The efficacy of platelet rich plasma as adjuvant therapy in the treatment of cryptosporidiosis in experimentally infected immunosuppressed rats

Original Article

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ABSTRACT

Background: *Cryptosporidium* species are zoonotic opportunistic coccidian parasites that could cause disseminated life-threatening infection in the immunocompromised host. Unfortunately, few available drugs effectively eradicate the infectious oocyst with limited availability in developing countries. Although Nitazoxanide (NTZ) is the drug of choice for the treatment of cryptosporidiosis, it has limited efficacy in malnourished and immunocompromised patients. Moreover, platelet-rich plasma (PRP) successfully ameliorated the hepatic granuloma size in patients with parasitic schistosomiasis *mansoni*.
Objective: This study aims to test the potential therapeutic effect of PRP versus the currently used NTZ, and/or using PRP as adjuvant therapy.
Material and Methods: Sixty-five immunosuppressed rats were divided into 5 groups: non-infected as negative control (GI), infected non-treated as the positive control (GII), infected with *Cryptosporidium* spp. and treated with either intraperitoneal PRP (GIII), or NTZ (GIV), or a combination of intraperitoneal PRP and NTZ (GV). Parameters used for evaluation of the therapeutic efficacy included parasitological examination, histopathological examination of ileocaecal and liver specimens, and quantitative analysis of reduced glutathione (GSH) and malondialdehyde (MDA) for evaluation of oxidative stress markers, glutamic-oxaloacetic transaminase (SGOT), and glutamic pyruvic transaminase (SGPT) for evaluation of liver functions.
Results: Parasitological and histopathological examinations revealed minimal improvement in GIII, marked improvement in GIV, and the best results were recorded in GV. The administration of PRP in GIII produced no significant changes in GSH, MDA, SGOT compared to positive control GII. Treatment with NTZ in GIV, and in addition to PRP in GV showed significant difference ($P<0.05$) compared to GII regarding serum results of GSH, MDA, and SGOT with the best results recorded in GV. GIV and GV showed reduction of serum levels of SGPT although there was statistically insignificant difference between the study groups.
Conclusion: PRP could be used as a potential adjuvant therapy with NTZ to ameliorate the pathologic and inflammatory effects of cryptosporidiosis on the ileocaecal region. It also improves liver function in the immunocompromised hosts.

Keywords: cryptosporidiosis; glutathione; liver function tests; malondialdehyde; nitazoxanide; platelet-rich plasma.

INTRODUCTION

The genus *Cryptosporidium* and its species belong to the phylum Apicomplexa. They are intestinal coccidian protozoa that mainly inhabit the brush border of the small intestine acquiring the unusual position of being intracellular, but extra-cytoplasmic[1]. Cryptosporidiosis is a globally distributed zoonotic disease-causing enteritis and aqueous diarrhea[2]. Implicated species are transmitted via consumption of contaminated food or drink with sporulated oocysts, inhalation of oocysts from the soil, and self-infection[3,4].

In 2015, 27 species of *Cryptosporidium* were characterized[5]. Despite reports on infection in man by several species, the majority of human cryptosporidiosis proved to be due to *C. hominis* and *C. parvum*[6-8]. Cryptosporidiosis may cause serious complications in immunocompromised patients including severe diarrhea with subsequent dehydration and electrolyte imbalance, hepatic and respiratory problems[9]. There are several major mechanisms responsible for the associated diarrhea, the most important of which is morphologic atrophy of small intestinal villi, blunting and fusion of the villi, in addition to inflammatory infiltrates in the lamina

propria of the intestinal wall[10]. It is noteworthy that cryptosporidiosis was shown to stimulate apoptosis in cultured epithelia of the intestinal and biliary tracts[11]. Additionally, cryptosporidiosis *parvum* induces an up-regulation in the expression of cyclooxygenase-2 (Cox-2) in the epithelial cells[12], besides stimulating the release of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-α (TNF-α)[13].

