibrosis related to *Schistosoma mansoni* (Egyptian strain) infection in murine model and correlating serum Hyaluronic acid (HA) to liver fibrosis. A pilot study was done first to ensure reaching of MSCs to the fibrosed liver tissues following infection with *S. mansoni* and secondly to detect the best route for MSCs inoculation. Then 70 female Swiss albino mice were divided into five groups. GI; uninfected untreated, (NC, n=10), infected mice were categorized into 4 groups each contained 15 mice regarding treatment at 6th week p.i; GII: Infected untreated, GIII: PZQ treated, GIV: received MSCs and GV: PZQ then MSCs treated. Sacrifaction of all mice at 14th week p.i. was done and mice were subjected to parasitological, biochemical, histological and immunohistochemical workup. We observed that the use of BM-MSCs and PZQ significantly decreased mean granuloma number, with significant reduction in fibrosis percent (Masson trichrome) staining than PZQ. Presence of fibrosis was associated with high serum HA level and reduction of fibrosis was linked to lowering its serum levels. Use of the combined treatment improved liver function tests. Intravenous route for administration of MSCs and the use of HA as a noninvasive marker of fibrosis are recommended. MSCs significantly alleviate liver fibrosis induced by *Schistosoma mansoni* infection.

Introduction

Schistosomiasis has global importance as a parasitic disease. It is a considerable reason for morbidity and mortality worldwide (Johnston, Teague, and Graham 2015). More than 220 million people are infected with various types of *Schistosoma*, about seven hundred million people are in danger of being infected in 52 countries (James *et al.,* 2018).The disease is usually represented pathologically by chronic inflammation, focal areas with excess extracellular matrix (ECM)that is deposited in peri-ovular granulomas and distributed in variable numbers at the portal venous system periphery (Colley *et al.,* 2014). Host reaction to parasite eggs laid in the venous portal system and then trapped in the liver and intestine is primarily responsible for the main pathology of *Schistosomiasis mansoni* which lead to hepatic fibrotic changes causing dangerous complications such as, hepatomegaly, portal hypertension and oesophageal varices with hemorrhage tendency that are frequently the main cause of death (Chuah *et al.,* 2014).

Praziquantel (PZQ) is the anti-*Schistosomiasis* drug used for more than forty years now; Its effectiveness against adult worms of all schistosome species is known to be more than against immature larvae (Cioli *et al.,* 2014). Besides, low capacity is shown to reverse tissue damage or liver fibrosis caused by schistosome eggs (Singh *et al.,* 2004).

A large number of drugs with known antifibrotic action *in vivo* and *in vitro* were tried to reverse liver fibrosis with no applicable results for clinical use (Liu *et al.,* 2015).Thus transplantation of healthy liver remains the sole regimen for end-stage liver disease. Multi-differentiation potential, regenerative activity, modulation of immune response and capability of self-renewal, putsmesenchymal stem cells (MSCs)in the focus of liver fibrosis treatment strategies (Zhang *et al.,* 2018; Zhao *et al.,* 2018). Trans-differentiation of hepatic stellate cells into myofibroblasts is a key factor in liverfibrosis (Carson *et al.,* 2018). An indicator of activation of hepatic stellate cells (HSC) is the expression of alpha-smooth muscle actin (α SMA) in the liver and is recognized as the main player in liver fibrogenesis (Sun *et al.,* 2015)

The potential role of stem cell transplantation was tested by some authors, with the capacity of transdifferentiation into hepatocytes which help in the regeneration of liver tissue. Immunohistochemical markers such as α SMA supported reduction of fibrosis (Xu *et al.,* 2012).

Hyaluronic acid is mainly synthesized by HSCs and its importance comes from avoidance of liver biopsy if used to detect degree of fibrosis (Tamaki *et al.,* 1996 and Suzuki *et al.,* 2005).

Herein, we aimed to reinforce the role of BM-MSCs transplantation in the management of *Schistosomiasis mansoni* related liver fibrosis and evaluate the role of HA as marker of fibrosis.

Materials and Methods

This study was conducted in the Department of Molecular and Clinical Parasitology National Liver Institute Menoufia University in collaboration with Bilharz Research Institute (TBRI), Giza, Egypt, from May 2017 to June 2019.

Ethics statement

The current study was performed in line with the international policy and acceptance of animal ethics committee Tudor Bilharz Research Institute (TBRI), Giza, Egypt.

Experimental mice

One hundred and eleven Swiss albino mice (*Musmusculus*), 6-8 weeks old, laboratory-bred weighing approximately 25 gm, (30 male mice selected as BM-MSC donors and 81 female mice) were used in the study. They were brought and housed in a designated and government- approved animal house at Theodor Bilharz Research Institute, Giza, Egypt.

All efforts were made to minimize animal suffering. The mice were kept in wired cages under appropriate housing and handling conditions.

They were given a standard eating regimen according to the regulations of animal ethical committee (TBRI).

