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Nitazoxanide, Ivermectin, and Artemether effects against cryptosporidiosis in diabetic mice: parasitological, histopathological, and chemical studies

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Abstract Human cryptosporidiosis is one of the most significant causes of water borne epidemics of diarrhea worldwide. It is extremely important in immunocompromised hosts and malnourished children as it could cause severe life-threatening diarrhea. Despite the global burden of the disease, there are only few available therapies against cryptosporidiosis. Diabetes mellitus is a common metabolic disorder that impair both the innate and adaptive immune responses of the patient. This study aimed to test the effect of Nitazoxanide, Ivermectin, and Artemether against cryptosporidiosis in diabetic mice. Sixty white albino mice were categorized into 6 groups; 10 mice each: **GI:** normal non-infected non-treated (healthy- control), **GII-GVI** (diabetic groups), **GII:** non-infected non treated (diabetic control), **GIII:** infected non treated (infected control), **GIV:** infected and treated with Nitazoxanide (NTZ), **GV:** infected and treated with Ivermectin (IVC), **GVI:** infected and treated with Artemether (ART). Parasitological, histopathological, and chemical examinations were done to evaluate the effect of NTZ, IVC, and ART against cryptosporidiosis in diabetic mice. Parasitological examination revealed maximum reduction of oocyst shedding in GVI, while histopathological examination showed the least pathologic changes in GV with mild vascular wall fibrosis and moderate lymphocytic infiltration of islets of Langerhans. Measurement of blood glucose level showed the best results with GIV. Nitazoxanide is effective against cryptosporidiosis in diabetic patients with minimal hyperglycemia, Artemether is especially effective in reducing the oocyst shedding in stool, whereas Ivermectin is associated with the least pathological changes in pancreatic islets of Langerhans.
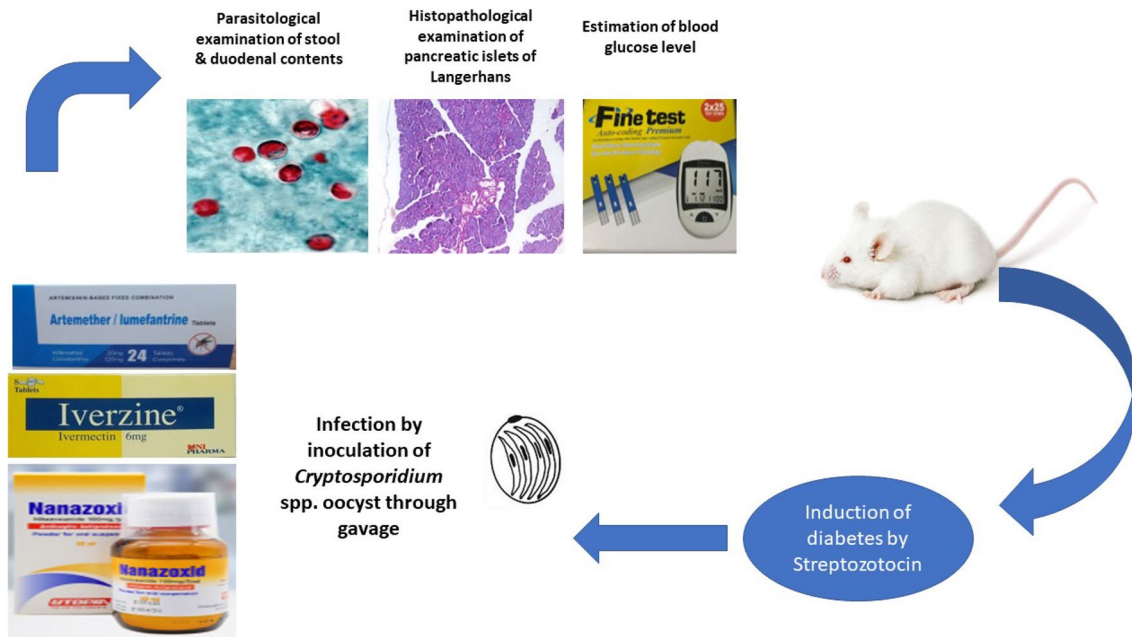
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Graphical abstract

Introduction

Cryptosporidiosis is considered as one of the most common causes of human diarrhea worldwide (Widmer et al. 2020) and 2nd to *Rotavirus*, as an important cause of water and food-borne diarrhea outbreaks in humans, especially children and patients with compromised immune system (Efstratiou et al. 2017; Ryan et al. 2018; Khalil et al. 2018).

