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Effect of ozone/oxygen gas mixture on bilharzial hepatic fibrosis induced in infected mice

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Abstract

Aim: on check whether ozone/oxygen gas mixture has an anti-inflammatory or anti-fibrotic effect in mice infected with Schistosoma mansoni.

Methods: This work was done on a total of 40 Swiss albino mice infected at 7 weeks of age with an average of 50 cercariae of Schistosoma mansoni. Twenty mice were given ozone treatment via the rectal route for 7 weeks starting from week 15, while the other twenty were used as controls. Both treated and untreated groups were sacrificed at 22 weeks. Physiological parameters, parasitological parameters together with histological and morphometric parameters were compared in both treated and untreated groups.

Results: At the end of therapy, the mean body weight increased, and the weight of the spleen diminished in the treated group but insignificantly, while the weight of the liver did not indicate change in both groups. As regards parasitological parameters worm load demonstrated a highly significant decrease ($P < 0.001$) where the number of couple worms decreased from 2.6 to 0.6, while the total worm burden decreased from 11.2 to 8.29 (percent reduction 25.98 %) on ozone treatment. The Oogram % showed a highly significant reduction in the number of immature ova in the treated group 14 versus 18.25 in the control group. Mature ova decreased from 26.75 to 23.71 and the number of dead ova increased from 57.5 to 62.9 on ozone treatment. As regards the histogram the number of ova per gram tissue of intestine showed highly significant decrease from 7146 to 3607($p<0.001$), while the number of ova per gram liver tissue decreased significantly from 7850 to 5964($P<0.05$). The total ova count in tissues decreased from 14996 to 9671. As regards the histological and morphometric parameters, there has been a highly significant decrease in the number and size of granulomata ($P<0.0001$) where the number of granulomata dropped from 45.0 to 35.0 and the diameter from 233.25 to 133.55 ($P< 0.0001$). There has been a highly significant reduction in the amount of collagen on Masson trichrome stain, where the fibrosis percent was 28.6 % in the treated group compared to 49.85 % in the control group ($P<0.0001$). The number of degenerated ova in the treated group increased significantly to 69.4 compared to 17.75 in the control group ($P<0.0001$).

Conclusion: Ozone/oxygen gas mixture has a strong anti-parasitic effect against all stages of Schistosoma mansoni parasite in mice. This effect is possibly through immune modulation by augmentation of Th2 immune response. It has a significant effect on granuloma number and size together with a significant effect on percent of fibrosis through this antiparasitic-immunomodulation effect and possibly also through a direct anti-inflammatory and anti-fibrotic effect.

Keywords: bilharzial granuloma, bilharzial fibrosis, immune modulation, ozone/oxygen gas mixture, Schistosoma mansoni.

Introduction

Liver fibrosis results from sustained injury, which can be inflicted by various factors such as viruses, drugs, alcohol, metabolic diseases, and autoimmune attacks [1]. it is characterized by the formation and deposition of excess fibrous connective tissue, leading to progressive architectural tissue alteration [2].Removing the causative agent is currently the most effective way to treat liver fibrosis. When instituted at early stages, it can reverse hepatic fibrosis, leading to the repair of normal liver histology. This strategy is effective in iron and copper overload, alcohol consumption, chronic hepatitis B virus or hepatitis C virus (HCV) infection, autoimmune liver disease, schistosomiasis, secondary biliary obstruction, and drug-induced liver disease [3, 4]. Most of these agents induce hepatocellular injury and subsequently hepatic inflammation, finally resulting in hepatic stellate cell (HSC) activation and collagen deposition. However, some factors (i.e., alcohol metabolites, ferritin, and bile acids) may directly stimulate HSCs and enhance their fibrogenic potential [5].

Schistosomiasis is a waterborne parasitic disease that threatens millions of people worldwide [6] and the second most prevalent common parasitic infection after malaria [7] with approximately 200 million infected and in sub-Saharan Africa alone, it is estimated that 200,000 die each year as a result of the infection [8, 9]. This infection is endemic in tropical and subtropical regions of Africa, Asia, the Caribbean, and South America [10]. Chronic infections can lead to significant morbidity including kidney damage, anemia, malnutrition, and infertility and growth impairment (Cribb et al. 2018). Schistosoma mansoni inhabits the mesenteries around the intestine.

Schistosomal involvement of the liver is an excellent model of the study of immunologic liver injury, fibrosis and hemodynamic disturbances in the absence of parenchymal injury [12]. The term Schistosomal cirrhosis is no longer used, as nodular renewal and diffuse distortion of hepatic lobular architecture are not the features of hepatic schistosomiasis. The parenchyma between fibrotic areas is typically well-maintained, correlating with the repairs of nearly normal hepatic function, one of the clinical hallmarks of hepatosplenic schistosomiasis (HSS) [13] .

The fact that the ozone is a potent oxidant accounts for its high reactivity. Ozone is simply an energized form of oxygen. It is created when electric or ultraviolet energy causes oxygen atoms to temporarily recombine in groups of three. The result is an unstable, highly reactive molecule. This gas dissolves in the plasma, extracellular fluids, the thin layer of water which covers the skin and in regions coated by mucosa, prompting reactions with a number of molecules present in biological fluids, such as antioxidants, proteins, carbohydrates, and, mainly, with polyunsaturated fatty acids. By these reactions, reactive oxygen species (ROS) and lipid oxidation products (LOPs) are generated, both responsible for successive and multiple

biochemical reactions that occur in different body cells [14].

