Journal of the Egyptian Society of Parasitology, Vol. 55, No. 2, August 2025
J. Egypt. Soc. Parasitol. (JESP), 55(2), 2025: 269 – 279
Online: 2090-2549

EVALUATION OF THE EFFECT OF NITROFURANTOIN ON EXPERIMENTAL *CRYPTOSPORIDIUM PARVUM* INFECTION IN IMMUNOSUPPRESSED MICE

By
ASMAA M. BARAKAT[1], DALIA S. ASHOUR[1], REDA.H. ELBAKARY[2] and WESAM M. A. ELGENDY[1]
[1]Department Medical Parasitology and [2]Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt
(*Correspondence: asmaabarakat1295@gmail.com)

Abstract

Cryptosporidium parvum, a worldwide zoonotic parasite and in immunosuppressed patients can be fatal. Nitazoxanide® (NTZ) is effective in immunocompetent patients, but unsatisfactory in immunosuppressed ones. This study evaluated nitrofurantoin® (NF) as an anti-cryptosporidial drug in immunosuppressed mice.

A total of 100 immunosuppressed mice were divided into 5 groups of 20 mice each: GI: neither infected nor treated, GII: infected non-treated, GIII: infected and NTZ (250mg/kg/day) treated, GIV: infected and NF 100mg/kg/day) treated, and GV: infected and NTZ & NF treated. All drugs were given orally on 4th day post infection (P.I.) for 7 consecutive days, and then mice were gently sacrificed for parasitological, histopathological, ultra-structural and biochemical assessment after one and two weeks post treatment (i.e. 11th & 18th day P.I.)

GV showed the highest oocysts reduction count (65.7%, & 80%), followed by GIV (48.6%, & 59.8%) but, GIII showed the least effect (25.4%, & 30.2%) on 11th, & 18th day P.I. respectively. Histopathological and TEM examinations showed marked restoration of normal intestinal structure in GV, followed by significant improvement in GIV, but GIII showed partial improvement with persistent dysplastic changes in enterocytes and marked ultra-structural abnormalities. Both NTZ &NF showed significant reduction of MDA level, however, NF was more effective in elevating GSH level than NTZ. Both NF & NTZ restored normal liver levels of SGOT & SGPT, compared to significantly increase positive control, normal kidneys levels of urea & creatinine.

Keywords: *C. parvum*, Immunosuppressed mice, Nitrofurantoin, Nitazoxanide, Evaluation.

Introduction

Cryptosporidiosis is one of the major public health problems with high morbidity, and mortality mainly among immunocompromised persons (CDC, 2025). There are about 20 species infect mammals, birds, fish and reptiles (Xiao *et al*, 2004). However, zoonotic *Cryptosporidium hominis*, and *C. parvum* are obligatory intracellular apicomplexan protozoan of global distribution including all the Arab Countries (El Bahnasawy *et al*, 2018). It is considered a culprit for numerous water-borne outbreaks, causing self-limited diarrhea in immunocompetent patients, or a life-threatening disease in immunocompromised patients with persistent watery diarrhea, respiratory and biliary cryptosporidiosis that could be fatal (Helmy and Hafez, 2022). Man is infected by ingestion of oocysts in contaminated food or water or even from animal contract (Youssef *et al*, 2008). Also, patient- to-person transmission occurred commonly particularly among the house-members, schoolchildren and/or day care centers, and even the healthcare workers (Musher and Musher, 2004)

Despite many decades passed since cryptosporidiosis discovery neither effective treatment nor vaccination was available (Innes *et al*, 2020). Nitazoxanide (NTZ) is considered only approved drug by FDA for cryptosporidiosis treatment, however, it is effective only in the immunocompetent patients with little or no effect at all in immunocompromised ones (Khan and Witola, 2023).

Nitrofurantoin (NF) is an effective antibacterial drug, approved for treatment of lower urinary tract infection. It is a broad-spectrum antibiotic effective against many gram-positive and gram-negative organisms, without development significant resistance till now, may be due to its various mechanisms of action. Reduction of the nitro group of nitrofurantoin by intracellular nitroreductases produces active form of the drug with highly active intermediate me-

tabolites, which bind to bacterial ribosomes causing inhibition of many bacterial enzymes involved in the synthesis of DNA, RNA and other metabolic ones (Huttner *et al,* 2015).

The nitrofurantoin has shown anti-parasitic agent against *Trypanosoma congolense*-induced animal African Trypanosomiasis (Suganuma *et al,* 2022), and as an effective therapy for acute and chronic toxoplasmosis (Yeo *et al,* 2016), but the exact mechanism as anti-parasitic action was not fully studied (Abdallah *et al,* 2022). This raised a question if nitrofurantoin has anti-*Cryptosporidium parvum* action (Ctrnacta *et al,* 2006) *Cryptosporidium* sp. contains a unique fusion protein pyruvate: $NADP^+$ oxidoreductase (CpPNO) composed of 2 distinct, conserved domains, a N-terminal pyruvate; ferredoxin oxidoreductase (PFO) the nitazoxanide target and the C-terminal cytochrome P450 reductase or CPR (Khan and Witola, 2023). Wang *et al.* (2008) reported that the role of cytochrome P450 reductase in nitrofurantoin-induced redox cycling. Besides, *Cryptosporidium*-induced oxidative stress was implicated in pathogenesis of immunosuppressed mice (Bhagat *et al,* 2017). The decreasing oxidative stress may allow the host to eliminate the pathogen and reduces the tissue damage (Atia *et al,* 2021).