Diabetes mellitus (DM) is one of the most common chronic metabolic disorders characterized by persistent hyperglycemia (Zheng et al. 2018). The innate and adaptive immune responses in diabetic patients are thought to be weakened because of hyperglycemia. That is why the immune system fails to control the spread of invading pathogens in diabetic patients. Therefore, diabetic patients are known to be more vulnerable to infections and are immunocompromised (Casqueiro et al. 2012; Berbudi et al. 2019). It is well known that diabetic patients are associated with greater incidence of bacterial and fungal infections (Robertson and Polk 1974), but clinical data on the association with parasites is extremely limited and not conclusive (Coovadia et al. 1993; Abaza et al. 1995; Nazligul et al. 2001).

Many parasitological research found higher prevalence of *Trichinella spiralis* larvae in mice with chemically-induced DM (Antonios et al. 1989) in addition to higher parasitemia and shorter survival rate in diabetic mice having *Trypanosoma brucei* (Amole et al. 1985) or *Trypanosoma cruzi* (Tanowitz et al. 1988; Nagajyothi et al. 2009). Many

studies reported the relation between DM and experimental schistosomiasis. Numerous studies used chemically induced DM in experimental mice, showed diminution in the size of the granulomas surrounding *Schistosoma* eggs in both the liver and the lung, because of the consequent impairment in the cell mediated immunity (Mahmoud et al. 1975; Mahmoud 1979). Human studies performed in Brazil gave us a clue supporting the postulate of a possible correlation between positive *Stongyloides stercoralis* serology and DM in humans (Mendonça et al. 2006).

Although the global burden of cryptosporidiosis on patients' health, the only FDA-approved antiparasitic treatment in immunocompetent patients is Nitazoxanide; however, it has failed to convince the researchers about its effectiveness among HIV-positive patients, immunosuppressed individuals, and malnourished children (Schneider et al. 2021). Besides, the drug is not recommended for use in children younger than 12 months (Amadi et al. 2002; Abubakar et al. 2007b).

Macrocyclic lactones constitute the big family to which the semi-synthetic compound Ivermectin (IVC) belongs to. It has wide range of activities against parasites and viruses beside its therapeutic effects against cancers (Laing et al. 2017; Momekov and Momekova 2020). It is recommended against large numbers of endoparasites as nematodes and ectoparasites like acarine and insects of humans and animals. Besides, IVC activity against protozoan parasites like *Giardia lamblia* and *Cryptosporidium* spp. was also reported

in many studies (Youssef et al. 1996; Zinada 2000; Hassan et al. 2001). Recently, IVC is used in mass drug administration for malaria (Smit et al. 2018). Moreover, IVC has been reported to enhance the cellular and humoral immune responses in rabbits (Sajid et al. 2007; Zhang et al. 2008; Omer et al. 2012). Ivermectin has been considered to possess anti-viral properties against COVID-19 (Horowitz and Freeman 2020).

Artemether (ART) is an extract of the qinghaosu "Chinese herbal remedy" that is active against multidrug resistant *P. falciparum* and are widely used in Asia by several routes like oral, parenteral, and rectal forms (Rosenblatt 1999). Artemether was formerly evaluated to suppress the growth of *Cryptosporidium* in cell culture (Wu et al. 2011).

With the increasing numbers of potential immune-altered diabetic patients, cryptosporidiosis may represent a major public health concern. However, there is a general lack of scientific research regarding the management of such protozoa infection in diabetic patients.

Therefore, the present study aimed to explore the anti-cryptosporidial action of NTZ, IVC and ART in diabetic mice and their effect on the blood glucose levels in these mice.

Material and methods

Laboratory animals

Animal source

Laboratory male, white Albino mice of CDI strain, about 4–6 weeks old, weighing 20-25 g, were obtained from Theodor Bilharz Research Institute (TBRI) animal house. The Biological Unit of TBRI was the venue for animal experiments. Animals were kept in a well-ventilated plastic cage with clean wood-chip bedding in conditioned rooms (24 ± 1 °C) and away from direct sunlight with good sanitary conditions. All animal experiments were done following the internationally valid guidelines after the approval of the institutional ethical committee of TBRI.

Experimental design

60 laboratory male white albino mice were used in this study. Because female mice are less sensitive to this islet-cell toxin, most (streptozotocin) STZ-induced diabetic mouse studies are conducted on male animals (Kolb 1987).