[15], reported protection against hepatic cellular damage induced via chloroform (CCL4) in an animal model by ten rectal insufflations of ozone before CCL_4 application. This protection occurs through the activation of antioxidative enzymes and radical scavengers [16].

Medical ozone is more bactericidal [17], fungicidal, and virucidal than any other natural substance. Some studies proved that ozone infused into donated blood samples can kill viruses 100% of the time. Ozone, because of its special biologic properties, has theoretical and practical attributes to make it a potent hepatitis C virus (HCV) inactivator, which suggests an important role in the therapy for hepatitis C [18] and is effective in patients with chronic hepatitis B [19]. [20], after a study in 141 hepatic patients concluded that ozone oxygen gas mixture with and without anti-oxidants is effective and safe in the treatment of hepatic fibrosis due to chronic viral hepatitis C.

[21], in an electron microscopic study on 26 patients of chronic hepatitis C given ozone therapy for 12 weeks, observed regression of hepatocyte apoptosis with diminution of hepatic stellate cells despite the continuation of viral replication. (Thabet et al., 2007) pointed to the importance of medical ozone as a promising agent to complement schistosomiasis mansoni specific treatment in mice, helping to attenuate infection morbidity.

Basic research from Italy [23] and [24] had led to a confirmation that ozone therapy modulated immune system by balancing its inflammatory/anti-inflammatory cytokines, increased the production of red blood cell (RBC) 2,3 diglycerophosphate (DGP) (greater oxygen release), and improved rheology properties of blood (increased RBC flexibility), and elevated of key antioxidant enzymes such as superoxide dismutase (SOD), and increased glutathione, achieving a redox cell balance.

Aim of the Work

The aim of this work is to study the effect and safety of ozone/oxygen gas mixture on the different stages of infection of schistosomiasis in mice. Also to see if Ozone-Oxygen Gas Mixture has an anti-inflammatory or anti-fibrotic effect in bilharzial hepatic fibrosis induced in infected mice.

Materials and Methods

Experimental Animals

Laboratory inbred CD1albino mice, aged 6-7 weeks and weighing 18-20 gm at the beginning of the experiment, were used in this study. Mice were bred on standard diet with free access to water at the Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Giza, Egypt.

Experimental Design

Forty Swiss albino mice are going to be included in this study and are going to be divided into 2 groups, group I or control group formed of 20 mice is infected by an average of fifty cercariae of Egyptian Schistosoma mansoni type, and sacrificed after 22weeks without treatment with ozone and group II which is infected by the same number of cercariae and given ozone at the 15^{th} week of infection and sacrificed at the 22^{nd} week. Ozone was taken rectally daily for 10 successive days and then twice weekly for the rest of the 7 weeks course of treatment.

Infection of Mice

An Egyptian strain of S. mansoni cercariae was used in all the experiments. The strain was maintained by a laboratory passage in an Egyptian strain of Biomphalaria Alexandrina snails (provided by the

Biological Unit of Theodor Bilharz Institute Cairo, Egypt).Infection was done by subcutaneous injection of mice with 50±5 S. mansoni cercariae suspended in 0.2 ml solution. Mice were infected subcutaneously according to the method of [25]. All the animal experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals and were approved by the Institutional Review Board of TBRI.

Parasitological parameters

Worm load and distribution [26]**.**

Worm counting

The perfusate was transferred into a Y-shaped flask and left for about 5 min. until all worms were settled on the bottom of the flask. The free supernatant was decanted and the settled worms were washed 3 times with 0.85% phosphate buffer saline (pH 7.4). The washed worms were transferred into divided Petri-dish and were counted using a stereomicroscope [27].

The degree of protection or the percentage of reduction in challenge to a live infection was calculated as follows: P = C – V / C x 100,Where: P is the percentage of protection, C is the mean number of parasites recovered from infected mice and V is the mean number of the parasites recovered from infected and treated mice. [28].

Ova count

The number of eggs/g liver and intestine tissue was assessed following digestion with 4% KOH according to the method of [29].

The Oogram pattern

The egg developmental stages in the small intestines of mice was evaluated according to [30].

Histopathological studies [31]**.**

After the sacrifice of animals, part of the liver from each animal was removed and immersed in 10% buffered formalin solution. Livers were kept in separate number small glass bottles. Livers were then embedded in paraffin, and sectioned. Five sections (5 microns in thickness) were taken from each liver specimen, each section being at a distance of at least 500 μm from the preceding one. Sections were stained with hematoxylin and eosin (H&E) and Masson trichrome.

Measurement of liver granuloma

Measurements were done only for liver lesions containing single eggs in their centers. The mean diameter of each liver granuloma (30/mouse) was measured in microns, from two diameters of the lesion taken at right angles to each other with the help of an ocular micrometer. Egg viability was assessed microscopically in the same liver sections and cell composition of granulomas was also investigated. First, the greatest diameter of the lesion was obtained, then the ocular micrometer was rotated 90 degrees and the diameter perpendicular to the first one was measured [31].The collagen content of the liver tissue sections was evaluated after staining with picrosirius red. According to [32], lesion counts between 50-100 were taken into consideration. Lesions from 6-7 animals were measured in each group. The volume of each liver granuloma was calculated from the mean diameter of each lesion on the assumption that they were spherical.