This study aimed to evaluate nitrofurantoin (NF) anti-cryptosporidial and antioxidant effects in *Cryptosporidium parvum* infected immunosuppressed mice as compared to NTZ alone or combined with NF.

Materials and Methods

Study design: One hundred parasite-free laboratories bred male Swiss Albino mice weighed 20-25g and aged six weeks were purchased from Theodor Bilharz Research Institute (TBRI), Giza. Mice were housed in the experimental animal house, Medical Parasitology Department and maintained on normal diet and water for a week to adapt the new conditions before being experimented with. Also, the *Cryptosporidium parvum* oocysts were purchased from TBRI.

Drugs: 1- Dexamethasone® (Dexazone; Al Kahira Pharmaceutical and Chemical Indudstries Co., Cairo). Tablet (0.5mg) was dissolved in distilled water and was given orally in a dose of 0.25µg/g/day to all mice 14 successive days before oocysts inoculation and continued to the study end to cause the immune sup-p ression (Rehg *et al,* 1988). 2- Nitazoxanide® (Nanazoxid; 100mg/5ml suspension; Future Pharmaceutical Industries for Utopia Pharmaceuticals, Egypt). NTZ was dissolved in distilled water and was given orally in a dose of 250mg/kg/day for 7 successive days (Fahmy *et al,* 2020). 3- Nitrofurantoin® (Uvamine Retard; 100mg capsule: Medical Union Pharmaceuticals, Egypt). NF was given orally in a dose of 100mg/kg/day for 7 successive days (Abdallah *et al,* 2022).

Experimental design: Mice were divided into five groups of 20 immunosuppressed mice each: GI: Neither infected nor treated (negative control), GII: Infected, but non-treated (positive control), GIII: Infected, and NTZ-treated, GIV: Infected, and NF-treated, & GV: Infected, and NTZ & NF combined treated. *C. parvum* oocysts were given by an oral gavage of about 10^4 oocysts each mouse (Love *et al,* 2017). Stool samples were collected from all infected mice on the 3rd day P.I. stained and microscopically examined for cryptosporidiosis infection. Drugs (NTZ and/or NF) were given to infected mice on the 4th day P.I. for seven successive days. Treated mice were gently sacrificed after one week (11th day P.I.) & two weeks (18th day P.I.) for parasitological, histopathological, ultrastructural and biochemical evaluations.

Parasitological study: Fresh stools collect- ed from each mouse were examined as stained direct smear and concentrated by formal ether concentration technique, and stained with modified Ziehl-Neelsen (Benamrouz *et al,* 2012). Oocysts number was counted per gram stool for each sample, and their mean was calculated (Esmat *et al,* 2022)

Histopathological study: A part of the small intestine was fixed in 10% formalin and processed in paraffin blocking, sectioning (5µm thick), hematoxylin and eosin staining and micrroscopically examined to the histopathological changes (Mangoud *et al,* 2004).

TEM study: A part of ileal tissues was preserved in 2.5% glutaraldehyde, divided with sharp razor blades into small pieces of 1 mm^3 in

size, processed for semithin and ultrathin sections (Winey *et al*, 2014).

Biochemical oxidative stress & antioxidant markers; A part of ileum was put at -80^{o}C for tissue homogenate preparation (Wang *et al*, 2012). Supernatant fluid was collected and examined by colorimetric kits for malondialdehyde (MDA), and reduced glutathione (GSH).

Liver function tests: Serum levels of SGPT & SGOT were colorimetrically measured by Mindray BS-120 Fully Automated Chemistry Analyzer by Biomed-GPT, and Biomed-GOT Kit respectively (Biomed. Co., Egypt). Prcedures were done after the manufacturer's instructions.

Renal function tests: **S**erum levels of urea and creatinine were colorimetrically measured by using Mindray BS-120 Fully Automated Chemistry Analyzer by Urease Berthelot method for urea and Jaffe colorimetric-kinetic method for creatinine (Diamond Diagnostic Co., Egypt). Procedures were done after the manufacturer's instructions.

Ethical consideration: The study was approved by the Ethics Committee, Faculty of Medicine, Tanta University (Approval code: 3626-4MS260/7/23).

Statistical analysis**:** Data were computerized and analyzed by IBM SPSS (Statistical Package for Social Studies) version 20 (Armonk, N Y: IBM Corp). Shapiro-Wilk test verified normal data distribution. Quantitative data were expressed as M$\pm$SD. One-way analysis of variance (ANOVA) compared the means of more than two groups, followed by Tukey test for pair-wise comparison. Paired T test assessed significance difference between 2 groups. Significant data was judged at 5% level. If *P* values < 0.05 were considered significant. The reduction % in treated groups was calculated by the following equation: (mean oocyst count of positive control group-mean oocyst count of treated group) / (mean oocyst count of positive control group) X 100 to assess the treatment efficacy.