Mice were divided into 6 main groups, each containing 10 mice; GI: nondiabetic, GII-GVI: diabetic:

- **GI:** Normal non-infected non-treated (healthy- control)
- **GII:** diabetic non-infected non treated (diabetic control)

- **GIII:** diabetic infected non treated (infected control)
- **GIV:** diabetic infected and treated with Nitazoxanide
- **GV:** diabetic infected and treated with Ivermectin
- **GVI:** diabetic infected and treated with Artemether

Induction of diabetes (**Donovan and Brown 2006a; Furman 2015**)

DM was induced by the administration of the drug STZ (Sigma, St. Louis, USA) in GII-GVI.

1. House 2 to 5 male mice per cage at temperature of 24 °C ± 1 °C and 55% ± 5% humidity, with a 12-h light–dark cycle (light on at 8:00 and off at 20:00) for at least 5 days before initiating the experiment. Free access to food and water was allowed to the mice.

The technique mentioned below is designed to reduce variability, group sizes of 10 mice are recommended given the morbidity associated with the STZ treatment.

2. Each group should contain the same number of mice.
3. On the first experimental day, food should be removed from all cages 4 hr prior to STZ. Give water as normal.

-Treat animals with STZ

4. Put 4 mg of STZ into a 1.5 ml microcentrifuge tube then use aluminum foil to cover the tubes; use one tube for 3 mice. Prepare the citrate buffer.
5. Immediately before the injection, dissolve the STZ in 50 mM sodium citrate buffer (pH 4.5) to reach a concentration of 4 mg/ml.

As STZ damages in 15 to 20 min after dissolving in the citrate buffer, the STZ solution should be prepared just before use and injected within 5 min of dissolution.

6. Administer the STZ solution through intraperitoneal injection at a dose of 40 mg/kg body weight (bw) (1.0 ml/100 g) using 1-ml syringes and 25-G needles.
7. The mice were returned to their cages. Allow free access to normal food and 10% sucrose water.
8. Steps 3 to 7 were repeated on days 2 to 5 (the next 4 days).
9. On day 6, replace the 10% sucrose water with regular water.
10. On day 14 (9 days after the last STZ injection), fast all mice for 6 hr (e.g., from 7:00-13:00). A blood glucose test was done from a tail-vein blood sample (Donovan and Brown 2006b). The blood drop was applied to a strip of "Fine test Auto- coding Premium strips" apparatus for measuring the blood glucose to ensure

hyperglycemia in the STZ-treated subjects. This was also repeated at 12th day post infection (dpi).

Animal infection

Cryptosporidium spp. oocysts were obtained from the Animal Research Institute in Giza governorate, Egypt. Mice were orally infected with Cryptosporidium *Cryptosporidium spp*. oocysts by gavage using esophageal tube at the 14th day of the experiment. The infection dose per mouse was a suspension of 10^3 oocysts dissolved in 200µL of PBS (Abdelmaksoud et al. 2020).

Drugs administration

- *Nitazoxanide (NTZ)* was given in a dose of 100 mg/kg bw daily starting on 7th dpi for 5 consequent days (Li et al. 2003). Nitazoxanide was obtained in the form of (Nanazoxid®) (100 mg/5 ml suspension) manufactured and provided by [Medizen Pharmaceutical industries for Utopia Pharmaceuticals].
- The doses were calculated by extrapolation of human therapeutic doses to animal doses according to the table of (Barnes and Paget 1965).
- *Ivermectin (IVC)* was given in a dose of 200 mg/kg bw once starting on 7th dpi through oral gavage (Zinada 2000). Ivermectin was obtained in the form of (Iverzine ®) produced by Unipharma (Universal Pharmaceutical Industries) Al Obour City, Cairo, Egypt, in the form of 6 mg tablets. IVC was dissolved in distilled water.
- *Artemether (ART)* was given in a dose of 400 mg/kg bw once starting on 7th dpi through oral gavage (Fayer and Ellis 1994). Artemether was purchased from Kunming Pharmaceutical Cooperation (Artemidine®; Kunming, People's Republic of China) in the form of 50 mg tablets. ART was suspended at a concentration of 20 mg/mL in a solution of 0.5% ethanol, 1% gum tragacanth and 7% Tween 80 in water.