Statistical analysis

Statistical analysis was performed using the SPSS.9 software computer program. Results were expressed as means ± standard deviation of the means (SD), one way ANOVA and LSD test was used for multiple group comparison. Data were summarized also using cross-tabulation. Statistical significance was established at p-value = $p< 0.05$. (Data that were considered to be significantly different were reported at probability levels of $P<0.05$ or $P<0.005$, as indicated.)

Results

This work was done on a total of 40 Swiss albino mice infected with an average of 50 cercariae of Schistosoma mansoni for 15 weeks. Twenty given ozone treatment via the rectal route while the other twenty were used as controls. The results were as follows

Physiological parameters

The mean body weight increased insignificantly in the treated group than the untreated group. The mean bodyweight of the treated group was 27.63 gm while the mean bodyweight of the untreated group was 26.7 gm.

No change in liver weight was detected between the treated and untreated groups. (2.80 gm versus 2.83). The mean weight of the spleen decreased insignificantly from 0.65 gm in the untreated group to 0.59 gm in the treated group (Table 1, figure 1)

Parasitological Parameters

A-Worm load

The number of female worms 3.0 versus 2.64 which was statistically insignificant. The number of male worms increased by 3.0 versus 4.36 which is also statistically insignificant. From the correlation table, there is a significant negative correlation between the number of male separate worms and the number of granulomas (P< 0.03) (table 5). The number of couple worms decreased, where the control group was 2.6 versus 0.64 in the treated group, which is statistically highly significant (P < 0.001). Total worm burden decreased from 11.2 to 8.29 which is highly statistically significant (percent of reduction 25.98 %) (Table1, figure 2).

B- Oogram pattern in intestine

Regarding Oogram there was a highly significant reduction in the number of immature ova in the treated group compared to the control group. The number of immature ova was 18.25 in the control group, while the number of immature decreased to 14.0 on treatment with ozone (P < 0.001). The number of mature ova was 26.75 in the control group and dropped to 23.71 in the treated group which is statistically significant (P < 0.05). The number of dead ova increased significantly from 57.5 to 62.9 which is statistically significant (<0.01) (Table 2, figure 3). From the correlation table, there is a positive correlation between the immature and mature ova and % of fibrosis (Table 5).

C-Ova count in hepatic and intestinal tissues

Regarding the number of ova per gram infected tissue, the number of ova per gram tissue of the liver was significantly reduced on treatment, while the number of ova per gram tissue of intestine was highly significantly decreased on treatment. The number of ova /gram tissue of the liver dropped from 7850 in the control group to 5964 in the treated group (P <0.05). The number of ova /gram tissue of intestine dropped from 7146 in the control group to 3607 in the ozone-treated group (P<0.001).The total ova count in tissue reduced from 14996 to 9571(Table3, figure 4). From correlation table there is a positive correlation between the number of ova /gram tissue of intestine and the number of granulomata, whereas a negative correlation was shown between the number of ova/gram tissue of intestine and number of degenerated ova. (Table 3).

Histological and morphometric studies

There is a highly significant decrease in the number of granulomata in the treated group compared to the controls (Table 4, figure 5).The number of hepatic granulomas dropped from 45.0 to 35.0 which is highly significant (P-value < 0.0001).

There is a highly significant decrease in the size of the granuloma in the treated group compared to the

controls (Table 4, figure 5). The diameter of granuloma dropped from 233.25 to 133.55 with P-value < 0.0001 .

There is a highly significant positive correlation between the size and the number of granulomata P < 0.001 .

There is a highly significant positive correlation between the granuloma diameter and % of fibrosis P < 0.001

There is a highly significant negative correlation between degenerated ova and the number of granulomata P < 0.007

There is a significant positive correlation between the % of fibrosis and the number of granulomata (P<0.05) (see table 5)

On Masson trichrome stain (Table 4, figure 5), there has been a highly significant reduction in the amount of collagen as determined by the percent of fibrotic areas in the field of the treated compared to the non-treated control group. The fibrosis % was 49.85 in the control group and dropped to 28.6 in the treated group (P< 0.0001).

Regarding the number of degenerated ova in the hepatic granuloma (Table 4, figure 5), there has been a highly significant increase in the number of degenerated ova from 17.75 in the non-treated control group to 69.4 in the group treated with ozone with a p-value < 0.0001.

Table (1): The effect of ozone on the mean weight of mice, liver, spleen and worm burden in mice infected with ± 50 S. mansoni cercariae, 15 weeks post-treated and sacrificed at 22 weeks after infection.

Animal group	Weight of mice	Weight of liver	Weight of spleen	Mean worm burden ± SE (liver and portomesentric vein)			Total worm burden	% reduction
				Male	**Female**	Couple		
Normal(n= 10)	34±1.4	2.8±0.6	0.25±0.1	0	0	0	0	
Infected(n =20)	26.7±3.8	3.0±0.4	0.65±0.3	3±1.1	3±1.1	2.6 ±0.7	11.2±1.8	0
Infected and ozone treated (n=20)	27.6±7.2	2.8±0.5	0.59±0.7	4.4±2.7	2.6±0.7	0.6±0.7 ***	8.3±2.0 ***	26

***denotes statistically significant difference from infected control at P<0.001.Results are expressed as mean (M) ± standard error (SE) of animals.

Table (2): The effect of ozone on Oogram, the number of ova per gram tissue (liver and intestine) in mice infected with ±50 S. mansoni cercariae, 15 week post-ozone treatment and sacrificed 22 weeks after infection.