Although, the worldwide nature of cryptosporidiosis is well established, there are still few limited options for treatment. Supportive therapy and specific treatment by NTZ, which is the only approved drug by the Food and Drug Administration (FDA) in the United States, are still the only available solution for serious infections. Unfortunately, NTZ has restricted efficacy in poorly nourished children and seems to have no efficacy in immunocompromised patients[14-17].

It was established that PRP is composed of a high concentration of autologous platelets in a small amount of centrifugated plasma[18]. Platelets have a basic role in hemostasis and are a natural origin for growth factors (GFs) production. Growth factors, stored within platelet α-granules, include platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), platelet-derived angiogenic factor (PDAF), and transforming growth factor-beta (TGF-β). Thrombin, calcium chloride, or collagen are important stimulants that could activate the platelets to release the GFs. These GFs are capable of stimulating angiogenesis and increasing fibroblast cell differentiation[18,19]. The rate of wound healing of soft tissues could be doubled or tripled under the influence of these GFs[20]. Furthermore, platelets produce many important factors like fibronectin, vitronectin, sphingosine 1-phosphate, that have an extremely significant role in wound healing[21]. These properties initiated the proposal of medical usage of PRP to promote soft tissue healing[18,19]. Although, there is no accessible effective anti-fibrotic therapy[22], PRP was shown to reduce chemically-induced liver fibrosis in experimental animals[23-25]. As a parasitic treatment, PRP proved to alleviate liver fibrosis in murine schistosomiasis *mansoni*[26]. Besides, PRP products are easy and inexpensive to prepare[27].

Oxidative stress occurs due to a change in the balance between oxidant and antioxidant cellular agents, and it is caused by overproduction of reactive oxygen substrates (ROS). A balance between ROS and primary antioxidant products, i.e., superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH), is needed for preventing damage by oxidative stress. There are very few reports in the literature documenting the role of ROS in the pathogenesis of *C. parvum* infection[28-30]. Experimental infection of immunocompromised Swiss albino mice with *C. parvum* caused an elevation in lipid peroxidation products like MDA and reduction of GSH,

CAT, and SOD at the peak of infection, which proves the role of ROS in the pathogenesis of experimental *C. parvum* infection in mice[31].

Keeping the previous layout in mind, our study aimed to evaluate the synergistic effect of PRP as an adjuvant therapy with NTZ, to revert the pathologic changes in the ileocaecal region, livers, and portal tracts of white albino rats that were subjected to immunosuppression then infection with *Cryptosporidium* oocysts. In addition to evaluation of liver functions and oxidative stress markers.

MATERIAL AND METHODS

This experimental study was performed in the biological unit of Theodor Bilharz Research Institute (TBRI) during the period from September 2020 to March 2021. Pathology sections were prepared and examined in the Pathology Department Laboratory at Ain-Shams University Hospital.

Animals: Sixty-five laboratory rats of Sprague Dawley strain, ~10 weeks old, weighing 130-150 gm, were purchased from the Animal House of TBRI. The animals were kept in plastic cages with clean wood-chip bedding, in well-ventilated, sanitary, conditioned rooms with the mean temperature (27 ± 2^0C), away from direct sunlight. Animals were provided by chow and water ad lib.

Study groups: Fifty immunosuppressed rats were divided into 5 groups; 10 in each. Groups I (non-infected) and II (infected with *Cryptosporidium* oocysts, non-treated) were used as negative and positive controls, respectively. Groups III-V included infected rats treated with intraperitoneal PRP, NTZ, and combined therapy, respectively. Fifteen non-immunosuppressed rats served for PRP preparation.

Immunosuppression: Rats were immunosuppressed by oral administration of dexamethasone sodium phosphate (Dexazone) with a dose of 0.25 ug/g/day for two weeks by gavage technique using an esophageal tube; and was maintained by a weekly dose during the whole experiment for all the study groups[32].

Infection: After two weeks, immunosuppressed rats were orally infected by a suspension of 10^5 *Cryptosporidium* oocysts in 200 μL PBS, by gavage using an esophageal tube[33]. *Cryptosporidium* oocysts were kindly obtained from the Animal Research Institute in Giza governorate, Egypt.