3. Parasitological examination

- *Stool examination* Stool samples were collected daily after infection throughout the experiment and subjected to parasitological examination after staining by modified Ziehl–Neelsen (MZN) stain according to (Henricksen and Pohlenz 1981) to ensure that mice have been infected and to count *Cryptosporidium species* oocysts per oil immersion lens ($\times$ 100). Faecal samples were collected from each mouse after transferred into an individual clean cage. The samples were then put into clean, wide-mouthed containers with tight-fitting covers and homogenized in PBS to evaluate *Cryptosporidium* species oocysts shedding. The number of oocysts was
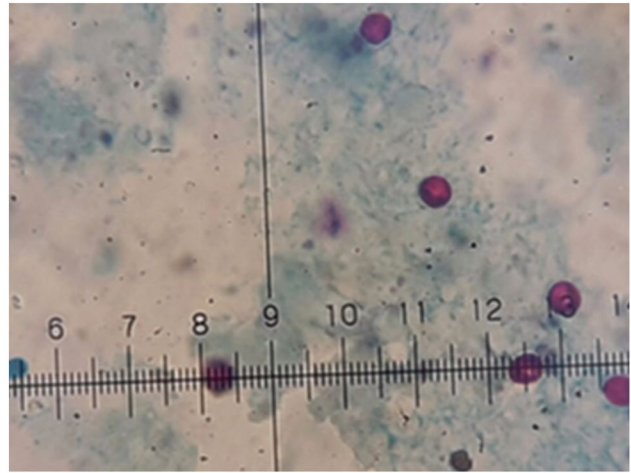

Fig. 1 Micrometer for measuring the size of oocysts

counted and then calculated/gm feces (Benamrouz et al. 2012). The mean oocyst shedding for each study group was calculated at 12th dpi.

- *Duodenal content examination* Scarification of mice was done on 12th dpi; their duodenal contents were subjected to duodenal examination. The duodenal contents were then put into clean, wide-mouthed containers with tight-fitting covers and homogenized in PBS to evaluate *Cryptosporidium* spp. oocysts shedding. The number of oocysts was counted and then calculated/ml by the MZN staining technique as described by (John and Petri 2006).

4. Histopathological examination

Scarification was done at the 12th dpi and euthanasia was performed by decapitation (Boivin et al. 2016). Pancreas was removed from each animal, fixed in 10% buffered formalin solution, embedded in paraffin wax blocks that were sectioned then stained in the pathology lab of TBRI, staining was done using Hematoxylin and Eosin (H&E) to assess the pathological changes and explore any abnormal patterns (Feldman and Wolfe 2014). Pancreatic architecture, vascular wall changes, and pathological changes in the islets of Langerhans were reported through subjective qualitative evaluation by an expert histopathologist.

5. Estimation of blood sugar level

A sterile lancet was applied on the tail vein of the mouse, then blood drop was applied to a strip of "Fine test Autocoding Premium strips" apparatus for measuring the blood glucose level that was imported by "Vanamedica Co., Exclusive Agent, Cairo- Egypt from OSANG Healthcare Co., Ltd-Korea".

Table 1 Post-Hoc test for comparison between the different study groups regarding *Cryptosporidium* spp. oocyst shedding in stool

Group		P-value
GIV vs	GIII	< 0.001
	GV	0.529
	GVI	0.861
GV vs	GIII	< 0.001
	GVI	0.491
GVI vs	GIII	< 0.001

[***]Statistically significant difference at $P < 0.001$ highly sig

6. Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. Data were presented as mean, standard deviations. The comparison between groups were done using One Way ANOVA test followed by post hoc analysis using LSD test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at the level of < 0.05.

Table 2 Comparison between the studied groups regarding the intestinal *Cryptosporidium* stages and number of oocysts in stools

Groups	Cryptosporidium stages in duodenal contents (Mean ± S)	% of reduction	Oocysts/ gm stool (Mean ± S)	% of reduction
GI	0		0	
GII	0.8 ± 0.74		350 ± 5.47	
GIII	16.82 ± 3.38		10,854 ± 28.94	
GIV	6.09 ± 2.75	63.79	1400 ± 22.16	87.10
GV	7 ± 3.54	58.38	1800 ± 25.18	83.42
GVI	5.82 ± 3.95	65.39	1250 ± 22.17	88.48