Animal group	% ova developmental stages ± SE			% Reduction	Number of ova/gm		ova count in tissue	% Reduction
	Immature ova	Mature ova	**Dead ova**		Liver	Intestine		
Control infected	18.3 ±2.4	26.8 ±2.4	57.5± 3.8		7850±2476	7146±1725	14996+4201	
Infected ozone treated	14±2.7 ***	23.7±3.5 *	62.3±4.7 **	- 8.3	5964±1805 *	1229±3607 ***	9571+3034	36.18

Values are expressed as the mean number (M) ± standard error (SE) of twenty animals. *** denotes statistically highly significant difference from infected control mice at P<0.001, ** P <0.01 and * P<0.05.

Animal group	No. of granuloma in 5 successive power fields	Mean granuloma diameter in µm	Fibrosis %	Ova Degenerated
Control infected	45.1500±1.0495	233.2500±8.4314	49.8500±3.1575	17.7500±1.6589
Infected ozone treated	35.7500±.9013 ***	133.5500±6.2763 ***	28.6000±2.3857 ***	69.4000±3.2658 ***

Table (4):The effect of ozone on the mean number, diameter of hepatic granulomas,% of fibrosis, and ova degenerated in mice infected with ±50 S. mansoni cercariae, 15week post-ozone and sacrificed 22 weeks after infection

Values are expressed as mean number (M) ± standard error (SE) of twenty animals. *** Statistically highly significant difference from infected, control mice at P<0.0001.

(Table 5): Correlations between parameters.

Parameter	R	P
Granuloma diameter Vs N° of granuloma	0.478**	0.001
Granuloma diameter Vs % of fibrosis	0.466**	0.001
Granuloma diameter Vs immature ova.	0.415**	0.008
Granuloma diameter Vs degenerated ova	-0.640**	0.0001
N° Of granuloma Vs degenerated ova	-0.421**	0.007
N° Of granuloma Vs male worms	-0.334*	0.035
N° Of granuloma Vs ova/(g) intestine	0.407**	0.009
% of fibrosis Vs degenerated ova	-0.553**	0.0001
% of fibrosis Vs immature ova	0.438**	0.005

% of fibrosis Vs mature ova	0.421**	0.007
Degenerated ova Vs immature ova	-0.501**	0.001
Degenerated ova Vs mature ova	-0.475**	0.001
Degenerated ova Vs Ova/(g) intestine	-0.438**	0.005
Immature ova Vs mature ova	0.315*	0.048
Immature ova Vs dead Ova	-0.379*	0.016

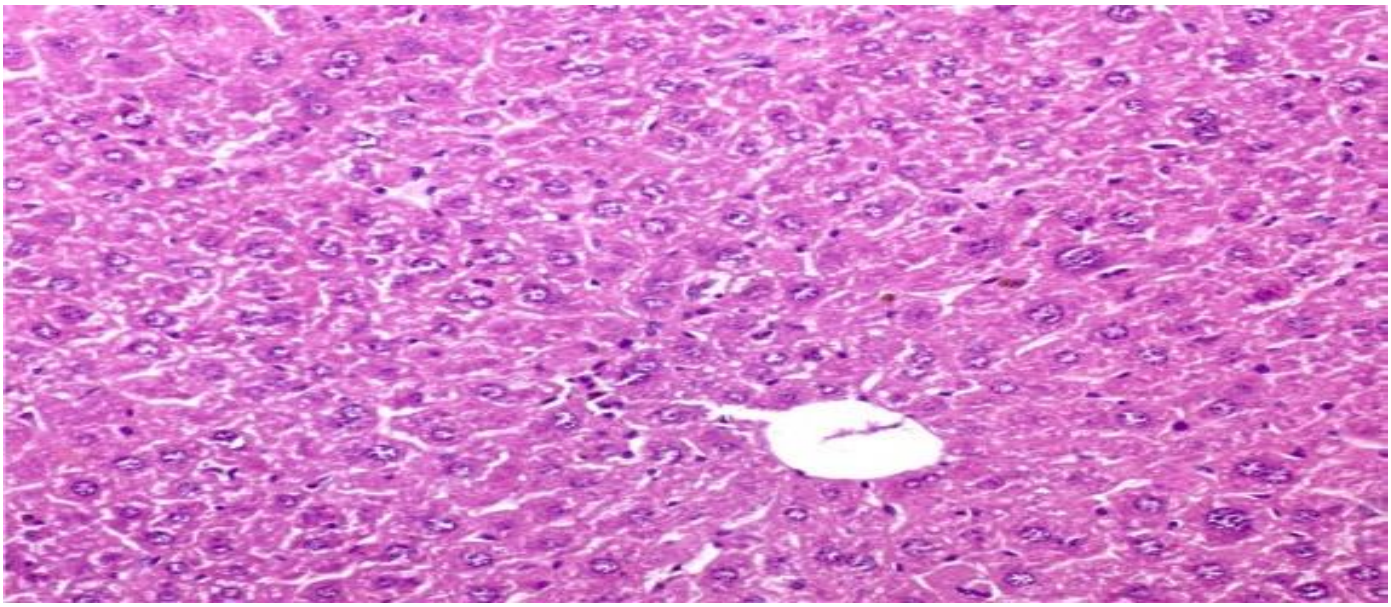

Fig.1: .Liver section from normal control mice. (H&E X400).

