Echinacea purpurea and its nano-formulation as antiparasitic agents in experimental cryptosporidiosis

Original Article

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ABSTRACT

Background: Available therapy options for the worldwide spread cryptosporidiosis are restricted. Although nitazoxanide (NTZ) is the preferred medication, its effectiveness in immunocompromised patients is not recommended.
Objective: To assess the effectiveness of *Echinacea purpurea* alone, or loaded on ZIF-8 nanoparticles (NPs) in treating immunocompromised mice infected with *Cryptosporidium* spp.
Material and Methods: Eighty male Swiss albino mice were immunosuppressed and divided into 8 groups: GI: the negative control group; GII: the positive control group; GIII: infected and given NTZ; GIV: infected and given *E. purpurea*; GV: infected and given combination of *E. purpurea* and NTZ; GVI: infected and given NTZ@ZIF-8; GVII: infected and given *E. purpurea*@ZIF-8; GVIII: infected and given combination of *E. purpurea*@ZIF-8, and NTZ@ZIF-8. Efficacy was evaluated by stool examination for oocyst shedding on 7th and 14th dpi. Blood was collected for measuring serum superoxide dismutase (SOD), and malondialdehyde (MDA). After mice sacrifice, intestinal dissection was performed for histopathological examination.
Results: The lowest number of *Cryptosporidium* oocysts shedding, improvement of histopathological features, and almost normal levels of SOD and MDA were observed in mice receiving *E. purpurea*/NTZ@ ZIF-8 NPs followed by mice receiving *E. purpurea*/NTZ with statistically significant difference when compared with the infected non-treated mice.
Conclusion: Combining *E. purpurea* with NTZ is beneficial not only for its anti-*Cryptosporidium* activity, but also for antioxidant activity. Furthermore, ZIF-8 is an effective potent delivery system that improved drugs efficiency.

Keywords: coneflower; cryptosporidiosis; delivery system; *Echinacea*; nanomedicine; nitazoxanide; oxidative stress, ZIF-8.

Received: 16 October, 2024; **Accepted:** 23 November, 2024.

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Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 17, No. 3, December, 2024.**

INTRODUCTION

Cryptosporidiosis is an enteric protozoan parasitic illness caused by numerous *Cryptosporidium* spp. The most frequently noticed species infecting humans are *C. parvum* and *C. hominis*[1]. Cryptosporidiosis is thought to be the second leading reason for diarrhea and child death, after rotavirus[2]. Human infections occur through ingestion of the infective oocyst stage with contaminated water or food[3]. Cryptosporidiosis usually presents as self-limiting watery diarrhea lasting several days in immunocompetent individuals. In contrast, life threatening diarrhea can happen in immunosuppressed patients, mainly in patients with HIV infection[4].

Currently, there are limited options for treating cryptosporidiosis. According to Food and Drug Administration (FDA), the recommended medication for treating cryptosporidiosis is NTZ[5] which has anti-helminthic and anti-protozoal activities[6]. However, it has a restricted effectiveness in immunocompromised patients and malnourished individuals[5]. Therefore, the development of novel anti-*Cryptosporidium* agents is advised.

On the other hand, *E. purpurea* known as coneflower, is a medicinal herb that belongs to the Asteraceae family[7]. While FDA classified it as a food, the commission E (Task Force E of the Federal Bureau of Health of Germany) categorizes it as a medication. It was used extensively for hundreds of years to treat a variety of infectious disorders because of its antimicrobial, antiviral, antiparasitic, antifungal, immunomodulatory, and antioxidant properties[8,9]. These effects are attributed to its chemical contents that include flavonoids, polysaccharides, alkylamides,

 DOI: 10.21608/puj.2024.328830.1272

phenolic acids, and essential oils[10]. It is used as a dietary supplement, assisting cure and prevention of a variety of infections, particularly in children, the elderly and individuals with impaired immune systems[11].

Recently, NPs attracted much interest in the field of medication delivery[12]. In fact, they are beneficial in treatment of several parasitic diseases for their ability to overcome the obstacle of low bioavailability. Besides, they augment cellular permeability, avoid non-specific distribution, and enhance excretion of toxins outside the body[13]. Different nanomaterials were evaluated to enhance treatment of cryptosporidiosis. One kind of porous hybrid organic-inorganic materials is called metal-organic frameworks (MOFs) that have unique physical and chemical properties as well as low toxicity, designating them a promising class of nanocarriers for drug delivery[14]. Zeolitic imidazolate framework-8 (ZIF-8) which is a class of MOFs that is highly porous, with simple modifications and excellent biocompatibility, has been extensively researched for drug delivery[15]. Besides, ZIF-8 is believed to have a high degree of biocompatibility and safety because the second most common metal in the human body is zinc and the amino acid histidine contains the imidazole group[16].