Infection of mice with *S. mansoni*

Female mice (71) mice were infected via injecting 50-60 cercariae delivered from infected *Biomphalaria alexandrina* snails and subcutaneously injected in each mouse(Peters and Warren 1969).

At 6th week post-infection (p.i.), one mouse was sacrificed and its liver was stained with Haematoxylin and Eosin (H&E) to find out whether the infection succeeded to produce fibrosis or not. Pathological examination of the liver tissue proved the presence of fibrosis.

Drugs

Praziquantel

Distocide (EPICO, Egypt) was administered for praziquantel (PZQ) group, the drug was administered in the form of aqueous suspension orally to each mouse by gavage through the mouth (Jiraungkoorskul *et al.,* 2005).

Mesenchymal stem cells (MSCs)

Bone marrow MSCs isolation and culture

Isolation of Bone marrow mesenchymal stem cells (BM-MSCs) was done according to Soleimani and Nadri (2009). The isolated cells were initially seeded at a density of 1.5×10^6 cells/well into 6-well plate utilizing complete DMEM that contains low glucose Dulbecco's Modified Eagle's Medium (DMEM) (Lonza, Visp, Switzerland) supplemented with 30% fetal bovine serum (Hyclone, USA), 1% penicillin/streptomycin (Biochrom, Berlin, Germany), and 1% L-glutamine (Lonza). Incubation was done in an atmosphere of 5% CO2 and 90% relative humidity at 37°C.

The wells were examined after ten days to detect colony formation. Media change for the first time was done after two weeks, then twice weekly.

After reaching the cells 95% confluency, trypsinization was done using 0.25% trypsin (Euro-lone, Milan, Italy). Cells were transferred after plating to the first passage by culturing at seeding density 5000 cells/cm^2.

When 80 - 90% confluency was reached, trypsinization was done and cells were cultured at the same density and transferred to the next passage till reaching senescence (Soleimani and Nadri 2009). MSCs were administered in a single dose of $1x10^6$ Cells/mouse.

MSCs immunophenotyping

Staining of Cells from the 4th passage with fluorescein isothiocyanate (FITC) rat anti-mouse CD90, CD 44, CD31 and CD 45 (BD Biosciences, San Jose, CA, USA) for 30 minutes, then final analysis using flow cytometer was done (Beckman Coulter Epics XL-MCL).

Experiment protocol

Pilot study

A pilot study was performed before proceeding this work aiming to; determine the route of MSCs administration either through hepatic or intravenous injection (I.V.) and ensure reaching of administered MSCs. to the liver.

250 microns of Labeled MSCs with red fluorochrome PKH26 (Sigma– Aldrich Co., USA) suspended in phosphate buffer solution (PBS) was injected in the tail vein of five mice and intrahepatically to another five mice 6 weeks p.i. with *S. mansoni* cercaria (50- 60 per mouse) which were injected subcutaneously. Mice were sacrificed at 14thweek p.i., and the livers were harvested and examined under a fluorescence microscope. The number of labeled BM-MSC was estimated after excision of parts of the livers, snap-frozen in liquid nitrogen and cut into 5 mm sections to be examined using a fluorescence microscopy (Yang *et al.*, 2009).

Statistical analysis was done and we found that there was no significant difference between intrahepatic and intravenous injection of MSCs as the route of infection, although intrahepatic route appeared to be more invasive and require more skilled personnel to minimize the suffering of animals thus in this study we depend on the intravenous injection of MSCs.

Mice were classified into; Group I; uninfected untreated, negative control, (NC, n=10), then the remaining infected mice (60 mice) were randomly categorized into; Group II: Infected untreated positive control group (PC, n=15), Group III: Infected, treated with PZQ at 6th week p.i. (PZQ, n=15), Group IV: Infected, received MSCs 6th week p.i. (MSCs, n=15) and Group V: Infected, treated at 6thweek p.i. with PZQ then received MSCs. And (PZQ+ MSCs, n=15). Cervical dislocation was done for all the mice 14thweek p.i. and blood was quickly gathered by heart's puncture. It was kept at room temperature for twenty minutes, there after samples were centrifuged at 2000 rpm for 15 minutes for collection of serum and were stored in aliquots at -80°C.

Parasitological and morphological parameters

Weighing the liver and spleen in addition to the body of each mouse was done. Liver and spleen indices were calculated according to the ratio of liver weight to whole body weight and spleen weight to whole body weight respectively. After mice scarification, the adult worms were perfused from the liver and porto-mesenteric vessels (Duvall and DeWitt 1967). Small portions of the liver and intestine were weighted, left overnight at 37 °C in five percent of KOH and calculated the mean number of eggs per gram of tissue (Cheever 1968).