Drug and PRP preparation: NTZ was obtained from a local pharmacy in the form of powder to prepare100 mg suspension/5 ml (Nanazoxide®). To prepare PRP, 15 non-immunosuppressed rats were anesthetized using ether. After laparotomy, four ml venous blood

was collected from the inferior vena cava in tubes containing 3.2% sodium citrate (Merck, Darmstadt, Germany). Centrifugation of blood samples was performed at 400 g for 10 min. The supernatant was centrifuged again at 800 g for 10 min. From each tube, two-thirds of the serum were discarded from the top, and the remaining pooled portions were accepted as PRP[34]. After the aforementioned procedure, ~15 ml of PRP was obtained.

Therapeutic doses: Rats of GIII received PRP in a dose of 0.5 ml/kg by intraperitoneal injection twice weekly for 4 weeks starting one week post-infection (pi)[24]. Rats of GIV received 65 mg of NTZ oral suspension. The drug was administered daily for 4 weeks starting one-week pi. The doses were adjusted by an extrapolation table for the therapeutic doses of man and animal[35]. Whereas GV received a similar regimen and doses of PRP and NTZ.

Parasitological examination: To ensure rats' infection, stool samples were obtained a week after inoculation with oocyst for microscopic examination using the oil immersion lens (x100) after staining the stool smears by modified Zeihl Neelsen (MZN) stain[36]. Parasite load was calculated by counting the number of *Cryptosporidium* oocysts in the fecal pellets collected from infected rats at 7th, 14th, 21st, 28th, and 35th day pi. One mg stool pellet was weighed, then preserved in 1 ml 10% formalin; the stool suspension was concentrated by centrifugation at 500 g for 10 min. The oocysts were counted in 1 ml of stool sample by staining 100 ul of stool sediment by MZN and examining microscopically (x100). The average of 3 counts multiplied by 10 equaled the number of oocysts in 1 ml of stool[37]. The number of oocysts was expressed per gram of feces[38]. Oocyst shedding one week after inoculation was considered a sure sign of viability and establishment of infection.

Measurement of serum liver enzymes and oxidative stress markers: One ml venous blood was collected from each rat from the retro-orbital vein 4 weeks after the establishment of infection at the end of the treatment regimen. Levels of GSH[39], MDA[40], SGOT and SGPT[41] were measured using Sigma Diagnostic kits (USA).
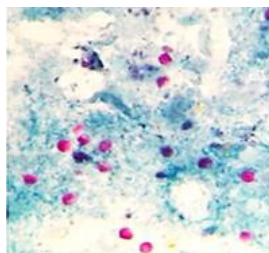
Histopathological examination: All rats were euthanized 4 weeks pi to remove the ileocecal region of the small intestine and liver with the portal tracts. The specimens were fixed in 10% buffered formalin, embedded in paraffin wax blocks, sectioned, and stained by Hematoxylin and Eosin (H&E)[42].

Statistical analysis: Data were expressed as mean ± standard deviation (SD). Differences between groups were determined using a one-way ANOVA and post hoc Tukey`s multiple comparison tests. The level of significance was accepted at P value < 0.05.

Ethical consideration: This study was approved by the Scientific Research Ethics Committee of TBRI. The internationally valid guidelines were applied for all animal experiments after acceptance by the institutional ethical committee.

RESULTS

Parasitological studies: After inoculation of rats with *Cryptosporidium* spp. oocysts (Fig. 1), fecal pellets were collected from GII (infected non-treated) at different days pi to determine the number of *Cryptosporidium* spp. oocysts/gm of feces on each day (Fig. 2). It was observed that oocysts shedding started from the 7th day pi with 715±48.65 x10³ oocysts/gm of feces.

Table (1) shows the therapeutic effects of drugs used on oocysts shedding in collected stool pellets. The number of *Cryptosporidium* spp. oocysts/gm of feces that were shed by GII rats on the 35th day of infection was 2292.8±227.32x10³ oocysts. There was 59.17%, 65.14% and 71.32% reduction of oocyst shedding in GIII, GIV and GV compared to GII, respectively. It is worth mentioning that the mortality rate was around 10% in all the study groups.