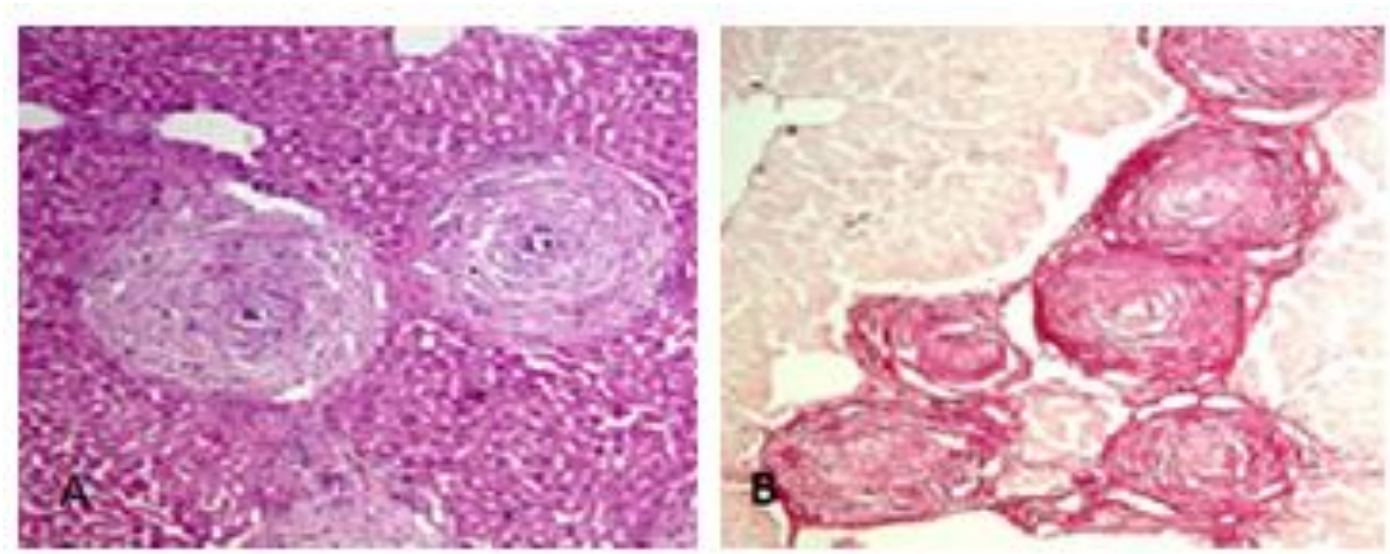

Fig. 2. A: Liver section from infected mice with S.mansoni (22 weeks post-infection) showing large fibrocellular granuloma with central active miracidia. (H&E X 200).

Fig.2. B: Liver section from mice infected with S.mansoni (22 weeks post-infection) showing large areas of fibrosis (red) representing granuloma fibrosis (Sirius Red X 200).