Histopathological analysis

After the standard procedure, the liver portions of the euthanized mice were stained with hematoxylin and eosin (H&E). The specimens were fixed in ten percent formalin and then dehydrated before washing with xylene in ascending alcohol grades. In molten paraffin wax, the dehydrated tissues were embedded and then cut into thin sections. The sections were mounted on clean glass slides and stained with H&E. By using a low magnification power the number of liver granulomas was counted in randomly picked out five microscopic field/ liver sections for each mouse. Sections usually were seen per mouse. The mean number of egg granulomas in the liver section was calculated (Ali and Hamed, 2006).

Measuring the degree of hepatic fibrosis by Masson's trichrome staining & α SMA

Liver sections stained with Masson's trichrome were utilized to estimate the degree of hepatic fibrosis. Paraffinized liver samples were stained following the manufacturer's instructions using Masson's trichrome staining kit (Sigma-Aldrich, St. Louis, USA).

By applying the image J software program, version 1.47v, the percentage of fibrosis was calculated using photos taken for mice group liver slides. In liver tissues of all mice groups, α SMA immunostaining was performed using the standard avidin-biotin immunoperoxidase immunohistochemical method.

Measuring some biochemical markers for liver function& fibrosis

Assessment of liver function tests including alanine aminotransferase (ALT), alkaline phosphatase (ALP) and albumin was done using Beckman Coulter Olympus AU480 automated chemistry analyzer (Beckman Instrument Inc. Fullerton, California USA) in the serum of mice of all groups. Using Enzyme-linked immunosorbent assay (ELISA) the serum level of HA was detected according to manufacturer instructions (R&D System, Inc, USA).

Statistical data analysis

Data were analyzed using the SPSS program (Statistical Package for the Social Sciences, version 20 for Windows) software (SPSS Inc., Chicago, Illinois, USA). Our results were conferred as mean± standard deviation (SD). Percentage of reduction (PR) in mice treated groups = (C– T/C) x 100, where C refers to mean of IU mice group and mean of the treated mice group represented as T. Mann Whitney U test was used as a non-parametric measurement of data for comparison between the studied groups as pairs. P-value of less than 0.05 was considered a significant result.

Results and Discussion

World Health Organization (WHO), has listed seventeen diseases as ignored tropical disease, one of them is *Schistosomiasis* which has lethal sequelae resulting in liver fibrosis with a high rate of morbidity and mortality (Hotez *et al.,* 2014 &Vos *et al.,* 2016). Scientists found that MSCs therapy represents a new era in the management of chronically diseased liver which has been under research lately.

Mainly immune modulation, inhibiting fibrogenesis and transformation are the possible ways by which regeneration of liver occurs (Kang *et al.,* 2019).

Success in treatment with MSCs could substitute liver transplantation which appears to be the sole treatment of liver cirrhosis but lack of donor's, surgical producers complication and high cost restrict this choice of treatment (Zhang *et al.,* 2019).

Bone marrow MSCs isolation and culture

Successful isolation of MSCs from mice bone marrow was done. After seeding, on the 14th day elongated adherent cells were observed (Fig. 1). BM-MSCs showed high expression levels of adhesion marker (CD44) and typical mesenchymal markers (CD90) whereas they were negative for endothelial cell marker (CD31), Leukocyte common antigen (CD45).

Using fluorescent microscopy, labeled cells with PKH26 dye were reported in the liver cells which indeed ensure the presence of MSCs that have been transplanted. Anan *et al.,* (2016) and Hegab *et al.,* (2018), also used the same method to emphasize the presence of MSCs in liver tissue following transplantation.

Parasitological and morphological parameters

PC group have a lower weight compared to NC group. Mice weights were increased in all treated groups (24.4±2.7, 22.94±2.14 and 27.4±1.67 in PZQ, MSCS and MSCS&PZQ respectively) compared to infected untreated group (18.8±1.48), P-values were 0.02, 0.02 and 0.009 respectively (Table 1).

The liver index was significantly increased in infected mice compared to the uninfected group (P= 0.009) and after treatment, it showed no significant changes except in the MSCS group (P=0.03) compared to untreated mice. Mice weight in MSCs group was lower than combined group (BM-MSCs/PZQ) which may explain the significant difference in our study.

Regarding the splenic index, it was highly elevated in the infected group than in uninfected one (P= 0.009) which was followed by a significant reduction in all treated groups PZQ, MSCs and BM-MSCs/PZQ (P-value was 0.009, 0.047 and 0.009 respectively) (Table 1). Splenic index was decreased in the present work in BM-MSCs/PZQ treated group more effectively may be due to combined anti*Schistosoma*l with antifibrotic activity this is going with the results of Xu *et al.,* (2012) who also reported a significant reduction in splenic index after BM-MSCs based therapy (Table 1).