Histopathological examination of ileocaecal and liver specimens: In GI, sections of the small intestine at ileocecal junction showed normal small intestinal mucosa with normal villous architecture length and width. A well-formed intact brush border was detected
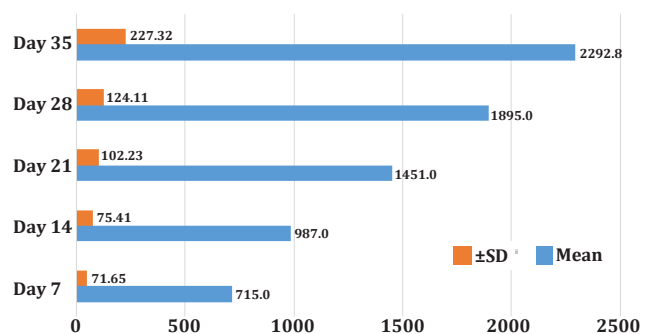

Fig. 1. *Cryptosporidium* spp. oocyst (red spots), stained by MZN (X1000).

Fig. 2. The number of shed *Cryptosporidium* spp. oocysts (mean ±SD) x 10³/gm of feces in the infected non-treated group (GII) on different days pi.

Table 1. The number of *Cryptosporidium* spp. oocysts (mean ± SD) x10^3/gm of feces at the 35th day pi in the different study groups with the estimation of the percentage of reduction compared to GII.

Groups	Number of oocysts shedding at day 35 pi Mean ± SD X 10^3	% of reduction
Group II	2292.8 ± 227.32	--
Group III	936.18 ± 29.38	59.17
Group IV	799.2 ± 45.19	65.14
Group V	657.6 ± 29.08	71.32

with an average number of goblet cells. Hepatic sections showed normal hepatic architecture with the hepatocytes arranged in thin regular plates around the central vein and unremarkable portal tracts (Fig. 3).

In GII, the ileocecal junction of the small intestine revealed marked histopathological changes as a result of cryptosporidiosis. Variable grades of villous atrophy in addition to decreased villous height to crypt ratio and shortened broad villi were detected. Foci of mucosal ulceration were observed together with the depletion of goblet cells. Significant inflammatory infiltrate was also seen in the lamina propria composed mainly of plasma cells, lymphocytes, and macrophages with lymphoid aggregates. Examination of liver sections showed expanded portal tracts with moderate lymphoid infiltrate. Dispersed spotty necrosis foci were detected together with mild interface activity and mild fibrosing reaction forming porto-portal bridges (Fig. 4).

In GIII, there was minimal amelioration of the histopathological changes at the ileocecal junction.

A mild reduction in villous height to crypt ratio with moderate villous blunting was detected. Foci of mucosal ulceration were seen with mild exhaustion of goblet cells. Lamina propria showed moderate inflammatory infiltration. Liver sections showed foci of lobular activity with mildly dilated portal tracts and moderate lymphocytic infiltrate (Fig. 5).

In GIV, marked amelioration of the histopathological changes occurred. Restoration of normal villous architecture was detected and the ratio between villous height and the crypt became close to normal. Regular brush border and normal goblet cells were seen. There were minimal surface mucosal erosions, and the mucosal lining showed a nearly normal pattern. The lamina propria showed a mild inflammatory reaction. The liver sections showed mild portal inflammatory infiltrate (Fig. 6).