Fig. 2 a, **b** Sections in pancreas of GII mice showing mild vascular wall fibrosis (yellow arrow) and nearly normal number and size of islets of Langerhans (red arrow) (H&E stain, A: X200, B: X400)
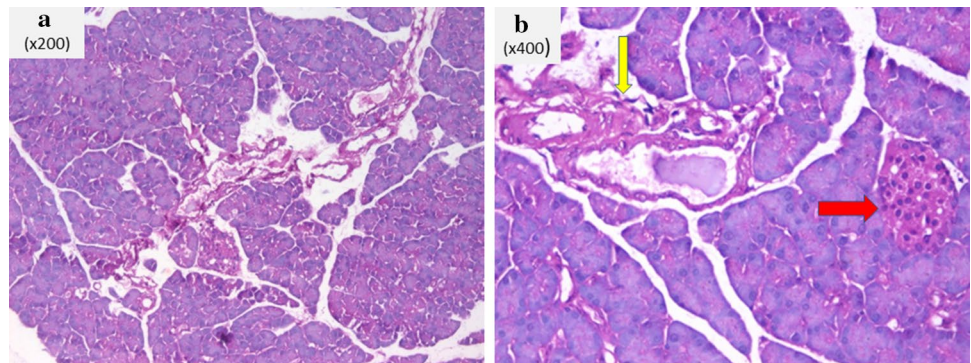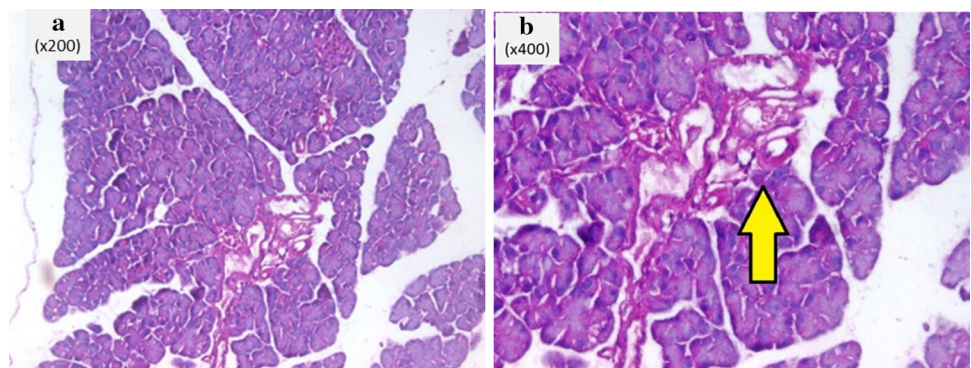

Fig. 3 a, **b** Sections in pancreas of GIII mice showing mild vascular wall fibrosis (yellow arrow) and decrease in number and size of islets of Langerhans (H&E stain, A: X200, B: X400)

7. Ethical consideration

This study was approved by Scientific Research Ethics Committee of TBRI for the project number 109 ع with title "Effect of synthetic and natural agents on molecularly characterized *Cryptosporidium* species: an experimental model".

Results

Parasitological examination

Oocysts of *Cryptosporidium spp*. were visualized at the 7th dpi as red spots measuring 4-6 μm by using a micrometry and with refractile round thick capsules on blue background when examined by light microscope with × 1000 objective (Fig. 1). The number of shed oocysts in the evacuated duodenal contents and in stool of infected mice of different treated study groups (GIV, GV, and GVI) showed a statistically significant difference compared to the infected control group; GIII (*P*-value < 0.001) (Table 1). The best result was observed in GVI (diabetic and treated with ART) in which the percentages of reduction was 65.39%, and 88.48% by duodenal content and stool examinations respectively compared to 63.79% and 87.10 for GIV, and 58.38% and 83.42% for GV, although there was no statistically significant difference between the GIV, GV, and GVI (Table 2).

Fig. 4 **a**, **b** Sections in pancreas of group GIV mice showing moderate vascular wall fibrosis (yellow arrow) and dense lymphocytic cellular infiltration of islets of Langerhans (red arrow) (H&E stain, A: X200, B: X400)
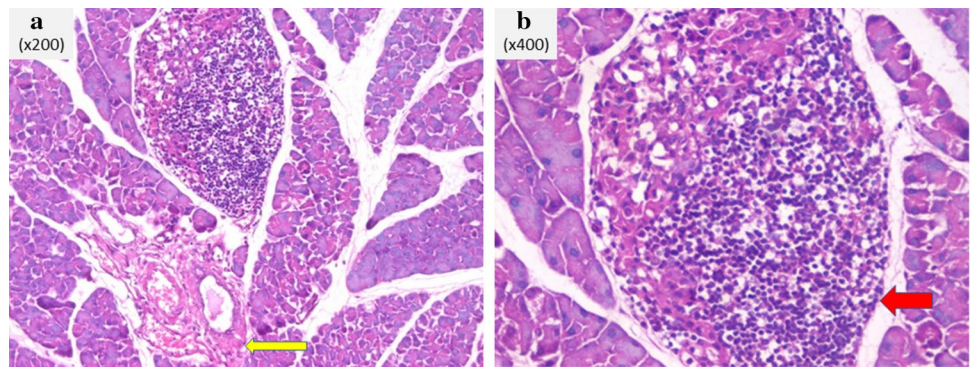