Results

On the 11th & 18th day P.I, both NTZ-NF treated (GV) and NF-treated (GIV) showed a significant reduction in mean *C. parvum* oocyst count compared to infected non-treated (GII), with highest reduction in GV (65.7%, & 80%, respectively), followed by GIV (48.6%, & 59.8%, respectively). The lowest reduction was in NTZ-treated mice 25.4%, & 30.2%, respectively, but without significant decrease in oocysts mean number compared to GII on 11th day P.I. There was a significant oocysts reduction in GV on 18th day compared to 11th day PI.

Ileum sections negative control (GI) showed normal architecture with intact brush border as the normal immunocompetent group, but with some edema and inflammatory cells in (GI) lamina propria. Positive control (GII) showed significant intestinal histopathological changes in architecture, on 11th day P.I. & 18th day P.I. in form of interruption brush border, villous atrophy with shortened broad villi, decreased villous/crypt ratio, goblet cell depletion, abnormal epithelial cells with dark shrunken nucleus, expansion of villous core by edema, excess inflammatory cellular infiltration in lamina propria, congested capillaries and disruption of muscle layer. Many basophilic *Cryptosporidium* oocysts were detected along brush border of villi, in intestinal lumen attached to surface epithelial crypt. After NTZ treatment (GIII), only mild improvement was detected on 11th day P.I with focal mucosal ulcerations, moderate decrease in villous/crypt ratio, mild goblet cell depletion and moderate inflammatory infiltration. Abnormal shaped dark nuclei of epithelial cells with dysplastic changes and abnormal proliferation of fibroblasts. On 18th day P.I, partial intestinal healed in GIII, but some villi still showed loss continuity of surface epithelium, few goblet cells with inflammatory cells infiltration. In NF-treated, significant histopathological improvement included a restoration of normal architecture of most villi with regular shape, well-preserved paneth cells, few inflammatory infiltrate and continuous muscle layer. More improvement was on 18th day P.I restored normal villus pattern, intact and elongated with normal epithelial cells, intact brush border, well preserved goblet cells without inflammatory cells.

The best results were in NTZ-NF treated (GV) in restoring normal intestinal architecture with intact brush border of villi and preserved villous/crypt ratio.

TEM of ileum negative control showed normal intestinal ultrastructure, well-developed apical microvilli, and intact intercellular junction. Positive control one showed marked ultrastructural abnormalities, such as discountinuity of brush border, severe distortion and atrophy of microvilli, apical blebbing of enterocytes with abnormal nuclei and cytoplasmic vacuolation, loss of microvilli of apical surface of paneth cell, numerous degenerated mitochondria, dilated RER, few goblet cells, and wide separation and discontinuity between cells with different *Cryptosporidium* stages, with total loss of normal intestinal architecture and extensive loss of the brush border 18 days P.I. NTZ-treated group, epithelial lining showed mild improvement compared to GII, 11 days P.I., but, areas of microvilli loss, some abnormal enlarged mitochondria and cytoplasmic vacuolation were observed. More improvement was observed 18 days P.I, but with some loss of microvilli, few vacuoles and partially degenerated mitochondria. In GIV, NF showed a significant regeneration of villi with average shape, density and number as well as dilated RER with very few areas of cytoplasmic vacuolation, 11 days P.I. while, regeneration of most cells with well-preserved brush border, intact multiple goblet cells and intact intercellular junction were shown on 18 days P.I. NTZ-NF treated 11^{th} days P.I. showed villi regeneration with normal brush border, well-preserved goblet cells, intact tight junction, mitochondria and dilated RER with few areas of cytoplasmic vacuolation. On 18^{th} day P.I., the best results were signs of cellular regeneration and mucosa renewal, complete repair of brush border with parallel tightly adherent microvilli, normal enterocytes with typical nucleus, mitochondria and RER and well-preserved goblets and intact tight junctions.

On 11^{th} &18^{th} days P.I, MDA mean level in ileal tissue homogenate showed a significant increase in GII compared to GI. But, a significant decrease in MDA level was in all treated mice compared to positive control, with the best reduction was in GV followed by GIV and least one was in GIII. GV didn't show significant difference compared to GI on 18^{th} day P.I. Mean level of reduced glutathione (GSH) in ileal tissue homogenate, showed a significant decrease in GII compared to GI on 11^{th} & 18^{th} days P.I. There was a significant GSH increase in level in GV followed by GIV compared to GII, but least increase was in GIII, but without a significant difference compared to GII.

On 11^{th} & 18^{th} days P.I, serum levels of SGOT & SGPT in GII showed a significant increase compared to GI. There was a significant decrease in SGOT & SGPT serum levels in all treated mice compared to positive control, with highest reduction in GV, followed by GIV and least one was in GIII. A significant decrease was in SGOT & SGPT mean levels on 18 days P.I in GIV & GV compared to 11^{th} day P.I.