In GV, the ileocecal junction showed remarkable amelioration of the histopathological changes, with preservation of the villous to crypt height ratio.
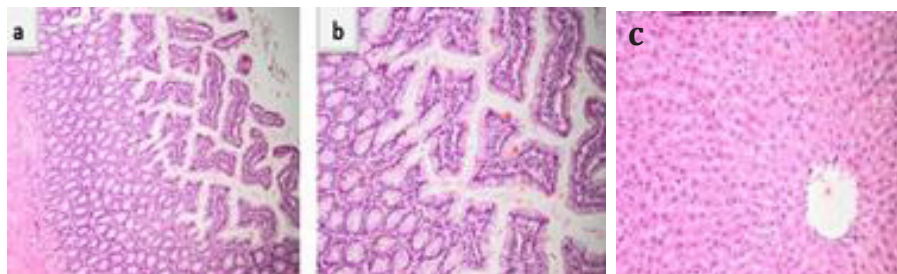
Fig. 3. (a, and b): Small intestine section of GI showing preserved architecture of the villi with intact brush border (H&E stain x200). **c):** Liver section of G1 showing normal hepatic architecture (H&E stain x 200).
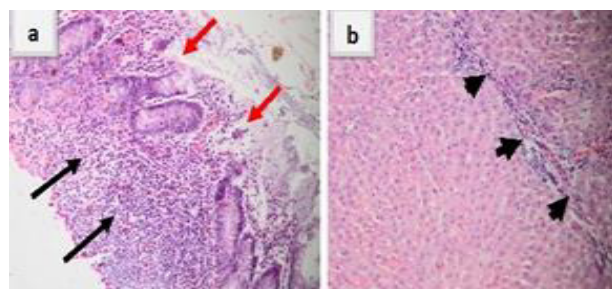
Fig. 4. a): Small intestinal section from GII showing variable grades of villous atrophy, mucosal ulceration, and goblet cell depletion (red arrows). The lamina propria contained significant inflammatory infiltrates with lymphoid aggregates (black arrows) (H&E stain x 200). **b):** Liver section of GII showing dilated portal tracts with severe inflammation and porto-portal bridges with interface activity and moderate fibrosis (black arrow heads) (H&E stain x200).
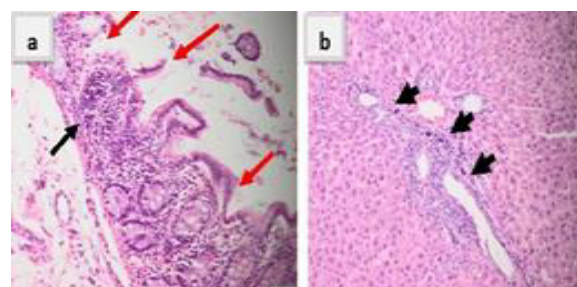
Fig. 5. a): Small intestinal section from GIII showing moderate villous blunting with foci of ulcerated mucosa (red arrows) and lamina propria with inflammatory infiltration (black arrow) (H&E stain x200). **b):** Liver section showing foci of lobular activity with mildly dilated portal tracts and moderate lymphocytic infiltrate (black arrowheads) (H&E stain x200).

The normal villous architecture was restored. The mucosal lining, brush border, and goblet cells were nearly normal. The lamina propria showed minimal

inflammatory infiltration. Liver sections showed maintenance of hepatic architecture with minimal inflammatory infiltrate (Fig. 7).
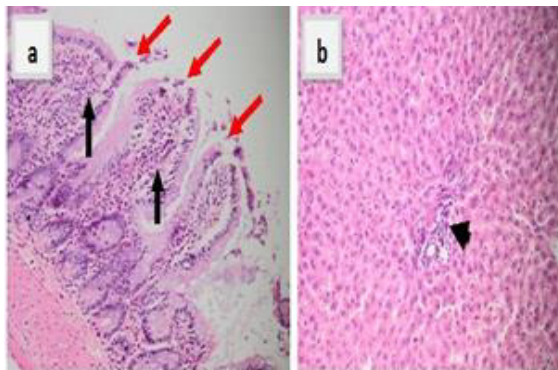