Therefore, the objective of the current study was to assess the effectiveness of *E. purpurea* alone or loaded on ZIF-8 NPs in treating immunocompromised mice infected with *Cryptosporidium* spp.

MATERIAL AND METHODS

This case-control experiment was carried out from December, 2023 to October, 2024 at the National Research Center's Zoonotic Diseases Department in Cairo, Egypt.

Study design: Mice were immunosuppressed for seven days before experimental infection with *Cryptosporidium* oocysts. After a week of infection, mice were treated orally for one week with NTZ, *E. purpurea*, NTZ/*E. purpurea* alone or loaded on ZIF-8 NPs. Stool samples were examined for oocyst counting on 1st and 7th days of treatment. One day after the last dose, blood samples were collected to measure SOD and MDA, then mice were sacrificed for small intestinal histopathological examination

***Cryptosporidium* spp. oocysts preparation:** *Cryptosporidium* oocysts were extracted from stool samples of naturally infected calves using Sheather's sugar floatation technique and identified by the modified Ziehl-Neelsen stain[17]. Positive samples were centrifuged at 2000g for 15 min after filtration through a 100-mesh sieve four times with distilled water. Intermittent sucrose gradients were used to concentrate sedimented *Cryptosporidium* oocysts, which were then preserved in 2.5% aqueous potassium dichromate solution and kept at 4°C. Before mice infection, *Cryptosporidium* oocysts were rinsed three times in phosphate buffered saline (PBS) to eliminate the potassium dichromate. A hemocytometer was used to count the oocysts which were adjusted to $3x10^3$ oocysts/ml PBS[18].

Experimental animals: Eighty male Swiss albino mice (6–8 w old and weighing 20–25 g) were obtained from the National Research Centre in Dokki, Egypt, and housed under observation in ventilated cages with perforated lids, and fed standard pellet food and water. Bedding was changed daily in ventilated cages with perforated lids.

Medications: *E. purpurea* (Immulant 175 mg capsules; Arab Company for Pharmaceuticals and Medicinal plants) was given at a dose of 100 mg/kg/day[19]. Nitazoxanide (Nanazoxid 100 mg/5 ml powder reconstituted for oral solution; Utopia Pharmaceuticals) was adjusted at a dose of 100 mg/kg/day[20]. The drugs were administrated on the 7th day post infection (dpi) for one week.

Immunosuppression, and infection of mice: Dexamethasone (Dexazone 0.5 mg tablets; Al Kahira Pharmaceutical & Chemical Industries Company, Egypt) was administered orally to the mice to suppress their immune systems. This was done seven days prior to infection and continued throughout the experiment at a rate of 0.25 mg/kg/day[21]. All mice, except those in GI (negative control), were infected orally with 0.1ml of the oocyst inoculum ($3x10^3$ oocysts/ml)[18].

Preparation of ZIF-8 NPs: The ZIF-8 was manufactured[22] according to the following steps: 100 ml of methanol was used to dissolve 2-methylimidazole (Hmim, 4.10 g, 50 mmole). The methanol solution of $Zn(NO_3)2{\cdot}6H_2O$ (2.97 g, 10 mmole) was added to a methanol solution of 2-methylimidazole containing lignin. The mixture was stirred continuously at room temperature for 24 h. After centrifugation, the remaining solids were separated, cleaned in methanol and deionized water, and dried at 60°C for twenty-four hours at reduced pressure. All used compounds were analytical-grade reagents obtained from Sigma (Ronkonkoma, NY 11779, USA).

Drug loading: The ZIF-8 NPs were loaded with NTZ and *E. purpurea* by dissolving them in ethanol (100 ml) at different gradients (100–1000 ppm). The drug solutions were mixed with one gram of ZIF-8 NPs and left to stand at room temperature for ninety minutes, using a magnetic stirrer set at 600 rpm. The solution was left undisturbed overnight and centrifuged for 5 min at 5000 rpm. The precipitated material and the supernatant were isolated. The loaded amount of each drug was determined by comparing drug concentration in the solution before and after loading. The following

equation was used to calculate the percentage of drug loading: Drug loading %= [(A−B)/A]×100; where A and B represent the initial and final concentration of each drug in the solution[23].