Fig. 5 **a**, **b** Sections in pancreas of GV mice showing moderate vascular wall fibrosis (yellow arrow) and moderate lymphocytic cellular infiltration of islets of Langerhans (red arrow) (H&E stain, A: X200, B:X400)
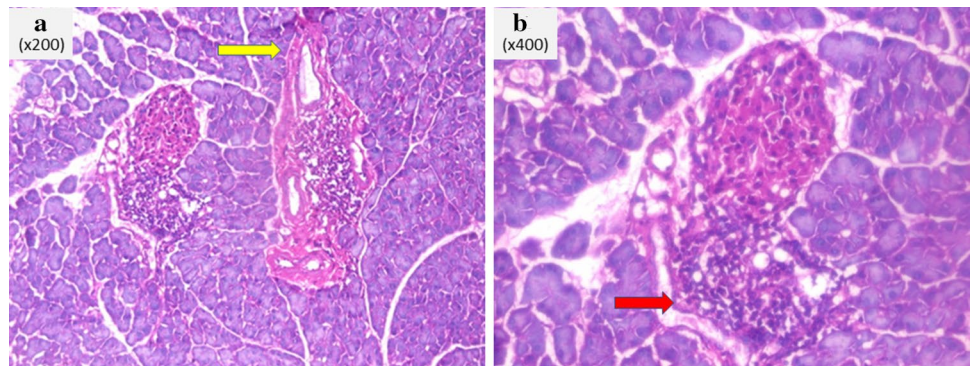

Fig. 6 **a**, **b** Sections in pancreas of GVI mice showing mild vascular wall fibrosis (yellow arrow) with hyperplastic islets of Langerhans (red arrow) (H&E stain, A: X200, B:X400)
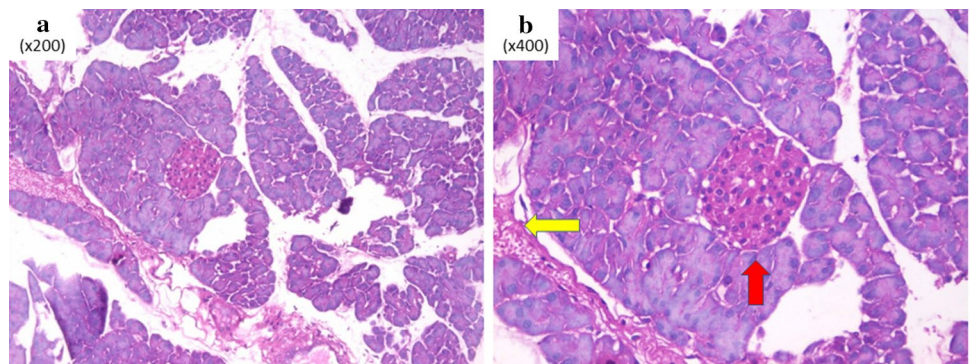
Table 3 Comparison between the different study groups regarding the blood glucose level

Groups	Blood glucose level (mg/dL)	Post-Hoc test	
		Group	*P*-value
GI (normal mice)	117 ± 6.68		
GII (diabetic non infected)	169.93 ± 22.64		
GIII (diabetic and infected)	171.43 ± 20.01		
GIV (diabetic, infected and treated with NTZ)	193 ± 56.76	Vs GIII	< 0.001
		Vs GV	< 0.001
		Vs GVI	< 0.001
GV (diabetic, infected and treated with Ivermectin)	246.75 ± 36.05	Vs GIII	< 0.001
		Vs GVI	< 0.001
GVI (diabetic, infected and treated with Artemether)	224.67 ± 67.63	Vs GIII	< 0.001

Histopathological examination

GII (diabetic control) showed normal pancreatic histological architecture with mild vascular wall fibrosis (Fig. 2 a, b). Pancreatic sections of GIII (infected control) showed mild vascular wall fibrosis and decrease in number and size of islets of Langerhans (Fig. 3 a, b). Pancreatic sections of GIV showed moderate vascular wall fibrosis, hyperplastic islets of Langerhans with dense focal lymphocytic infiltration (Fig. 4 a, b). Pancreatic sections of GV mice showed mild vascular wall fibrosis and moderate lymphocytic cellular infiltration of islets of Langerhans (Fig. 5 a, b). Sections in pancreas of GVI mice showed mild vascular wall fibrosis with hyperplastic islets of Langerhans (Fig. 6 a, b).