Urea and creatinine serum levels on both days in positive control and mice treated with NTZ, NF, & NTZ-NF were not significant compared to negative control.

Details were given in table (1) and figures (1, 2 & 3).

Table 1: Mean *C. parvum* oocyst count/gm. stool among groups.

Variations	GII	GIII	GIV	GV	F	
11^{th} day P.I						
Mean± SD.	6086.8± 25100	4982.5 ± 18700	7995.3±12900	2219.2± 8600	7.856	
Reduction %	--	25.4%	48.6%	65.7%		
P_1	--	0.323	0.018*	0.002*	--	
P_2	--	--	0.404	0.057		
P_3	--	--		0.642	--	
18^{th} day P.I						
Mean± SD.	3297.7± 21500	4401.7± 15000	2407.7± 8625	1036.8± 4300	30.426	
Reduction %	--	30.2%	59.8%	80%		
P_1	--	0.018*	<0.001*	<0.001*	--	
P_2	--	--	0.021*	<0.001*		
P_3	--	--	--	0.153	--	--
P_4	0.374	0.271	0.277	0.010*	--	--

P1: Compared GII & each other, *P2:* Compared GIII & each other, *P3:* Compared GIV & GV, *P4:* Compared mean values on 11^{th} &18^{th} day P.I, *Significant at P < 0.05

Discussion

Although NTZ and its two metabolites, tizoxanide and tizoxanide-glucuronide were found to suppress the growth of *C. parvum*, its effectiveness was limited to immuncompetent patients (Cai *et al,* 2005). This indicated need for safe effective drug mainly for the immunocompromised patients. NF®, a member of the nitrofuran family is a broad-spectrum antibiotic (Mahdizade-Ari *et al,* 2023) that showed the anti-parasitic action against *Plasmodium* species (Adikwu *et al,* 2021). Besides, it presents an excellent safety profile and managed to avoid resistance problems, despite several decades ago of its use (Shafi *et al,* 2023).

In the present study, the positive control showed huge number of *C. parvum* oocysts in feces (25100±6086.8 & 21500±3297.7), respectively at both scarification times. This agreed with Taha *et al.*(2023) and Elmansory *et al.* (2024), who reported many *C. parvum* oocysts shaded in infected immunocompromised mice, as well as in immunosuppressive ones (Fox and Saravolatz, 2005; Ben amrouz *et al,* 2012).

In the present study, NTZ decreased oocysts shedding (25.4%, 30.2%) on 11th & 18th days P.I, respectively. This agreed with Madbouly *et al.* (2017) and Mostafa *et al.* (2018), they reported that NTZ-treated mice gave least oocysts reduction. But, Fahmy *et al.* (2020) and Nageeb *et al.* (2024) reported that use of NTZ for a prolonged duration or with high doses caused higher *C. parvum* oocysts reduction (39% & 47.8% respectively). Atia *et al.* (2016) reported that NTZ inhibited pyruvate ferrodoxin oxidoreductase (PFOR) the anaerobic energy metabolism, but its efficacy was limited with high doses in immunocompromised patients.

In the present study, nitrofurantoin given to infected mice led to a significant reduction in oocysts count (48.6%, & 59.8%) on 11th & 18th day P.I, respectively, compared to positive control showed its effectiveness in immunocompromised mice. NF anti-*Cryptosporidium* efficacy was due to the nitro group reduction by cytochrome P450 reductase existed in C-terminal of *C. parvum* pyruvate NADP+ oxidoreductase (Wang *et al,* 2008; Khan and Witola, 2023). Moreover, NF disrupted energy metabolism by inhibition of several enzymes participate in the carbohydrate metabolism in the Krebs cycle (Munoz-Davila, 2014; Huttner *et al,* 2015; Abdallah *et al,* 2022). Munsimbwe *et al.* (2021) reported that nitrofurantoin analogs with high hydrophilicity were used for in-vivo assessment to determine if they were promising in trypanocidal drugs development. Elkholy *et al.* (2023) reported a significant *Toxoplasma gondii* reduction without cysts in the liver, kidney, and uterus.

In the present study, ileal tissues of negative control showed normal histological picture but, positive control showed marked intestinal pathological changes This agreed with some authors (AbuEl Ezz *et al,* 2011; Soufy *et al,* 2017; Abdelhamed *et al,* 2019; Moawad *et al,* 2021; Yahia *et al,* 2023). *Cryptosporidium* direct cellular invaded, which toxins damaged epithelial cells (Zhang *et al,* 2000; Moawad *et al,* 2021; Yahia *et al,* 2023), but dysplastic changes were due to increased parasitic load, severity, infection duration, and without parasitic clearance in immunosuppressed mice (Abdou *et al,* 2013). Also, the heaviest burden of intestinal cryptosporidiosis in immunocompetent and immunosuppressed mice was due to the specific receptors (TLR2 & TLR4) and suitable biochemical conditions in the infected ileum (Hug *et al,* 2018).