S. mansoni eggs were recognized in mice feces before starting treatment. The total worm burden recovered by perfusion of sacrificed animals was counted and analyzed. The mean worm count was statistically reduced in all groups compared to infected the untreated group with *P* = 0.008. MSCs treated group mean worm count was 7.6±0.89 followed by combined treated group 1.0±1.0 and lastly PZQ treated group with mean worm count of 0.8 ±0.82 however, it was 20.6±1.67 in the untreated group. Egg count per gram in the liver and intestine tissues was also reduced with a significant difference in all treated groups p value< 0.001(Table 2). These results were consistent to results obtained by El-Mahdi *et al.,* (2014), they stated that egg number correlated to severity of disease and is associated with either decrease with decreasing liver fibrosis. Percent of reduction in total worm count, egg count in liver tissue and egg count in intestinal tissue was higher in PZQ and combined treated groups than in MSCs alone treated group (Table 2), which could be explained by lack of direct anti*Schistosoma*l effect on adult worm compared to the effect PZQ treatment.

Serum liver biomarkers

Infected untreated mice showed a significant rise in serum ALT (95.0±23.95) than uninfected control (27.8±3.7) together with a significant decrease in serum Albumin levels (1.98 ±0.7) and increase in serum ALP (60.2±16.16). Levels of these biomarkers in serum were affected in different values after treatment regimens, as shown in Table 1. ALT level was decreased with PZQ therapy (40.2±2.8), with significant statistical difference compared with the infected untreated control (*P* = 0.009). A significant reduction in the ALT level (37.8±3.03) was reported in mice given PZQ/MSCs as well as MSCs only (38.8±2.59) *P* = 0.009. ALP level in serum decreased in the PZQ group (41.2±3.83), MSCs therapy (40.2±5.93) and combined PZQ/MSCs group (39.8±1.92). Moreover, a significant increase in albumin level was also noted in groups treated either with PZQ alone, MSCs alone or combined treatment referred to infected group (3.56±0.38, 4.05±0.39, and 3.86±0.06 respectively) (Table 1).

Table.1 Comparison between the studied groups regarding Lab tests

	The studied groups					U	P Value
	NC N = 10	PC N = 15	PZQ N = 15	MSCS N = 15	MSCS&PZQ N = 15		
WT						2.64	0.008[1]
X ±SD	26.2±0.76	18.8±1.48	24.4±2.7	22.94±2.14	27.4±1.67	2.41	0.02[2]
Range	25.5 – 27	0.17 – 0.21	20 – 27	20.8 – 25.4	26 – 30	2.31	0.02[3]
						2.63	0.009[4]
ALT						2.61	0.009[1]
X ±SD	27.8±3.7	95.0±23.95	40.2±2.8	38.8±2.59	37.8±3.03	2.61	0.009[2]
Range	23 – 32	61 – 120	38 – 45	36 – 42	34 – 42	2.61	0.009[3]
						2.61	0.009[4]
Albumin						2.63	0.009[1]
X ±SD	3.42±1.48	1.98 ±0.7	3.56±0.38	4.05±0.39	3.86±0.06	2.63	0.009[2]
Range	3.2 – 3.6	1.2 – 2.3	3.1 – 3.9	3.5 – 4.6	3.8 – 3.91	2.63	0.009[3]
						2.64	0.008[4]
ALP						2.62	0.009[1]
X ±SD	38.6±1.67	60.2±16.16	41.2±3.83	40.2±5.93	39.8±1.92	2.42	002[2]
Range	36 – 40	47 – 86	39 – 48	32 – 48	38 – 43	2.19	0.03[3]
						2.41	0.02[4]
Liver						2.61	0.009[1]
index	0.05±0.005	0.07±0.01	0.07±0.02	0.09±0.008	0.06 ±0.009	0.31	0.75[2]
X ±SD	0.04 – 0.06	0.07 – 0.09	0.05 – 0.09	0.08 – 0.11	0.06 – 0.08	2.19	0.03[3]
Range						0.94	0.35[4]
Spleen						2.63	0.009[1]
index	0.006±0.002	0.018±0.003	0.007±0.003	0.012±0.003	0.009 ±0.002	2.61	0.009[2]
X ±SD	0 – 0.01	0.01 – 0.02	0 – 1.0	0.01 – 0.02	0.01 – 0.01	1.99	0.047[3]
Range						2.62	0.009[4]

Liver index= Liver/Wt.
Splenic index= Spleen/ Wt.
U= Mann Whitney U test
1= Comparing negative control with positive control group; 2=Comparing PC with PZQ;
3=Comparing PC with MSCS; 4= Comparing PC with MSCS&PZQ

Table.2 Comparison between the studied groups regarding egg and worm count

	The studied groups				U	P Value
	PC N =15	PZQ N = 15	MSCS N = 15	MSCS&PZQ N = 15		
Egg count/gram intestine					2.61	0.009[1]
X ±SD	18429.4±4269.2	1514.6±392.6	6845.0±2147.0	1617.4±326.5	2.61	0.009[2]
Range	12345 – 23546	1109 – 2123	4356 –9765	1190 – 1979	2.61	0.009[3]
PR		91.78%	62.86%	91.22%		
Egg count/gram liver					2.61	0.009[1]
X ±SD	16485.8±4666.7	1382.4±378.7	6038.8±786.7	1441.8±265.4	2.61	0.009[2]
Range	11234 - 21765	987 – 1917	4963 – 7102	1132 – 1754	2.61	0.009[3]
PR		91.61%	63.37%	91.25%		
Total worm count					2.64	0.008[1]
X ±SD	20.6±1.67	0.8 ±0.82	7.6±0.89	1.0±1.0	2.66	0.008[2]
Range	19 – 23	0 – 2	7 – 9	0 – 2	2.64	0.008[3]
PR		96.12%	63.11%	95.14%		