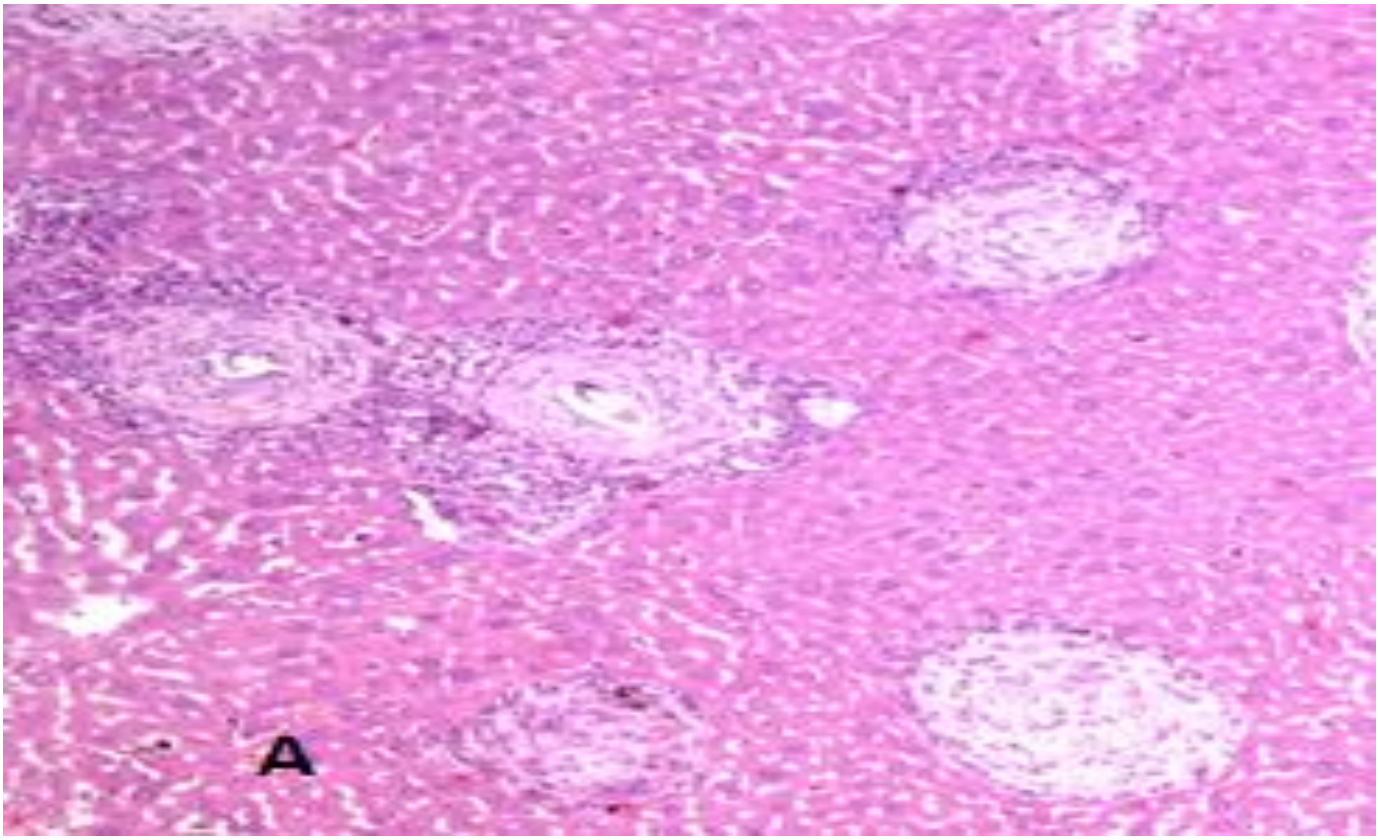

Fig.3.A: Liver section from mice infected with S.mansoni and treated with ozone gas at 15 weeks post-infection and sacrificed at 22 weeks post infection showing a small fibrocellular granuloma with degenerated ova (H&E X 200).

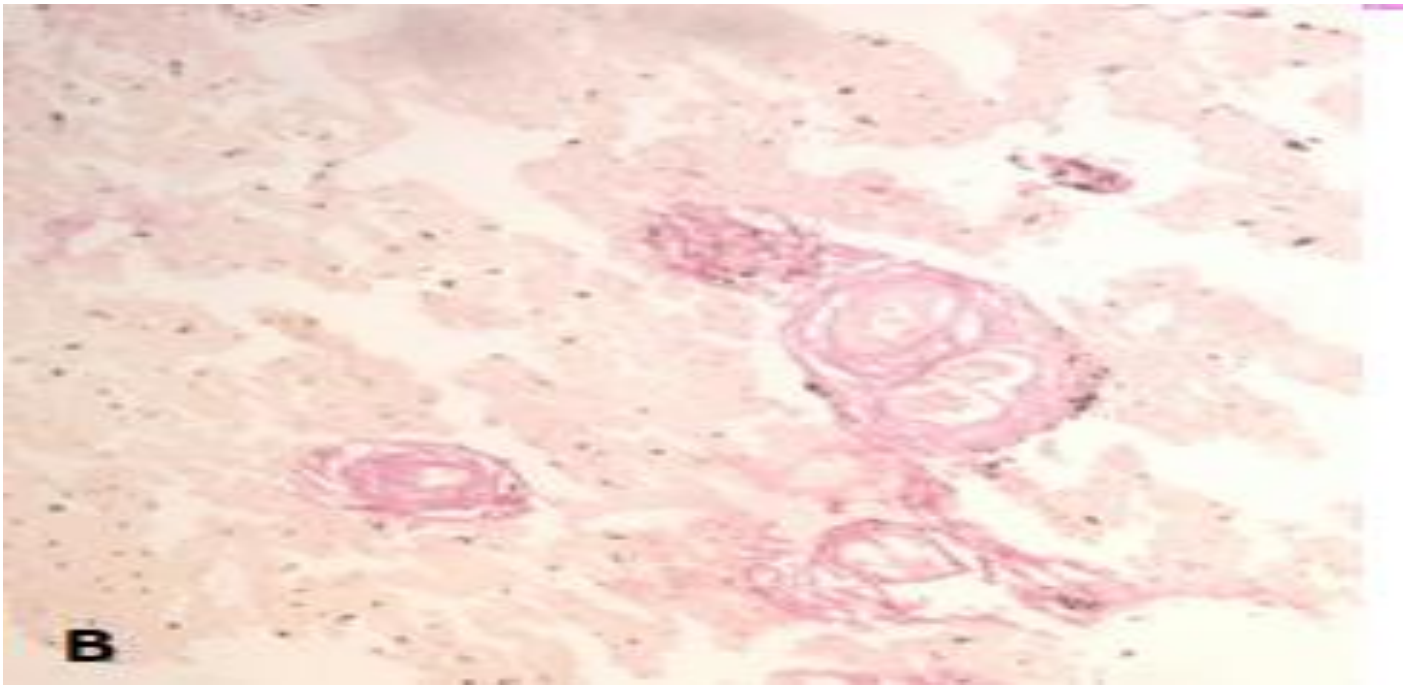

Fig.3.B: liver section from mice infected with S.mansoni treated with ozone gas at 15 weeks post-infection and sacrificed at 22 weeks post-infection showing small fibrocellular granuloma stained with Sinus red stain (%fibrosis)(Sirius red X 200).

Discussion

Hepatic fibrosis is the final result of most types of chronic liver injury [33].It is a scarring response to liver damage [34], which may be considered beneficial since it can encapsulate injury. However, in doing so liver function may ultimately become impaired [35].Removing the underlying cause of liver injury is the most effective way to prevent fibrosis. This approach can be highly effective when instituted early. Examples include removal of excess iron or copper in genetic hemochromatosis or Wilson's disease, respectively, abstinence in alcoholic liver disease, anti-helminthic therapy in schistosomiasis, clearance of HBV or HCV in chronic viral hepatitis, and biliary decompression in bile duct obstruction [36, 37]. Schistosomal involvement of the liver is an excellent model of the study of immunologic liver injury, fibrosis and hemodynamic disturbances in the absence of parenchymal injury [12].