Results of measuring the blood glucose level for the mice of different study groups

Blood sugar level for mice of the different study groups showed a statistically significant difference with GI (normal mice). Nitazoxanide, Ivermectin, and Artemether caused significant elevation of blood glucose in the treated groups when compared to the infected control group (*P*-value < 0.001) with mean blood glucose levels (193 ± 56.76, 246.75 ± 36.05, and 224.67 ± 67.63) respectively (Table 3).

Discussion

In the present study, a mice model was used to evaluate the effects of NTZ, IVC, and ART against cryptosporidiosis in diabetic hosts. It is noteworthy that animal models have always played an important role in the exploration of disease pathophysiology and in the evaluation of novel therapeutic agents in vivo (Al-Awar et al. 2016).

Streptozotocin (STZ) was used to induce diabetes in the mice model in our experiment to establish immunosuppression in mice which was needed to ensure the continuity of *cryptosporidium* spp. infection. In the present study, we used male mice as an animal model for STZ-induced diabetes as female mice are less sensitive to this islet-cell toxin (Kolb 1987). Streptozotocin compounds were considered the most powerful diabetogenic chemicals used in diabetes research until now (Lenzen 2008). Deeds et al (2011) stated that STZ-induced DM offers an extremely cost-effective and rapid technique that can be used in the majority of rodent strains. In spite of the common use of STZ in small animal models, the data regarding the preparation, dosing, and administration of the drug, in addition to the time of induction and severity of DM, and subsequent morbidity and mortality are still limited. Accordingly, researchers with poor experience with STZ-induced DM may find many difficulties in accurately designing new research using STZ with its potential toxicity. Till the advancement of research about finding alternatives to STZ as a DM inducer, trials should focus on how to overcome the shortcomings of the current STZ-induced DM models (Deeds et al. 2011).

Nitazoxanide is still the only chemotherapeutic agent against cryptosporidiosis with the approval from the Food and Drug Administration (FDA) in the United States. It helps the parasite clearance through inhibiting its anaerobic metabolism which is important for energy production (Abaza et al. 2016). It is mainly effective against cryptosporidiosis in immunocompetent patients but its efficacy is frustrating in malnourished children, children below 12 years old, immunodeficient and AIDS patients (Abubakar et al. 2007a, b; Amadi et al. 2009; Cabada and White 2010).

In the present study, IVC and ART were tested as alternative therapies against cryptosporidiosis in diabetic mice and the results were compared to the golden standard drug (NTZ). The side effects and the increasing resistance to the available antiparasitic drugs used in the treatment of *C. parvum* make the researchers face a great challenge to find alternatives that are not toxic and easily obtained in adequate

quantities to replace the standard drugs in the market (Rayan et al. 2005).

El Wakil et al. tested the prophylactic and therapeutic effects of Mefloquine (MQ) on experimental cryptosporidiosis in immunocompromised mice. The study revealed a significant prophylactic effect of MQ on cryptosporidiosis in immunosuppressed mice with 77% reduction of oocyst shedding with only 42% reduction in the group prophylactically treated with NTZ. They also reported 100%, and 53% clearance of oocyst when MQ and NTZ were administered at 14th dpi respectively (El-Wakil et al. 2021). Medicinal herbs were used for the treatment of various parasitic infections all over the world so many years ago and provided good alternatives to ordinary drugs (Behnke et al. 2008). Methanol extract of the natural herb Asafoetida effectively reduced *Cryptosporidium* parasites in experimentally infected mice and ameliorate the histopathological changes of small intestinal villi at the ileocaecal region with preservation of liver architecture (Abdelmaksoud et al. 2020). *Verbena officinalis* was also used against cryptosporidiosis in the immunosuppressed mice with recorded efficacy in reducing *Cryptosporidium* oocyst shedding by 74% compared to 66% for NTZ (El-Wakil et al. 2022). Coconut oil (CO) was also tried against cryptosporidiosis in immunocompromised mice. Parasitological studies revealed anti-cryptosporidial activity of CO that was better than NTZ with a statistically significant difference ($P = 0.003$). Histopathological examination of specimens from the ileocecal regions of treated groups showed dramatic improvement of the pathological changes with a preserved villous pattern, and very mild villous core inflammation in CO treated group that exceeded the healing effect induced by NTZ. Immunohistochemical examination showed a promising ability of CO to reduce the incidence of dysplastic changes in chronic cases (Abdelmaksoud et al. 2022a). Wheat germ oil (WGO) gave extremely promising results against cryptosporidiosis in immunosuppressed mice as it significantly reduced the shed of *Cryptosporidium* oocyst in the stool of infected mice compared to the group treated with NTZ (P-value < 0.001). Besides, WGO was proved to decrease intestinal villi inflammation with prevention of dysplasia and malignancy of chronically infected immunosuppressed mice with cryptosporidiosis (Abdelmaksoud et al. 2022b).