U= Mann Whitney U test; 1=Comparing PC with PZQ; 2=Comparing PC with MSCS; 3= Comparing PC with MSCS&PZQ; PR= Percent of reduction

Table.3 Comparison between the studied groups regarding histopathological parameters

	The studied groups				U	P Value
	PC N = 15	**PZQ N = 15**	**MSCS N = 15**	**MSCS&PZQ N = 15**		
Mean numberof granuloma	13.62±3.24	6.9±0.16	17.08±3.61	4.28±1.82	2.61 1.26 2.61	0.009[1] 0.21[2] 0.009[3]
X ±SD	11.2 – 19.2	6.7 – 6.9	11.5 – 20.4	1.3 – 6		
Range						
Mean size of granuloma	272.2±11.17	265.2±0.0	254.9±70.65	243.64±58.8	0.56 0.52 0.31	0.56[1] 0.60[2] 0.73[3]
X ±SD	261 – 286	265.2 – 265.2	177.6- 360.2	188.2 – 314.2		
Range						
Percent of fibrosis by Masson trichrome stain	19.51±3.48	14.80±6.94	12.3±0.0	12.63±1.97	1.27 2.59 2.40	0.21[1] 0.005[2] 0.02[3]
X ±SD	15.17 – 23.14	5.35 – 24.24	12.3 – 12.3	10.65 –15.58		
Range						
H-score of SMA/300	100.0±30.82	65.0±15.81	32.0±14.4	15.0±0.0	2.01 2.61 2.80	0.045[1] 0.009[2] 0.005[3]
X ±SD	70 – 140	50 – 90	15 – 50	15 – 15		
Range						

U= Mann Whitney U test
1=Comparing PC with PZQ
2=Comparing PC with MSCS
3= Comparing PC with MSCS&PZQ

Table.4 Hyaluronic acid level in different studied groups

		The studied groups				U	P Value
	NC N = 10	**PC N = 15**	**PZQ N = 15**	**MSCS N = 15**	**MSCS&PZQ N = 15**		
Hyaluronic acid	10.74±1.18	44.6±14.84	35.0±5.96	25.2±4.20	22.4±2.7	2.88 0.95 2.51 2.61	<0.001[1] 0.34[2] 0.01[3] 0.009[4]
X ±SD	9 – 12	30 – 65	30 – 45	20 – 30	18 – 25		
Range							

U= Mann Whitney U test
1= Comparing negative control with positive control group
2=Comparing PC with PZQ
3=Comparing PC with MSCS
4= Comparing PC with MSCS&PZQ

Fig.1 MSCs appearing as spindle shaped fibroblast-like under inverted tissue microscope

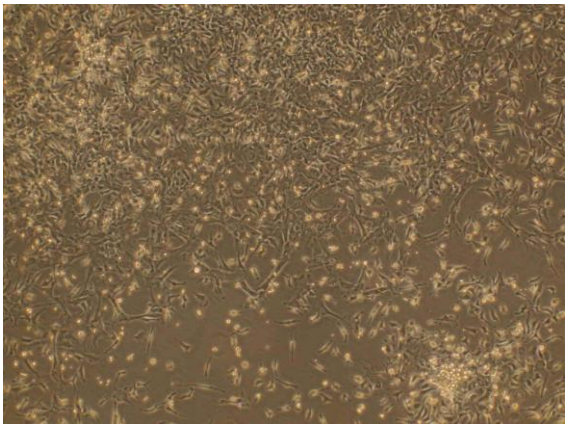


Fig.2 Section of liver tissue treated with PZQ showing multiple variable sized and shaped bilharzial granuloma (circles) with central calcified bilharzial ova surrounded by dense fibrosis and chronic inflammatory infiltrate (H&E x100)

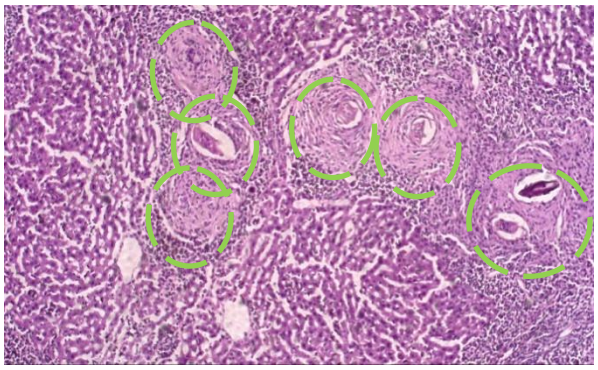


Fig.3 A; Section of liver tissue of PZQ group showed multiple bilharzial granuloma with areas of fibrosis highlighted by Masson trichrome stain (Masson trichrome x100). **B**; SMA immunostaining of the same group showed moderate fibrosis and mesenchymal stromal cells (SMA x100)