In the present study, NTZ treated mice showed partial pathological changes improvement. This agreed with Sadek and El-Aswad (2014); Oshiba *et al.* (2018) and Hassan *et al.* (2022), they reported a moderate improvement in NTZ treated mice. The partial NTZ treated mice were due to drug insufficient activity (Yahia *et al,* 2023) or immunodeficiency decreased efficacy (Atia *et al,* 2016; Dhal *et al,* 2022). But, in the present study, marked improvement was achieved with NF treated mice. This agreed with Yeo *et al.* (2016); Abdallah *et al.* (2020); Abdallah *et al.* (2022) & Elkholy *et al.* (2023), who reported that NF has anti-protozoa effect on other coccidian parasites. Also, the present infected mice given combined NTZ & NF showed marvelous intestinal improvement. This was not only due to NF ability to reduce parasitic burden, but also antioxidant effect decreased the oxidative stress and allowed the host to produce the necessary immune response for NTZ to be more effective in *C. pavum* (Abdelhamed *et al,* 2019; Abdelmaksoud *et al,* 2020;

Abdallah *et al,* 2022).

In the present study, TEM showed that the intestine was normal in negative control, but severe distorted microvilli changes were in positive one. This agreed with Nürnberger *et al.* (2012), and Beshay *et al.* (2023), who reported those mitochondria were degenerated and RER was dilated due to a synthetic function failure or the loss of osmotic equilibrium. Also, in the present study, NTZ-treated mice showed the least impact on intestinal cellular regeneration. This agreed with El-Wakil *et al.* (2021) and Esmat *et al.* (2022), they reported that ileocecal sections from positive cryptosporidiosis mice showed loss of brush borders with marked villous atrophy and a moderate improvement of pathological changes in NTZ-treated groups.

In the present study, *C. parvum* infection caused marked oxidative stress by significant elevated lipid peroxidation product (MDA), but reduced GSH as compared to negative control. This agreed with Abd El-Aziz *et al.* (2014), Bhagat *et al.* (2017), El-Sayed and Fathy (2019), and Elmahallawy *et al.* 2020). Formerly, Wang *et al.* (2009) reported that decreased antioxidant level was related to increased susceptibility of mice for *C. parvum* infection. But, Pawłowska *et al.* (2023) reported that excess production of reactive oxygen species (ROS) can occur as a host defense mechanism against parasitic infection to eradicate pathogens, over production of ROS can damage the immune cells and host tissues as well.

In the present study, NTZ-treated mice showed a significant decrease in MDA level, but insignificant increase in GSH level in ileal tissue homogenate compared to positive control. This agreed with Atia *et al.* (2021); Abd El-Hamed *et al.* (2021) and Fahmy *et al.* (2021), who reported that NTZ caused a significant decrease in MDA levels and a mild increase in GSH level compared to other treated mice. NTZ is strong antioxidant agent (Mahmoud *et al,* 2024).

In the present study, NF caused an antioxidant significant reduction in MDA level and significant increase in GSH level. This agreed with Yeo *et al.* (2016), and Abdallah *et al.* (2022), who reported that NF-treated mice showed significant inhibition of *T. gondii*-induced MDA levels, and increased GSH levels. NTZ-NF combined treatment gave the best significant reduction in MDA levels and a significant increase in GSH ones as compared to positive control, proving that NTZ & NF have synergistic effect on energy metabolism disruption in many ways with a great overall effect.

In the present study, SGOT & SGPT showed significant increase in positive control compared to negative one; *Cryptosporidium*-induced injury of hepatocyte membranes and mitochondrial damage of liver tissue. This agreed with Gupta *et al.* (2018); Aboelsoued *et al.* (2019), and Elmahallawy *et al.* 2020), who found that NTZ produced significant reduction in liver enzymes compared to positive control. Also, in this study, NF showed a significant best reduction in liver enzymes as compared to positive one. This agreed with Yeo *et al.* (2016), and Abdallah *et al.* (2022), who found that NF-treated *T. gondii*-infected mice caused marked reduction in liver enzymes. Also, NTZ-NF combined treatment strongly ameliorated cryptosporidiosis risky effect. This agreed with Atia *et al.* (2021), who found that NTZ & NF antioxidant properties inhibited lipid peroxidation.

In the present study, serum urea and creatinine levels were within normal ranges in all infected mice compared to control one, but neither NTZ nor NF was toxic kidneys. This agreed with Zaki and El-Amir (2018), who found that neither cryptosporidiosis nor dexamethasone's affected renal functions. But, Beshay *et al.* (2023) reported that high dose (1×10^5 oocysts) significantly increased serum urea and creatinine levels by the severe watery diarrhea.

Conclusion

The NF gave a superior result as anti-cryptosporidial and antioxidant activities, with same hepatoprotective and safe effects on kidneys compared to the NTZ.

Recommendation

The nitrofurantoin is a promising anti-cryptosporidial treatment of immunosuppressed patients with synergistic effects of the NF-NTZ co-mbined therapies.

Authors' Declaration: All authors neither have any conflict of interest nor received any funds.

Authors' Contribution: All authors equally well writing the manuscript and approved its publication.