Fig. 6. a): Small intestinal section from GIV showing resumption of the normal villous architecture; the ratio between villous height and crypt were close to normal with focal minimal surface mucosa erosions (red arrows). The lamina propria showed mild inflammatory infiltration (black arrows) (H&E stain x200). **b):** Liver sections showing mild portal inflammatory infiltrate (black-arrow head) (H&E stain x200).
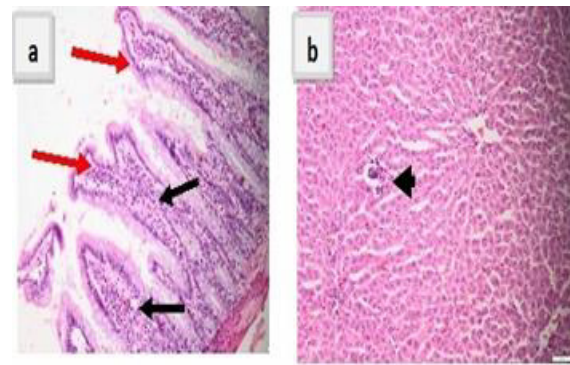

Fig. 7. a): Small intestinal sections in GV showing normal surface epithelium, with preserved goblet cells (red arrows). The lamina propria contains mild inflammatory infiltration (black arrows) (H&E stain x200). **b):** Liver section showing normal architecture with mild dilatation and inflammatory infiltration of the portal tracts (black-arrow head) (H&E stain x200).

Biochemical analysis of serum GSH, MDA, SGOT, and SGPT: There was a significant difference between all the study groups regarding serum GSH, and SGOT ($P<0.01$), and serum MDA ($P<0.05$), with a non-significant difference regarding serum SGPT ($P=0.1257$). Compared to GI, there was a significant difference regarding serum GSH, MDA, SGOT ($P<0.001$) in GIII. However, when compared to GII, there was a non-significant change regarding the same parameters. The difference between GIV and GI regarding serum GSH, MDA, SGOT, and SGPT was significant ($P<0.001$), and there was also a significant difference when compared to GII regarding the same parameters

($P<0.001$). Similarly, GV versus GI showed a significant difference for the results of serum GSH and SGPT ($P<0.001$) and a non-significant difference regarding serum MDA and SGOT, while GV versus GII showed a significant difference for the serum levels of GSH, MDA, SGOT, and SGPT ($P<0.001$), also, there was a significant difference between GV and GIV regarding serum GSH, SGOT, SGPT ($P<0.001$) and MDA ($P<0.05$) (Table 2). The administration of PRP in GIII produced no significant changes in GSH, MDA, SGOT compared to positive control GII. Treatment with NTZ in GIV, and in addition to PRP in GV showed significant difference ($P<0.05$).

Table 2. Biochemical changes of the different study groups.

Groups	GSH	MDA	SGPT	SGOT
I	136.3±5.467	2.060±0.2875	47.07±0.8512	51.09±0.9893
II	91.71± 2.080[a]	3.920±0.5865[a]	59.08±1.405[a]	52.03±1.239
III	93.91±3.527[a]	3.780± 0.5473[a]	61.17±3.029[a]	68.19±1.686[ab]
IV	110.2±4.626[ab]	2.950±0.3923[ab]	41.09±1.166[ab]	44.11±1.453[ab]
V	123.1±1.567[abc]	2.390± 0.2331[bc]	49.03±1.467[bc]	40.06±0.7011[abc]
P value[@]	0.0027*	0.0400*	0.0019*	0.1257

I: Non-infected, non-treated (negative control); **II:** Infected, non-treated (positive control); **III:** Infected and received PRP; **IV:** Infected and received NTZ; **V:** Infected received PRP and NTZ. Values are presented as mean ± SD. **@:** One-way ANOVA and post hoc Tukey`s multiple comparison test. **a:** Significant difference between all groups and the negative control. **b:** Significant difference between GIII, GIV, and GV versus the positive control. **c:** Significant difference between GIV in comparison to GV. ***:** Significant $P < 0.05$.

DISCUSSION

Cryptosporidium spp. are well-known parasites causing waterborne epidemics all over the world[43]. The outcome of cryptosporidiosis is greatly dependent on the immune status of the patient, giving clinical manifestations that vary greatly from mild self-limited diarrhea within two to

three weeks in the immunocompetent patients, to severe life-threatening illness with dehydration and electrolyte imbalance in the immunocompromised patients[44-46]. Cryptosporidiosis is associated with high mortality among immunodeficient patients[47,48]. Also, cryptosporidiosis may lead to atypical clinical manifestations in immunodeficient patients such as pancreatitis, respiratory and biliary tract infections[49].