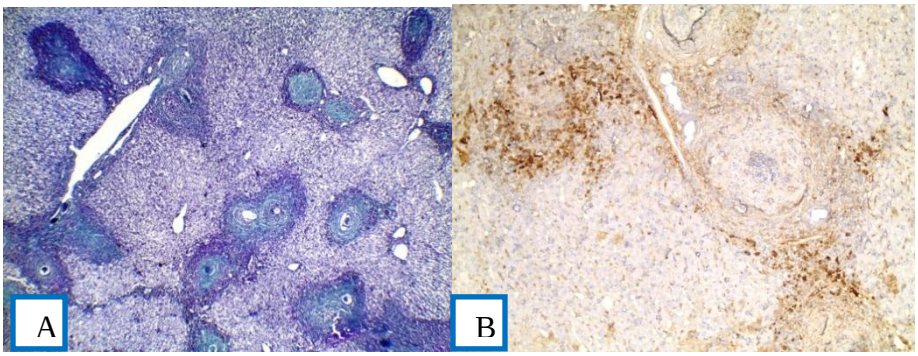


Fig.4 A; Section of liver tissue of positive control group showed large number of bilharzial granuloma with wide areas of fibrosis highlighted by Masson Trichrome stain (Masson Trichromax100). **B;** α SMA immunostaining of the same group showed high percent of fibrosis and mesenchymal stromal cells (α SMA x100)

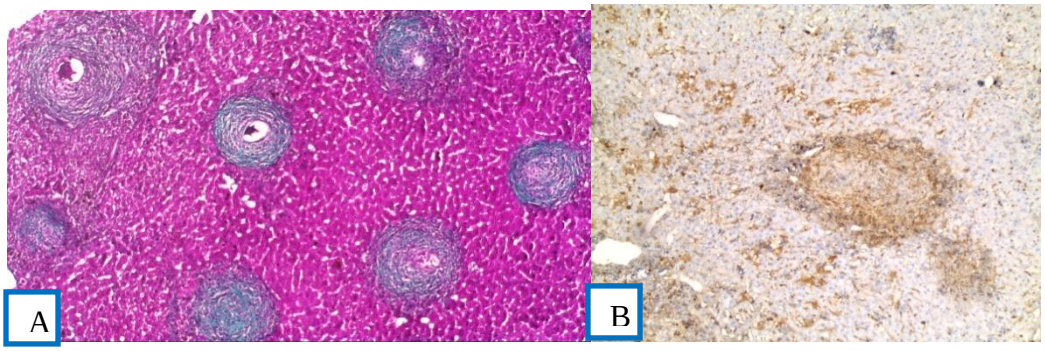


Fig.5 A; Section of liver tissue of MSCs group showed large number of bilharzial granuloma with scattered areas of fibrosis highlighted by Masson Trichrome stain (Masson Trichromax100). **B**; α SMA immunostaining of the same group showed granulomas with moderate fibrosis and many mesenchymal stromal cells (αSMA x100)

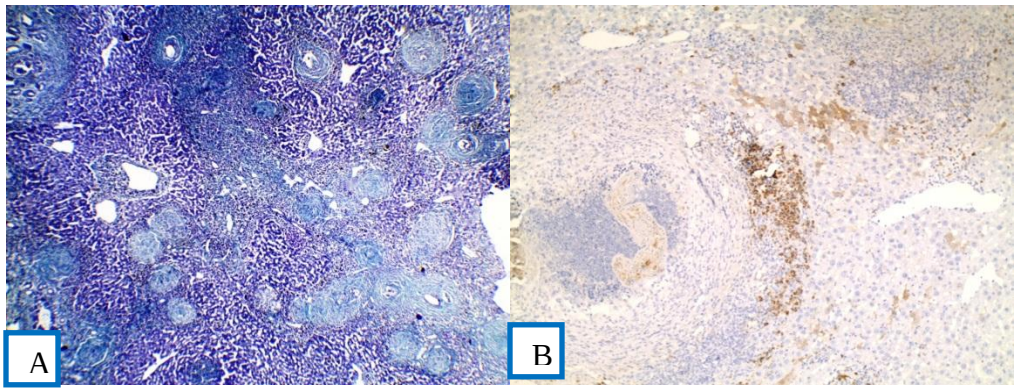


Fig.6 A; Section of liver tissue of (MSCS & PZQ) group showed few number of bilharzial granuloma with small areas of fibrosis highlighted by Masson trichrome stain (Masson Trichromax100). **B;** α SMA immunostaining of the same group showed moderate fibrosis and mesenchymal stromal cells (α SMA x100)