Parasitological examination of the evacuated duodenal contents and stool examination revealed the best results observed in GVI (diabetic and treated with ART) in which the efficacy for reducing the oocyst shedding was 65.39%, and 88.48% for duodenal content and stool examinations respectively compared to GIII (P-value < 0.001) and better than G IV (diabetic and treated with NTZ) and GV (diabetic and treated with IVC) although, there was a non-statistically significant difference between them. GVI showed better efficacy of reducing the oocyst shedding than GV both in the

duodenal contents (63.79%, and 58.38% respectively) and stool examinations (87.10% and 83.42% respectively). Our results are comparable to (Fahmy et al. 2021) who reported reduction of *Cryptosporidium* oocyst shedding in the stool of infected mice treated with NTZ by 63% whereas the group of mice treated with IVC showed reduction by 82%. In the present study, mice were inoculated with 10^3 oocysts while in their study the inoculum was 10^4 oocysts. In the present study, NTZ was used in a dose of 100 mg/kg/bw for 5 consecutive days and IVC was used in a dose of 200 mg/kg bw once whereas NTZ was used in dose of 250 mg/kg / day for 10 consecutive days and IVC was used as 2 mg/ kg once in their study. All the previous factors may affect the efficacy of the tested drugs. Parasitological examination results of the current work were against a study that recorded marked reduction of *Cryptosporidium andersoni* growth on the invitro cultures (HCT-8 cells) after exposure to NTZ for 48 h, but the number of parasites did not significantly decrease when exposed to ART (Wu et al. 2011).

Histopathological and chemical examinations showed detrimental effect of the tested drugs on the islet cells of Langerhans in the form of mild to moderate vascular wall fibrosis, lymphocytic infiltration, with hyperplastic changes. Using NTZ, IVC, or ART caused a statistically significant elevation of blood glucose level of the corresponding study groups compared to the infected control group (P-value < 0.001). Our results are comparable to a study reported that a significant improvement was noted on the histopathological changes at the ileal region of different groups of infected immunosuppressed mice treated with IVC alone or combination of NTZ and IVC or NTZ with Selenium, or IVC with selenium but no significant improvement was noted when NTZ was used alone (Fahmy et al. 2021).

We concluded that, every one of the three tested drugs has its own advantage in the treatment of cryptosporidiosis in diabetic mice. Nitazoxanide is effective against cryptosporidiosis in diabetic patients with minimal hyperglycemia, ART has a superior effect in reducing the oocyst shedding in stool, while IVC is associated with the least pathological changes in pancreatic islets of Langerhans. It is highly recommended to take an advanced care when using these drugs in diabetic infected patients as they were proved to elevate the blood glucose level in these patients. Further studies should evaluate the blood glucose level of the diabetic study groups in a daily manner to report the changes of the glycemic status of the tested mice throughout the experiment. It is also recommended for the following research to make a close observation for the weight, clinical appearance, and the rate of maximal survival for the studied animal groups. Additional studies should also focus on evaluating the efficacy of combination for the aforementioned drugs with adjustment of the proper dose to maximize the benefits and avoid the undesired side effects. Further studies should

build on the findings of this study and compare the results of this research with experimental trials including controlled diabetic animal groups.

Author contribution AHF, SM, HS, NG, WEK, and EA designed the plan of work and performed the parasitological and chemical studies. ATS performed histopathological studies of the pancreatic sections. EAM shared in designing the plan of work and analyzing the data. All authors shared in writing and revising the manuscript. The manuscript was read and approved by all named authors. We further confirm that the order of authors listed is approved by all authors.

Declarations

Conflicts of interest The authors declare that there is no conflict of interest.

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