Characterization of ZIF-8 NPs: By X-ray diffraction (XRD) patterns (X'Pert MPD Philips diffractometer; the used monochromate was Cu K, phase purity and crystallinity of prepared materials were characterized. Using a scanning electron microscope (SEM: Hitachi SU-70, JP), ZIF-8 NPs' nanostructure morphology was examined[24].

Study groups: Mice were divided into eight groups (G), 10 mice each, among which GI was the negative control group, and GII was the infected, non-treated positive control group. The remaining mice were all infected and then treated; GIII was treated with NTZ (100 mg/kg/d), GIV was treated with *E. purpurea* (100 mg/kg/d), GV was treated with a combination of *E. purpurea* and NTZ, GVI was treated with NTZ loaded on NPs (NTZ@ZIF-8), GVII was treated with *E. purpurea* loaded on NPs (*E. purpurea*@ ZIF-8), and GVIII was infected and treated with combination of *E. purpurea* and NTZ loaded on NPs.

Assessment of drug efficacy

Oocyst shedding: Mice stool samples were obtained from each group on 7th and 14th dpi which represents 1st and 7th days of the treatment. In modified acid-fast stained smear oocysts were counted in ten microscopic fields (X1000) and the mean number for every group was calculated. The effectiveness of the treatment was assessed using the following equation: Efficacy (%) = 100× (the difference between the mean number of oocysts in infected controls and the mean number of oocysts in treated mice)/mean number of oocysts in infected controls[18].

Histopathological assessment: At the end of the experiment, specimens were cut from different parts in the small intestines of mice in all groups, and immediately preserved in 10% formalin. The preserved samples underwent the following steps: dehydration, xylene cleaning, paraffin embedding, sectioning at a thickness of 5 *u*m, and hematoxylin and eosin (H&E) staining[25].

Assessment of oxidative stress and antioxidant activity: Serum samples (one ml) were used to measure the activity of the SOD antioxidant enzyme, and the concentration of the MDA oxidative stress marker. The SOD was measured using the Misra and Fridovich method, based on SOD's capacity to prevent the autoxidation of epinephrine to adrenochrome in an alkaline medium (pH 10.2)[26], and the values were expressed as unit/ml. The MDA was measured using the modified Ratty and Das method based on thiobarbituric acid and MDA reaction[27], and the values were expressed as mmol/l. Biochemical parameters were measured using a spectrophotometer and commercial kits (Biodiagnostic, Dokki, Giza, Egypt).

Statistical analysis: Data were analyzed using SPSS software package version 27.0. The mean ± standard deviation (SD) was used to represent the data. Student *t*-test, Wilcoxon Rank test and analysis of variance (ANOVA) were used to identify the statistical significance among the study groups. Significance is considered at $P<0.05$.

Ethical consideration: The study procedure was permitted by the Research Ethics Committee, Faculty of Medicine, Benha University, Egypt (Code number: RC10-2-2024). Mice were handled according to National Research Centre guideline[28].

RESULTS

Characterization of NPs: The SEM micrographs of NTZ@ZIF-8 and *E. purpurea*@ZIF-8 clearly showed comparable cubic-like morphology and 80 to 150 nm size range (Fig. 1). Furthermore, the X-ray diffraction spectra of *E. purpurea*@ZIF-8 and NTZ@ZIF-8 shared the same peak positions with different diffraction intensities (Fig. 2). These noticeable diffraction peaks indicated that all loaded ZIF-8 had good crystallinity. Notably, ZIF-8 NP loading efficiency depended on NTZ and *E. purpurea* concentrations as well as ZIF-8 NPs ratio. *E. purpurea* and NTZ loading percentage increases with increase in these parameters. It increases and becomes constant at a particular level. The loading amounts were 180 and 250 mg/g for *E. purpurea*@ZIF-8 and NTZ@ZIF-8, respectively.

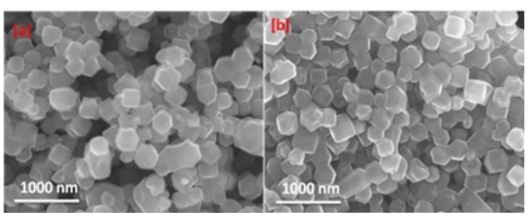

Fig. 1. SEM micrographs of NTZ @ZIF-8 **(a)**, and *E. purpurea* @ZIF-8 **(b)**.