Tissue sections of the small intestinal ileocaecal junction from immunocompromised mice infected with cryptosporidiosis revealed short, broad, and atrophied intestinal villi with the reduction of the villous to crypt height ratio. Mucosal ulceration, goblet cell depletion, and inflammatory infiltration of the lamina propria were noted. Liver sections showed congested sinusoids with dilated fibrotic portal tracts[50].

According to results of the present study, following cryptosporidiosis under immunocompromised conditions, treatment with the PRP showed significant improvement in the histopathological changes of small intestinal villi at the ileocaecal region and restored the liver architecture with reduction of portal tracts pathology. These results were marked when PRP was used in combination with NTZ giving results better than PRP injection alone, or NTZ administration alone.

Marx *et al.*[51] were the pioneers in studying PRP prepared by centrifugation of blood then separation of the supernatant that is very rich in platelets. Platelet-rich plasma proved to contain many important GFs. It is a breakthrough in the field of medicine to discover the ability to use PRP in tissue regeneration as platelets contain proteins required for hemostasis as well as numerous GFs[52] such as basic fibroblast growth factor (BFGF), platelet-derived growth factor (PDGF), transforming growth factor beta TGF-β, vascular endothelial growth factor (VEGF), endothelial growth factor (EGF), and insulin-like growth factor (IGF)[53,54]. Platelet-rich plasma also releases special chemokines and cytokines (e.g., IL-1 and platelet factor 4) in addition to important proteins and peptides (e.g., fibrinogen, fibronectin, osteonectin, osteocalcin, vitronectin, and thrombospondin). Therefore, many PRP types have been used by clinicians for years due to their enhancing effects on wound healing, cellular mitogenesis, osteogenesis, and angiogenesis[55]. Moreover, advocates of PRP treatment prefer PRP because of its beneficial effects in increasing tissue regeneration. Accordingly, it was used in reconstructive surgery to decrease the rate of postoperative necrosis and enhance healing, as well as lowering infection due to its antibiotic-like-action of contained leukocytes; in addition to decreasing pain and blood loss. Platelet-rich plasma is a cheap easily obtained product without any risk for rejection or harmful immune responses against it[56,57]. It was reported that PRP has a strong synergistic effect with adipose-derived stem cells on amelioration and healing in a mouse with induced pressure injury[58]. The use of PRP has been reported in many fields of medicine, including maxillofacial surgery and the treatment of problematic soft tissue ulcers[59,60]. Also, PRP became one of the best treatment options in the field of sports medicine for the treatment of tears in the rotator cuff, and the ulnar collateral ligament, hamstring injuries, in addition to knee osteoarthritis[61]. Also, transfusion of platelets can improve liver function in patients with chronic liver disease and cirrhosis due to their ability to produce many types of GFs in addition to the antifibrotic properties[62]. Platelet-rich plasma proved to reduce chemically induced liver fibrosis in experimental animals as it significantly diminished fibrotic areas and hepatic hydroxyproline in carbon tetrachloride (CCl4) induced hepatic fibrosis[52]. Also, Takahashi *et al.*[23] found that human PRP lessened significantly the fibrotic index and hepatic hydroxyproline content in CCl4-initiated liver fibrosis in severe combined immunodeficient mice. Moreover, a recent study revealed that rat PRP markedly improved dimethylnitrosamine-induced liver fibrosis as demonstrated by significant lowering of liver hydroxyproline content[25,45].

On the other hand, there is increasing evidence that PRP may be serious in patients with tumors as it may lead to enhancement of tumor progression due to the release of a large amount of GFs[63]. In the field of Parasitology, PRP successfully ameliorated the hepatic fibrosis in murine schistosomiasis *mansoni*. Although it did not significantly affect mean hepatic granuloma number, it caused a significant reduction in mean hepatic granuloma diameter in comparison with both infected untreated mice and praziquantel (PZQ) only treated mice. When PRP was combined with PZQ, the treatment produced the most noticeable decrease regarding granuloma diameter. The reducing effect of PRP on the granuloma diameter could be attributed to its anti-fibrotic action, besides, it may induce secretion of some mediators such as insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF). The former, greatly stimulates hepatocyte proliferation, and is, therefore, considered the most significant mediator for liver regeneration[26,64].