Hepatic Stellate Cell (HSC) activation participates in the maintenance of cell attachment and the architecture of liver tissue via extracellular matrix production and assists liver regeneration by producing growth factors [38].Inflammatory mediators may stimulate stellate cell activation in chronic liver diseases such as viral or autoimmune hepatitis and drug-induced liver injury [39], Thus, anti-inflammatory medications might be beneficial in preventing fibrosis in these conditions [40].

Emerging antifibrotic therapies are aimed at inhibiting the accumulation of fibrogenic cells and/or preventing the deposition of extracellular matrix proteins or inhibition of the accumulation of activated HSCs by modulating either their activation and/or proliferation or promoting their apoptosis [41].Many approaches for treating schistosomiasis-induced fibrosis involve the co-administration of interleukin-12 and worm egg antigen to modulate the host immune response [42]. The inhibition of fibrosis in this model is accompanied by replacement of the Th2-dominated pattern of cytokine expression, which is characteristic of S. mansoni, by one dominated by Th1 cytokines, which has a more protective profile. This approach could have implications for other human liver diseases in which the host immune responses play a role in fibrogenesis, including viral hepatitis, primary biliary cirrhosis, and autoimmune hepatitis. Another strategy in the treatment of hepatic fibrosis by using anti-TGF-β1 intervention, since hepatic fibrogenesis depends largely on fibroblast growth factors, mainly TGF-β1 to prevent hepatic fibrosis [43]. Targeting of antifibrotic drugs to HSCs and fibrogenic cytokines is another promising strategy to block the fibrotic process (Khalifa et al., 2014).

Based on the pharmacological implications and clinical evidences, it can be concluded that the use of medical ozone can be advantageous in the treatment of various diseases [45].(Sadek et al., 2006) in a study on the effect of ozone therapy in chronic hepatitis C patients, have found significant improvement of liver enzymes and histopathology despite rise of PCR count in a study on 141 patients.

In our study, we have found a highly significant decrease in the number of worm couples from 2.6 in untreated versus 0.64 in the treated group with $P<0.001$, with a highly significant decrease in the total number of worms from 8.29 in treated group versus 11.2 in control group ($P<0.001$). Percent of reduction of worm burden is 25.98 % which is statistically highly significant $P<0.001$. There is an insignificant increase in the number of male worms from 3.0 in the untreated group versus 4.36 in the treated group together with

insignificant decrease in the number of female worms from 3.0 in untreated control group versus 2.64 in the treated Ozone group.

This coincides with the results of [22], who claimed that administration of medical ozone to mice infected with S. mansoni resulted in significant reduction in worm burden evident by significant decrease in total number of worms as well as total number of females, males and couples. They explained this by an anti-Schistosomal effect of ozone causing reduction of worm load by the hypothesis that ozone oxidizes mono and polyunsaturated fatty acids present in the lipoprotein coat of many structures [46]. at the same regard, [47] attributed The mechanism of antimicrobial action of ozone to the membrane lysis of the agents, after the oxidation process. Ozone could possibly react with the same constituents of the tegumental layer of the adult worm perhaps destroying the highly resistant outer layer of the tegument which confers effective evading mechanisms for humoral and cellular immune responses of the host. In this way, ozone can transform the parasite tegument into a porous structure to become an easy victim for cell-mediated immunity. Damage to the parasite proteins and lipids was reported to contribute to the decrease in their survival [48]. In fact her explanation that ozone combines with the mono and polyunsaturated fatty acids in the tegument and destroys it, is not logic simply because ozone does not enter into the body but only the products of its reaction as hydrogen peroxide and lipid oxidation products which would not do the same [14]. Also there is discrepancy between the compared groups in her work where the treated group was sacrificed at the end of 13 weeks infection while the non-treated group was sacrificed at only 11 weeks post-infection.

Though this apparently anti-parasitic effect appears for the first look conflicting with the results of (Sadek et al., 2006) when they gave ozone to chronic hepatitis C patients and found significant increase of PCR count despite significant improvement of inflammation and fibrosis emphasizing the absence of anti-viral effect, and explained this effect by possible shift of the immune system from Th1 to Th2 response, yet we think that the effect is coinciding rather than contrasting. Actually, immunity against viruses is mainly a TH1 response while immunity against parasites is mainly a TH2 response. Generally, Th1 responses are more effective against intracellular pathogens (viruses and bacteria that are inside host cells), while Th2 responses are more effective against extracellular bacteria, parasites, and toxins [49]. We think that the augmentation of the Th2 response initiated by ozone is against immunity to viruses but with immunity against parasites. An inability to make Th2 response renders mice acutely sensitive to infection with schistosomes and highly susceptible to intestinal helminth infections [50].

In many pathological situations, the balance between Th1 and Th2 immune response determines the outcome of different immunologically-mediated clinical syndromes including infectious, autoimmune, and allergic diseases [51].

In allergic inflammation and helminthic infections, where eosinophils participate, a type-2 pattern of cytokines is seen, which includes the synthesis and release of IL-4, IL-5. IL-6, IL-10, and IL- 13. Interferon-γ and IL- 12 (from cells with type-1 activity) inhibit the activity of cells with a type-2 profile, whereas IL-4 and IL-10 inhibit type-1 activity. In this way, a delicate balance between the two systems can be maintained [52].