References

Abd El-Aziz, TH, El-Beih, N, Soufy, H, *et al,* 2014: Effect of Egyptian propolis on lipid profile and oxidative status in comparison with nitazoxanide in immunosuppressed rats infected with *Cryptosporidium* spp. Glob. Vet. 13:17-27.

Abd El-Hamed, WF, Yousef, NS, Mazrou, YS A, *et al,* 2021: Anti-*Cryptosporidium* efficacy of *Olea europaea* and *Ficus carica* leaves extract in immunocompromised mice associated with biochemical characters and antioxidative system. Cells 10:241-9.

Abdallah, K, Saleh, M, Kishik, S, *et al,* 2020: Assessment of nitrofurantoin efficacy on treatment of murine model of toxoplasmosis. Benha J. Appl. Sci. 5:253-6.

Abdallah, KF, Saleh, MH, Kishik, S, *et al,* 2022: A new approach in treatment of chronic murine models of toxoplasmosis using nitrofurantoin antibiotic. Zagazig Univ. Med. J. 28:1169-75.

Abdelhamed, EF, Fawzy, EM, Ahmed, SM, *et al,* 2019: Effect of nitazoxanide, artesunate loaded polymeric nano fiber and their combination on experimental cryptosporidiosis. Iran. J. Parasitol. 14:240-9.

Abdelmaksoud, HF, El-Ashkar, AM, Elgohary, SA, *et al,* 2020: Potential therapeutic and prophylactic effects of *Asafoetida* in murine cryptosporidiosis. J. Parasit. Dis. 44:646-53.

Abdou, AG, Harba, NM, Afifi, AF, *et al,* 2013: Assessment of *Cryptosporidium parvum* infection in immunocompetent and immunocompromised mice and its role in triggering intestinal dysplasia. Int. J. Infect. Dis. 17:e593-600.

Aboelsoued, D, Abo-Aziza, FAM, Mahmoud, MH, *et al,* 2019: Anti-cryptosporidial effect of pomegranate peels water extract in experimentally infected mice with special reference to some biochemical parameters and antioxidant activity. J. Parasit. Dis.43: 215-28.

AbuEl Ezz, NT, Khalil, FA, Shapaan, RM, 2011: Therapeutic effect of onion (*Allium cepa*) and cinnamon (*Cinnamomum zeylanicum*) oils on cryptosporidiosis in experimentally infected mice. Glob. Vet. 7:179-83.

Adikwu, E, Joseph, FA, Georgewill, UO, *et al,* 2021: In-vivo anti-plasmodia effect of dihydroartemisinin-piperaquinenitrofurantoin on *Plasmodium berghei*-infected mice. J. Adv. Biomed. Pharm. Sci. 23:12-20.

distributed in the field and practical work as

Atia, A, El Sobky, M, Harba, N, *et al,* 2021: Evaluation of potential prophylactic and therapeutic effect of azoximer bromide (polyoxidonium) on experimental cryptosporidiosis in immunocompromised mice. PUJ, 14:293-304.

Atia, MM, Abdul Fattah, MM, Abdel Rahman, HA, *et al,* 2016: Assessing the efficacy of nitazoxanide in treatment of cryptosporidiosis using PCR examination. J. Egypt. Soc. Parasitol. 46: 683-92.

Benamrouz, S, Guyot, K, Gazzola, S, *et al,* 2012: *Cryptosporidium parvum* infection in SCID mice infected with only one oocyst: qPCR assessment of parasite replication in tissues and development of digestive cancer. PLoS One 7: e51232.

Beshay EVN, Nassef NE, El Shafei OK, *et al,* 2023: Therapeutic efficacy of proton pump inhibitor (omeprazole) on cryptosporidiosis *parvum* in immunosuppressed experimental mice. J. Parasit. Dis. 47: 535-49.

Bhagat M, Sood S, Yadav A, *et al,* 2017: Alterations in oxidative stress parameters and its' associated correlation with clinical disease on experimental *Cryptosporidium parvum* infection in Swiss albino mice. J. Parasit. Dis. 41:707-12.

Cai, X, Woods, KM, Upton, SJ, *et al,* 2005: Application of quantitative real-time reverse transcription-PCR in assessing drug efficacy against the intracellular pathogen *Cryptosporidium parvum* in vitro. Antimicrob. Agents Chemother. 49:4437-42.

CDC, 2025: About Crypto Infections/Cryptosporidium (Crypto) https://www.cdc.gov/cryptosporidium/ about/index.html.

Ctrnacta, V, Ault, JG, Stejskal, F, *et al,* 2006: Localization of pyruvate: NADP+ oxidoreductase in sporozoites of *Cryptosporidium parvum.* J. Eukaryot. Microbiol. 53:225-31.

Dhal, AK, Panda, C, Yun, SI, *et al,* 2022: An update on *Cryptosporidium* biology and therapeutic avenues. J. Parasit. Dis. 46:923-39.

El-Bahnasawy, MMM, Morsy, ATA, Morsy, TA, 2018: A mini-overview on zoonotic cryptosporidiosis. JESP 48, No. 1:35-44.