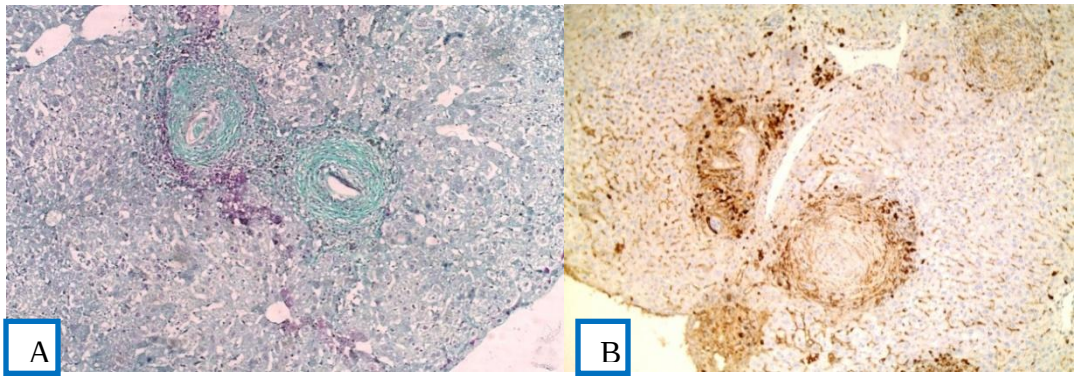


Fig.7 Showing HA level between different studied groups

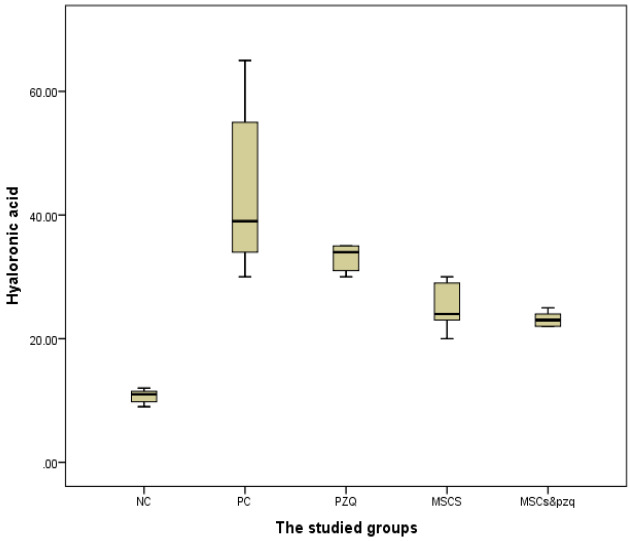
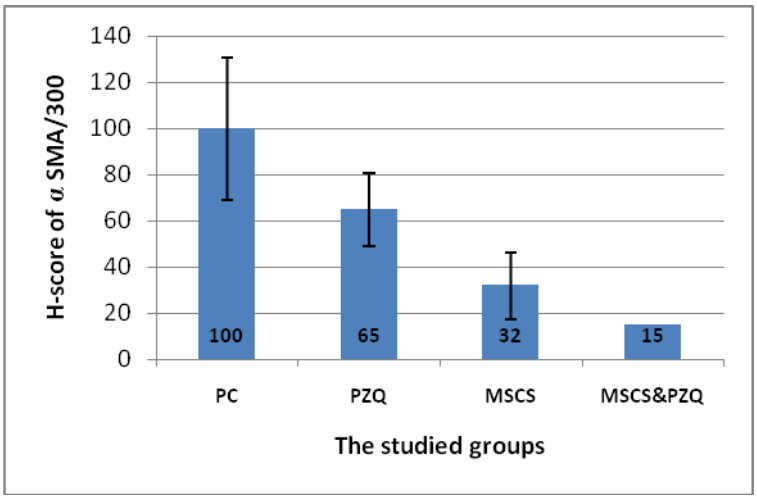


Fig.8 Comparison between studied groups regarding α SMA



In the current study level of alanine transaminase (ALT), alkaline phosphatase (ALP) and albumin were assessed as functional parameters of the injured liver. Hanna *et al.*, (2003) also reported injury of liver tissue as a result of *S. mansoni* infection which was accompanied by an increase in transaminases enzymes and decrease albumin level in serum**.** El-Rigal and Hetta (2006), referred the increase in liver enzymes to the cellular injury of the liver following deposition of eggs. El-mahdi *et al.*, (2014) also, measured liver injury, as consequence to infection by *Schistosomiasis*, by elevation of ALT, ALP and decrease in albumin noting the reactive inflammatory response to infection. A significant decrease in ALT, significant increase in ALB and a significant decrease in ALP was recorded following treatment with BM-MSCs (Table 1). These result were in accordance to Mehrabani *et al.*,(2019) finding who found that the level of ALT was decreased signifying the effect of BMSCs on liver injury repairing in a study used the rat as an animal model.