Glutathione has a significant role in the detoxification of oxygen free radicals produced at the inflammation sites[65]. A low level of GSH is of pathogenic importance in hepatic parasitic infections as in schistosomiasis. Accordingly, elevated GSH levels in target cells and the plasma might have a beneficial effect. Inhibition of oxidative stress and decreasing the intracellular peroxides level depend mainly on elevating the level of GSH and fosters its metabolizing enzymes to face the liberated free radicals[66]. In our study involving immunocompromised rats infected with *Cryptosporidium* spp., the MDA lipid peroxidation product was elevated, whereas the GSH level was reduced. This concurs with many studies in which there was liver affection in experimentally infected mice with *S. mansoni*[67-70]. In our study, biochemical measurements of GSH, MDA, SGOT and SGPT confirmed the beneficial therapeutic effect of NTZ against cryptosporidiosis. This concurs with the study stating that NTZ is the only Food and Drug Administration-approved therapy, although it is not consistently effective for therapy of cryptosporidiosis in the majority of vulnerable patients[71]. In the present study, GV versus GI showed a non-significant difference regarding

serum MDA and SGOT indicating the strong effect of the combination therapy in lowering their levels near to the normal values; and a significant difference regarding decreased serum GSH and SGPT, ($P<0.001$). However, when compared to GII, there was a significant change in decreasing serum GSH, and SGPT with increasing serum levels of MDA and SGOT ($P<0.001$).

Results of GV were better than GIV, being very similar to the negative control with a non-significant statistical difference regarding results of serum MDA and SGOT and had the best results between the study groups regarding serum GSH and SGPT compared to the negative control. This denotes the strong therapeutic effect for the PRP/NTZ combination that is superior to the effect of NTZ alone where there was a significant difference between GV and GIV regarding the results of serum GSH, SGOT, SGPT ($P<0.001$), and MDA ($P<0.05$). Accordingly, we could conclude that GV showed the best results due to statistically significant elevation of GSH with reduction of MDA, and SGOT indicating improvement compared to the GII. Also, GV showed a reduction of serum SGPT although there was statistically insignificant difference between the study groups. This is in agreement with the study that proved the strong synergistic effect of PRP with the adipose-derived stem cells in inducing healing of tissues in mice exposed to pressure injury[58]. This also concurs with the usage of PRP-gel as an adjuvant treatment in human fibrosarcoma, to stimulate tissue repair and speed up recovery, thus impairing tumor growth and slowing tumor progress[72]. Similar conclusions were reported regarding the results of serum GSH, MDA, and SGOT when ginger (*Zingiber Officinale*) loaded nanoparticles were used in the treatment of murine schistosomiasis *mansoni*[70]. Hence, it seems that combination therapies will play an important role to ameliorate the pathologic effects of parasitic infections.

In conclusion, results of former studies[26,58,64,72] in addition to the findings of the current research elucidate the important effects of PRP on tissue healing and fibrosis reduction that could be considered a breakthrough in the treatment of the pathologic changes following parasitic infections. Although the PRP therapy had no direct effect on the parasite, our results indicated that PRP could be considered as a novel potential adjuvant therapy with NTZ to ameliorate the pathologic and inflammatory effects of cryptosporidiosis on the small intestinal villi and on the liver and portal tracts in the immunocompromised hosts.

Author contribution: El-Kholy WAMS designed the plan of work, performed the practical part, and shared in writing and revising the manuscript. Elgohary SA performed the histopathological study. El-Ashkar AM shared in designing the plan of work, analyzing the data, writing, and revising the manuscript. The manuscript has been read and approved by all named authors. We further confirm that the order of authors listed has been approved by all of us.

Conflicts of interest: None.

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