Elkholy, A, Wassef, R, Alsaid, O, *et al,* 2023: Evaluation of mono and combined nitrofurantoin therapy for toxoplasmosis in vivo using murine model. Pathog. Glob. Hlth. 117:664-73.

Elmahallawy, EK, Elshopakey, GE, Saleh, A A, *et al,* 2020: S-Methylcysteine (SMC) ameliorates intestinal, hepatic, and splenic damage induced by *Cryptosporidium parvum* infection via ta-

rgeting inflammatory modulators and oxidative stress in Swiss Albino mice. Biomedicines 8:423-9.

Elmansory, BM, Zalat, RS, Khaled, E, *et al,* 2024: Efficacy of nitazoxanide, ivermectin and albendazole in treatment of cryptosporidiosis in immunosuppressed mice. J. Parasit. Dis. 48:1-8.

El-Sayed, NM, Fathy, GM, **2019:** Prophylactic and therapeutic treatments' effect of *Moringa oleifera* methanol extract on *Cryptosporidium* infection in immunosuppressed mice. Anti-infect. Agents 17:130-7.

El-Wakil, ES, Salem, AE, Al-Ghandour, AMF, **2021:** Evaluation of possible prophylactic and therapeutic effect of mefloquine on experimental cryptosporidiosis in immunocompromised mice. J. Parasit. Dis. 45:380-93.

Esmat, M, Abdel-Aal, AA, Shalaby, MA, *et al,* 2022: Efficacy of clofazimine and nitazoxanide combination in treating intestinal cryptosporidiosis and enhancing intestinal cellular regeneration in immunocompromised mice. Food Water-borne Parasitol. 27:e00161.

Fahmy, AM, Alshenawy, AM, El-Wakil, EA, *et al,* 2021: Efficacy of *Cyperus rotundus* extract against cryptosporidiosis and toxoplasmosis in murine infections. Egypt. Pharm. J. 20:242-8.

Fahmy, MA, Abdelaal, AA, Hassan, SI, *et al,* 2020: Potential immunomodulatory effect of single and combined therapies against *Cryptosporidium* infection in immunosuppressed mouse model. JESP 50, 2:673-82.

Fox, LM, Saravolatz, LD, **2005:** Nitazoxanide: A new thiazolide antiparasitic agent. Clin. Infect. Dis. 40:1173-80.

Gupta, S, Johnson, A, Meyrick, S, *et al,* 2018: A case of hepatobiliary infection secondary to *Cryptosporidium* in a patient on tacrolimus. JMM Case Rep. 5:e005159.

Hall, BS, Bot, C, Wilkinson, SR, 2011: Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. J. Biol. Chem 286:13088-95.

Hassan, ZR, Salama, DE, Ibrahim, HF, **2022:** Apoptotic changes in the intestinal epithelium of *Cryptosporidium*-infected mice after silver nanoparticles treatment versus nitazoxanide. J. Parasit. iDis. 46:101-12.

Helmy, YA, Hafez, HM, **2022:** Cryptosporidiosis: From prevention to treatment, a narrative review. Microorganisms 10:24-56.

Hug, H, Mohajeri, MH, La Fata, G, 2018: Toll-like receptors: Regulators of the immune response in the human gut. Nutrients 10:203-9.

Huttner, A, Verhaegh, EM, Harbarth, S, *et al,* 2015: Nitrofurantoin revisited: A systematic review and meta-analysis of controlled trials. J. Antimicrob. Chemother. 70:2456-64.

Innes, EA, Chalmers, RM, Wells, B, Pawlowic, M, 2020: A one health approach to tackle cryptosporidiosis. Trends Parasitol. 36, 3:290-303.

Khan, SM, Witola, WH, **2023**: Past, current, and potential treatments for cryptosporidiosis in humans and farm animals: A comprehensive review. Front. Cell Infect. Microbiol. 13:1115522.

Love, MS, Beasley, FC, Jumani, RS, *et al,* 2017: A high-throughput phenotypic screen identifies clofazimine as a potential treatment for cryptosporidiosis. PLoS Negl. Trop. Dis. 11:e000537.

Madbouly, NT, Salah, AYHA, El-Sayed, SH, *et al,* 2017: Atorvastatin repurposing for the treatment of cryptosporidiosis in experimentally immunosuppressed mice. Exp. Parasitol. 181:57-69.

Mahdizade-Ari, M, Dashtbin, S, Ghasemi F, *et al,* 2023: Nitrofurantoin: properties and potential in treatment of urinary tract infection: A narrative review. Front. Cell. Infect. Microbiol. 13: 1148603.

Mahmoud, NM, Elshazly, SM, El-Shaarawy, F, *et al,* 2024: Nitazoxanide mitigates methotrexate hepatotoxicity in rats: Role in inhibiting apoptosis and regulating endoplasmic reticulum stress. Front. Pharmacol. 15:1491249.

Mangoud, AM, Eissa, MH, Sabee, EI, Ibrahem, IA, Ismail, A, *et al,* 2004: New concept in histopathological grading and staging of chronic hepatitis C infection at Sharkia Governorate, Egypt. J. Egypt. Soc. Parasitol. 34, 1:S385-400.