Measuring *Schistosoma*l liver fibrosis

Histopathological examination revealed that the number of granulomas was significantly reduced in PZQ and PZQ/MSCs treated groups (6.9±0.16 and 4.28±1.82), while the increase in granuloma number was recorded in MSCs treated group (17.08±3.61) compared to untreated mice (13.62±3.24) (Fig. 2). Considering the mean size of granuloma, there was a non-statistical significant difference between different groups with the smallest granuloma size was represented in PZQ/MSCs (243.64±58.8), followed by MSCs (254.9±70.65) and larger granuloma in PZQ treated group (265.2±0.0). Using Masson trichrome staining, percent of fibrosis was reduced in MSCs and PZQ/MSCs treated groups (P = 0.005 and 0.02 respectively)while in PZQ treated group it was reduced but statistically not significant (P = 0.21). Immunostaining by using α SMA revealed that fibrosis was reduced in MSCs (32.0±14.4; P= 0.009), PZQ/MSCs (15.0±0.0; P= 0.005) and in PZQ treated group (65.0±15.81; P=0.045) compared to infected untreated mice (Fig. 3, 4, 5, 6 and Table 3).

Regarding histopathological results, significant recovery of fibrosed liver tissue was observed using H&E which was manifested by decrease granuloma number and which was measured by some authors before. They noted the same effect of MSCs on granuloma (Aziz *et al.,* 2007)**.** Also, Mohammed *et al.,* (2014) noted the improvement of liver architecture after the use of MSCs. Collagen deposition and fibrosis of the liver were assessed using Masson Trichrome stain with the resultant formation of blue-stained collagen fibers. Our work stated that collagen fibers amount was markedly increased in *S. mansoni* infection and significantly decreased after the use of PZQ and MCSs (Table 3).

Wu *et al.,* (2018), observed that the reduction of fibrosis was accompanied by a lower number of activated HSCs which are the main cells responsible for creating ECM components in the liver, and must be activated and then differentiated into a myofibroblast-like cell.

In addition to histopathological work up immunostaining marker alpha-smooth muscle actin was investigated to detect degree of fibrosis and to analyze the method by which MSCs alleviate fibrosis (Fig. 8). Jang *et al.,* (2015) found that decrease in the number of α-SMA+ cells in liver tissues treated with BMMCs was probably due to modulation of HSCs by specific cytokines and growth factors, including TGF-β1, TNF-α, and ROS, which resulted from liver injury and mediated by the autocrine and paracrine signaling activation of HSCs. Zhang *et al.,* (2019) found that BM-MSCs inoculation diminished α-SMA expression in liver tissues.

Measuring serum level of HA in our study revealed that serumhyaluronic acid was significantly elevated in infected untreated mice than uninfected ones (P=<0.001).

Comparing between treated groups and untreated mice showed that no statistical significant difference between infected untreated mice and PZQ group (P= 0.34), however, MSCS group [P=0.01] or MSCS & PZQ group [P=0.009] showed statistically significant lower levels of hyaluronic acid when compared with infected untreated mice (Table 4 and Fig. 7).

HA is considered a strong noninvasive marker of the grade of liver fibrosis (Popper and Kent, 1975 &Shigemori *et al.,* 2002). We examined HA titers in mice following *S. mansoni* infection and the association between the HA titers and the liver fibrosis degree was assessed. Our results showed that liver fibrosis

were positively correlated with the serum level of HA in mice infected with *S. mansoni.* other studies confirmed this finding, the concentrations of serum markers of liver fibrosis were significantly lower in the infected mice with *S. jabonicum* and treated with MSCs than those in the untreated infected mice which confirmed the anti-fibrotic effects of the administration of stem cells (Qiu *et al.*, 2014) (Table 4).

This study has experimentally explored the role of MSCs in the alleviation of liver fibrosis induced by *Schistosoma mansoni* (Egyptian strain) infection. Moreover, it highlights the use of HA as a marker for evaluating fibrosis giving nearly the same results reported by immunostaining and histopathological examination which facilitate the coming researches in noninvasive measuring of fibrosis.

Acknowledgment

The authors would like to thank Dr/ Mohamed Salah (animal house department) and Dr/ Mohamed ElZallat (stem cell department) TBRI for their helpful assistance through the work. No assistance was received in writing.

Financial support

This work received funding from Menoufia university project research unit.

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How to cite this article:

Marwa Ahmed Gouda, Asmaa Shams El Dein Mohamed, Fatma Eldesoky Ahmed, Ghada Samir Amer and Dalia Shafey. 2019. Antifibrotic Alleviative Effect of Bone Marrow-Derived Mesenchymal Stem Cells on Experimentally induced *Schistosoma mansoni* Related Liver Fibrosis. *Int.J.Curr.Microbiol.App.Sci.* 8(12): 1136-1151.
doi: https://doi.org/10.20546/ijcmas.2019.812.143