Regarding the number of ova per gram infected tissue, the number of ova per gram tissue of intestine was highly significantly decreased on treatment (7146 versus 3607 with P<0.001). Also, the number of ova per gram tissue of the liver was significantly decreased on treatment (7850 versus 5946 with P<0.05). Regarding the Oogram in the intestine, it showed highly significant reduction of the percent of immature ova (18.25 versus 14.0 P<0.001) and a significant reduction in the mature ova (26.75 versus 23.71 P<0.5) with significant increase of dead ova (57.0 versus 62.29 P<0.01). Also the number of degenerated ova in the liver was highly significantly increased (17.75 versus 69.40 P<0.0001).

Science there is a reduction in the number of worm pairs, and a marked increase in the % of dead ova, marked increase in the % of degenerated ova, this means that there is a positive effect on all forms of the parasite. Concerning the drug potential antischistosomal activity, it is that worm load, Oogram patterns, and tissue egg count are criteria for assessing antischistosomal activity of any tested compound and /or drug [53]. The highly significant reduction in the ova per gram intestines with only significant reduction in the ova per gram liver may reflect a possible parasite shift. Hepatic shift denoting change in the distribution of schistosomes within the hepatic portal system is one of the important parameters reflecting drug activity [54].

Experimental evidence suggests that parasite elimination in vivo may require additional host-dependent immunologic events [55]. Drug treatment of T cells deficient mice infected with S.mansoni has been shown to be less effective than therapy of immunologically intact mice [56].More recent observations suggest an even more complex relationship between host immune response and the effects of praziquantel in eliminating S.mansoni adult worms in vivo [57].

As regards the effect ozone/oxygen gas mixture on granuloma, we found a highly significant reduction in the number (45.15 versus 35.75 P<0.0001) and diameter (233.25 versus 133.55 P<0.0001) of granuloma in the treated group than the untreated group. Also, the percent of fibrosis was highly significantly reduced in the ozone-treated group as evidenced by the marked reduction of collagen on Masson trichrome stain of liver sections to 28.6 versus 49.85 in the untreated group with P<0.0001. This effect is related to the supposed anti-parasitic effect of ozone on the treated mice, but also an immune-modulating effect can be suggested. The significant reduction in the size of hepatic granulomas in the ozone-treated group might be beneficial for the host. [58] reported that, in mice, the diminution in granuloma size and number were directly correlated with reductions in rates of portal hypertension and morbidity.

The parasite is known to induce hepatic oxidative stress by the production of reactive oxygen species (ROS) [59, 60]. ROS initiate fibrogenesis cascade in the liver [61, 62], resulting in liver fibrosis responsible mainly for morbidity and the mortality associated with schistosomiasis [63]. Repeated administration of ozone in atoxic doses is able to induce an adaptation to oxidative stress thus enabling the animals to maintain hepatocellular integrity after CCl4 poisoning. Low doses of ozone increased antioxidant endogenous systems such as glutathione, superoxide dismutase and catalase [14, 64]. In an EM study done by (Sadek et al., 2008), they suggested that liver tissue exposed to ozone treatment revealed an increase in peroxisomes. In general, peroxisomes mainly contain enzymes against

oxidative stress. Their key enzymes are catalase and peroxidase; further they have D-amino acid oxidase, uratoxidase, and superoxid-dismutase.

In murine models of S. mansoni infection, the egg production was associated with a switch from a Th1 to Th2 response with subsequent dominance of a Th2 response [65–67], while inhibiting the Th1 component. In the study of [22] they claimed that according to [68], administration of ozone in a dose greater than 40μg/ml was found to suppress Th2-type lymphocytes, directly or indirectly, an effect that could be exhibited in their present study as ozone was administrated in a dose of 50μg/ml. Also, they claimed that ozone could produce the production of interferon –γ which is essential to mount a Th1 response to tolerate the infection better [69]. Being an inhibitor of collagen synthesis [70], IFN-γ could have contributed to the reduction in granuloma size and to decrease in the amount of fibrosis .IFN-γ and the Th1 response were reported to protect against severe fibrosis by preventing alternative macrophage activation and thereby limiting the fibrosis-enhancing effects of the Th2 response.

The study showed a highly significant positive correlation between the percent of mature and immature ova and percent of fibrosis, while a highly significant negative correlation exists between the percent of fibrosis and number of degenerated ova. Still, that does not exclude a possible direct anti-fibrotic effect as mentioned in the work of Sadek et al 2006, when they claimed a highly significant anti-fibrotic and anti-inflammatory effect of ozone during treatment of chronic hepatitis C patients without affecting the viral count.

Conclusion: Ozone has marked an antiparasitic effect against all stages of S.mansoni parasite in mice. This effect is possibly due to immune-modulation through augmentation of Th2 type of immune response. It has a significant effect on granuloma size and number together with a significant effect on percent of fibrosis through this anti-parasitic immune-modulating effect and possibly also through direct anti-inflammatory and anti-fibrotic effect.

Recommendations

1. Medicine, with a well-known chemical structure, which has its pharmaceutical effects, its optimal dosage, its toxic dose, and its sub-therapeutic dose.
2. To study the effect of ozone on more advanced stages of murine Schistosomal infection (22 weeks), where granuloma disappears and is replaced only by fibrous bands to study its anti-fibrotic effect.
3. To study the effect of ozone on different immunologic parameters related to murine Schistosomal infection especially Th1, and Th2 immune responses.
4. To study markers of fibrogenesis and fibrolysis to define the mechanism of ozone possible anti-fibrotic effect.
5. Pharmacists should deal with ozone as a drug rather than an alternative or complementary

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