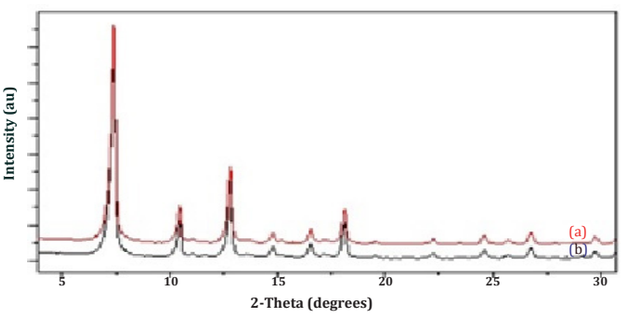

Fig. 2. PXRD patterns of NTZ@ZIF-8 **(a)**, and *E. purpurea*@ZIF-8 **(b)**. **au:** Arbitrary unit that indicates the relative intensity of a particular peak in the pattern.

Oocyst shedding: The control positive group displayed an increase in oocyst shedding ranging from 291.0±29.70 on 7th dpi to 342.0±47.80 on 14th dpi. The least quantity of 43.0±18.89 oocysts was detected on 7th day of treatment in mice given *E. purpurea*/NTZ combination loaded on ZIF-8 NPs (GVIII) with a reduction rate of 87.4%, followed by 75.0±16.50 in mice given *E. purpurea*/NTZ combination (GV) with a reduction rate of 78.1% and significant difference compared with the positive control group (P<0.001). Regarding administration of *E. purpurea* as a monotherapy (GIV), the oocyst count decreased to 127.0±18.89 without statistically significant change in contrast to the positive control group, but showed significant difference (P= 0.016) when loaded on ZIF-8 NPs in group GVII (104.0±18.38) (Table 1).

Table 1. *Cryptosporidium* oocysts shedding on different days of treatment among the study groups.

Groups	*Cryptosporidium* oocysts count						Statistical analysis# Test value (*P*)
	1st day of treatment			7th day of treatment			
	Mean ± SD	*P*@	Efficacy%	Mean ± SD	*P*@	Efficacy%	
I	0.0±0.0	-	-	0.0±0.0	-	-	2.075 (0.038*) 2.805 (0.005*)
II	291.0±29.70	-	-	342.0±47.80	-	-	
III	279.0±25.58	0.633	4.1	93.5±20.82	0.003*	72.7	
IV	284.0±27.57	0.776	2.4	127.0±18.89	0.199	62.9	
V	235.0±14.34[a,b,c]	0.002**	19.2	75.0±16.50[a,c]	<0.001**	78.1	
VI	239.0±22.34[a,b,c]	0.006**	17.9	83.0±13.37[a,c]	<0.001**	75.7	
VII	251.0±29.98[a]	0.036*	13.8	104.0±18.38[a]	0.016*	69.6	
VIII	201.0±13.70[a,b,c,f]	<0.001**	30.9	43.0±18.89[a,b,c,f]	<0.001**	87.4	

I: Negative control group; **II:** Positive control group; **III:** Treated with NTZ; **IV:** Treated with *E. purpurea*; **V:** Treated with *E. purpurea*/NTZ; **VI:** Treated with NTZ@ZIF-8; **VII:** Treated with *E. purpurea*@ZIF-8; **VIII:** Treated with *E. purpurea*/NTZ@ZIF-8; **@:** *P* value versus control groups; **#:** Wilcoxon signed ranks test. *: Significant (P<0.05); **: Significant (P<0.001); **[a]:** Significant versus positive control group, **[b]:** Significant versus group III, **[c]:** Significant versus group IV, **[d]:** Significant versus group V, **[e]:** Significant versus group VI, **[f]:** Significant versus group VII.

Histopathological examination: Intestinal tissue sections from non-infected control negative mice showed normal structure with average villi width and length and no pathological changes to the mucosa or lamina propria. The control positive group displayed *Cryptosporidium* various developmental stages at the brush border of the villi, with loss of villous architecture as well as villous shortening, widening, and even sloughing. In addition, inflammatory cells were seen in the center of the villi infiltrating the submucosa, along with hyperplasia and a decrease in goblet cells in the covering epithelium. Signs of improvement were detected in the treated groups, including a reduction in parasite load at the brush borders and a decrease in inflammation. The mice treated with NTZ only or *E. purpurea* only or combination of both showed moderate histopathological changes. Regarding treatment with NTZ or *E. purpurea* loaded ZIF-8 NPs or their combination; the histopathological alterations were reduced with a return to the typical villous pattern and minimal inflammation (Fig. 3).

Oxidative stress and antioxidant activity: Cryptosporidiosis induced oxidative stress by a reduction of SOD activity (82.89±6.12) and an elevation of MDA level (18.27±8.17) in the infected non-treated mice compared with the non-infected mice (P<0.05). The levels of SOD (109.05±13.01) and MDA (3.65±2.13) were almost normal in the group treated with combination of NTZ and *E. purpurea* loaded

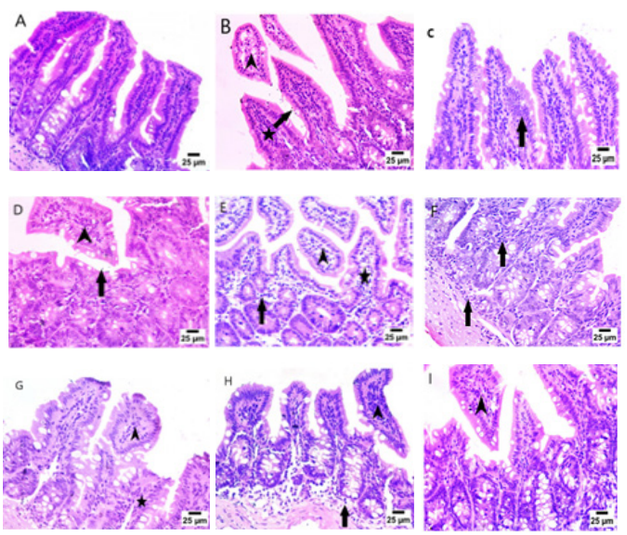

Fig. 3. Histopathological results of the H&E-stained small intestinal sections: **(A)** Normal villus pattern with no pathological lesions (negative control group); **(B)** *Cryptosporidium* developmental stages at the villi's brush border (arrow), shortage and thickening of the villi (star) with sloughing of the upper tips of some villi (arrow head) (positive control group); **(C)** Hyperplasia in epithelium of intestinal villi (arrow) (positive control group); **(D)** Different developmental stages of *Cryptosporidium* at the brush border of epithelial cells of the villi (arrow), and sloughing of the upper tips of some villi (arrow head) (NTZ treated group); **(E)** Villi are shortened and thickened (star) and some of them sloughed at their tips (arrow head) with infiltration by mononuclear inflammatory cells (arrow) (*E. purpurea* treated group); **(F)** Mucosal infiltration by inflammatory cells mainly lymphocytes and eosinophils (arrows) (Combined NTZ and *E. purpurea* treated group); **(G)** Villi are shortened and thickened (star) and some of them sloughed at their tips (arrow head) (NTZ @ZIF-8 treated group); **(H)** Sloughing off villi's upper tips (arrow head) (arrow head) with infiltration of submucosa by mononuclear inflammatory cells (arrow) (*E. purpurea* @ZIF-8 treated group); **(I)** Sloughing off villi's tips (arrow head) (Combined NTZ and *E. purpurea* @ZIF-8 treated group).

on ZIF-8 NPs (GVIII) compared to positive control group (P<0.001) followed by the group that received a combination of NTZ and *E. purpurea* (GV) with high SOD (103.79±9.85) and low MDA levels (4.17±1.26) (P<0.001). The mice treated with *E. purpurea* alone or loaded on ZIF@8 NPs (GIV, GVII) showed high SOD level (P<0.001) and low MDA level (P<0.05) compared to positive control group (Table 2).

Table 2. Serum levels of the antioxidant enzyme superoxide dismutase and the oxidative stress marker malondialdehyde among the studied groups.

Groups	SOD			MDA		
	Mean ± SD	$P^{@}$	%$^{\#}$	Mean ± SD	$P^{@}$	%
I	116.27±12.04	-	-	2.01±0.49	-	-
II	82.89±6.12^{a}	-	-	18.27±8.17^{a}	-	-
III	86.57±5.09^{a,d}	0.593	-4.4	15.45±6.56^{a}	0.637	15.4
IV	99.54±7.43^{a,b}	<0.001**	-20.1	12.22±4.17^{a}	0.202	33.1
V	103.79±9.85^{b,d}	<0.001**	-25.2	4.17±1.26^{b,c,d}	<0.001**	77.2
VI	91.10±4.91^{a,e,g}	0.122	-9.9	14.82±4.92^{a,e}	0.554	18.9
VII	102.97±8.55^{b,c}	<0.001**	-24.2	11.65±6.45^{a,e}	0.116	36.2
VIII	109.05±13.01^{b,e,f}	<0.001**	-31.6	3.65±2.13^{b,c,d,f,g}	<0.001**	80.0

I: Negative control group; **II:** Positive control group; **III:** Treated with NTZ; **IV:** Treated with *E. purpurea*; **V:** Treated with *E. purpurea*/NTZ; **VI:** Treated with NTZ@ZIF-8; **VII:** Treated with *E. purpurea*@ZIF-8; **VIII:** Treated with *E. purpurea*/NTZ@ZIF-8; **SOD:** Superoxide dismutase; **MDA:** Malondialdehyde; **$^{@}$:** P value versus control groups; **$^{\#}$:** Changes from positive control group (GII); ****:** Significant (P<0.001); **^{a}:** Significant versus positive control group, **^{b}:** Significant versus group III, **^{c}:** Significant versus group IV, **^{d}:** Significant versus group V, **^{e}:** Significant versus group VI, **^{f}:** Significant versus group VII.

DISCUSSION

Although public sanitation services have significantly improved, protozoan parasite-induced gastrointestinal diseases still represent a serious threat to the health of people and animals. A major obstacle to the control of cryptosporidiosis is the absence of effective medications and vaccinations[29]. *Echinacea* plant utilized as a dietary supplement, assists in the prevention and treatment of numerous infections, particularly in children, the elderly, and individuals with impaired immune systems[11]. Therefore, the current study evaluated the effectiveness of *E. purpurea* alone or loaded on ZIF-8 NPs in treatment of *Cryptosporidium* infected immunocompromised mice.

This study revealed that *E. purpurea* significantly reduced oocyst shedding with a reduced rate of 62.9%. Furthermore, addition of *E. purpurea* to NTZ significantly reduced stool oocyst count (78.1%) compared to positive control group which is better than NTZ alone (72.7%). These findings agree with Atia *et al*.[19] who reported that treating cryptosporidiosis with *E. purpurea* alone significantly reduced the mean oocyst shedding (6.94±3.77) when compared to the positive control group. Additionally, the investigators claimed that the cure rate for combined *E. purpurea* and NTZ treatment was the greatest (90%) with a statistically significant difference in comparison to the group treated with *E. purpurea* only. Sarkari *et al*.[30] stated that *E. purpurea* extract decreased the size of cutaneous leishmaniasis lesions in experimentally infected mice compared with positive control mice, but without statistical differences. As reported by Junior *et al.*[31], *E. purpurea* significantly lowered the quantity of *Toxoplasma* tachyzoites in liver imprints and peritoneal fluid of mice infected with the RH strain. However, the number of brain cysts in mice infected with the ME49 strain was significantly increased by prolonged treatment. Although the exact mechanism of action of *E. purpurea* against these parasites is unknown, its caffeic acid content may be responsible for this effect. Chlorogenic acid for which numerous biological actions have already been described is one of the derivatives of caffeic acid[32-34]. Furthermore, it has been previously documented that derivatives of caffeic acid exhibit well-established antioxidant action[35]. Thus, taking *E. purpurea* supplements during infections may offer further host protection. An additional probable benefit of *E. purpurea* for infection control is *via* indirect induction of immunological changes during pathogen host interaction[8].

Regarding results of histopathology, the intestinal tissues of infected non-treated mice exhibited injury of villous architecture, and even villous sloughing which are caused by both the parasite's released toxins leading to injury of the intestinal epithelium and the host's released inflammatory metabolites as a response to infection[36]. However, adding *E. purpurea* to NTZ retained the normal villus pattern with minimal inflammation. These results agreed with Atia *et al*.[19] who documented the amelioration of histopathological abnormalities by *E. purpurea* in intestinal tissue sections of immunosuppressed *Cryptosporidium*-infected mice. These findings may be explained by the polysaccharide components of *Echinacea*, as they have the capacity to promote tissue regeneration[37].

Oxidative stress is the consequence of an imbalance between pro-oxidants and antioxidants that results

in cell damage and tissue injury. This disturbance produces reactive oxygen species (ROS). If ROS are not eliminated in a safe and effective manner, oxidative stress may have negative effects on health[38]. One of the main factors of intestinal tract infection-related cellular damage is the peroxidation of the lipid layer in the cell membrane caused by free radicals. Fatty acid peroxidation produces MDA[39]. On the other hand, the harmful superoxide radical is converted into less harmful hydrogen peroxide by the enzyme SOD[40].

Our study showed an elevation of MDA value and a reduction of SOD activity in the infected non-treated mice compared with the non-infected mice. These results clearly link the pathogenesis of cryptosporidiosis to the occurrence of oxidative stress. This is consistent with Mahran *et al*.[41] who documented significant reduction in SOD and total antioxidant capacity in *Cryptosporidium*-infected buffalo calves. Furthermore, it was noted that *C. parvum* infection of Swiss albino mice resulted in an elevation in MDA and a reduction in SOD levels[42,43]. Our study showed decrease in MDA by 33.1% and increase in SOD by 20.1% with NTZ treatment which agrees with several studies[18,44,45]. Addition of *E. purpurea* to NTZ showed better results as MDA decreased by 77.2% and SOD increased by 25.2%. Several studies discussed *E. purpurea*'s antioxidant properties[10,46,47]. *E. purpurea*'s active ingredients, which include caffeic acid and polyphenolics including cichoric acid, glycosylated flavonoids, and polysaccharides, may have an antioxidant effect[48]. The mechanism of antioxidant activity of *E. purpurea* is by the scavenging of the free radicals 1,1-diphenyl-2-picrylhydrazyl radical, hydroxyl radical, transition metal chelating and ABTS measured radical[49]. According to our study's findings, *E. purpurea* appears to be a promising medication for preventing oxidative damage to intestinal tissue caused by cryptosporidiosis because of its ability to stimulate superoxide dismutase and inhibit lipid peroxidation.

To our best knowledge, this is the first study examining the potential efficacy of *E. purpurea* and NTZ loaded on ZIF-8 NPs in the treatment of cryptosporidiosis. In this study, ZIF-8 was used as a drug-carrier due to its unique nontoxic, biocompatible properties and thermal and hydrothermal stability[50]. Our results revealed that ZIF-8 increased the drug efficacy; *i.e*., it increased the efficacy of NTZ, *E. purpurea,* and their combination by decreasing oocyst shedding. There was also a successful improvement of the histopathological changes, and oxidative stress in comparison to other treatment groups. This is consistent with the findings of Abdelhamid[51], who found that antibacterial drugs carried by ZIF-8 had higher biological activity than free agents. NPs have been shown to allow delivery of the drug to the target sites with controlled release. Furthermore, this targeted delivery causes decrease in the side effects of the drug and improvement of the patient's response[52]. Ahmed *et al*.[53] reported that loading drugs (antinal, septrin, and NTZ) on MOFs (copper-benzene tricarboxylate) reduced oocyst shedding in *Cryptosporidium*-experimentally infected mice. Studies documented that ZIF-8 nanocarrier was beneficial against *Trypanosoma* parasite infections *in vitro*[54], and ZIF-8/KIT-6/resveratrol and curcumin had anti-blastocystosis activity[55]. Both studies confirmed the potential ZIF-8 functions as an antiparasitic agent in addition to its delivery system.

In conclusion, *E. purpurea* has anti-*Cryptosporidium* and antioxidant activity. The best results were for *E. purpurea*/NTZ combination loaded on ZIF-8 NPs followed *E. purpurea*/NTZ combination. Accordingly, we suggest that addition of *E. purpurea* to NTZ in treatment of cryptosporidiosis is beneficial not only for its anti-*Cryptosporidium* activity, but also for its antioxidant activity. Additionally, ZIF-8 has been demonstrated as an effective drug delivery system.

Acknowledgement: The authors express their appreciation to Dr. Rania M. Waheed, Manager of Chemistry and Hematology Unit in the Central Laboratory, Faculty of Veterinary Medicine, Banha University for her help in measuring SOD and MDA.

Author contribution: Ali AT and El-Sayed FA suggested the topic and designed the study plan. All authors participated in performing the practical work, and writing the manuscript. All authors accepted the authorship and the final version before publication.

Conflict of interest: All authors state no conflict of interest.

Funding statement: There has been no funding.

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