Moawad, HSF, Hegab, M, Badawey, MSR, *et al,* 2021: Assessment of chitosan nanoparticles in improving the efficacy of nitazoxanide on cryptosporidiosis in immunosuppressed and immunocompetent murine models. J. Parasit. Dis. 45: 606-19.

Mostafa, NE, Abdel Hamed, EF, Fawzy, EM, *et al,* 2018: The new trend in the treatment of experimental cryptosporidiosis and resulting intestinal dysplasia. Colorectal Cancer 7:CRC06.

Musher, DM, Musher, BL, 2004: Contagious acute gastrointestinal infection. N. Engl. J. Med. 351:2417-22.

Munoz-Davila, MJ, **2014:** Role of old antibiotics in the era of antibiotic resistance: Highlighted nitrofurantoin for the treatment of lower urinary tract infections. Antibiotics (Basel), 3:39-48.

Munsimbwe, L, Seetsi, A, Namangala, B, *et al,* 2021: In vitro and in vivo trypanocidal efficacy of synthesized nitrofurantoin analogs. Molecules 26:

33-72.
Nageeb, MM, El Fakahany, AF, Abdel Maaboud, AI, *et al,* 2024: Efficacy of atorvastatin loaded on nanoparticles on *Cryptosporidium parvum* in experimentally infected mice. Benha Med. J. 41:87-100.
Nürnberger, S, Miller, I, Duvigneau, JC, *et al,* 2012: Impairment of endoplasmic reticulum in liver as an early consequence of the systemic inflammatory response in rats. Am. J. Physiol. Gastrointest. Liver Physiol. 303:G1373-83.
Oshiba, SF, Yaseein, RI, El-Shennawy, AM, *et al,* 2018: In vivo effect of pomegranate (*Punica granatum*) extracts versus nitazoxanide drug on ileum of experimentally infected mice with *Cryptosporidium parvum* oocysts. J. Am. Sci. 14:27-39.
Pawłowska, M, Mila-Kierzenkowska, C, Szczegielniak, J, *et al,* 2023: Oxidative stress in parasitic diseases-reactive oxygen species as mediators of interactions between the host and the parasites. Anti-oxidants (Basel), 13:38-44.
Rehg, JE, Hancock, ML, Woodmansee, DB, *et al,* 1988: Characterization of a dexamethasone-treated rat model of cryptosporidial infection. J. Infect. Dis. 158:1406-7.
Sadek G, El-Aswad B, **2014:** Role of COX-2 in pathogenesis of intestinal cryptosporidiosis and effect of some drugs on treatment of infection. J. Parasitol. Res. 9:21-40.
Shafi. S, Gupta, S, Jain, R, *et al,* 2023: Tackling the emerging artemisinin-resistant malaria parasite by modulation of defensive oxido-reductive mechanism via nitrofurantoin repurposing. Biochem. Pharmacol. 215:115756.
Soufy, H, El-Beih, NM, Nasr, SM, *et al,* 2017: Effect of Egyptian propolis on cryptosporidiosis in immunosuppressed rats with special emphasis on oocysts shedding, leukogram, protein profile and ileum histopathology. Asian Pac. J. Trop. Med. 10:253-62.
Suganuma, K, N'Da, DD, Watanabe, KI, *et al,* 2022: Therapeutic efficacy of orally administered nitrofurantoin against animal African trypanosomosis caused by *Trypanosoma congolense* infection. Pathogens 11:331-6.
Taha, NM, Zalat, RS, Khaled, E, *et al,* 2023: Evaluation of the therapeutic efficacy of some essential oils in experimentally immunosuppressed mice infected with *Cryptosporidium parvum*. J. Parasit. Dis. 47**:**733-43.
Wang, C, Wu, Y, Qin, J, *et al,* 2009: Induced susceptibility of host is associated with an impaired antioxidant system following infection with *Cryptosporidium parvum* in Se-deficient mice. PLoS One 4: e4628.
Wang, J, Wang, YY, Lin, L, *et al,* 2012: Quantitative proteomic analysis of okadaic acid treated mouse small intestines reveals differentially expressed proteins involved in diarrheic shellfish poisoning. J. Proteomics 75:2038-52.
Wang, Y, Gray, JP, Mishin, V, *et al,* 2008: Role of cytochrome P_{450} reductase in nitrofurantoin-induced redox cycling and cytotoxicity. Free Radic. Biol. Med.44:1169-79.
Winey, M, Meehl, JB, O'Toole, E, *et al,* 2014: Conventional transmission electron microscopy. Mol. Biol. Cell. 25:319-23.
Yahia, SH, El Gamal, RL, Fathy, GM, *et al,* 2023: Potential therapeutic effect of *Nigella sativa* and *Zingiber officinale* extracts versus nitazoxanide drug against experimentally induced cryptosporidiosis in laboratory mice. J. Parasit. Dis. 47: 329-39.
Yeo, SJ, Jin, C, Kim, S, *et al,* 2016: In-vitro, & in-vivo effects of nitrofurantoin on experimental toxoplasmosis. Korean J. Parasitol. 54:155- 61.
Youssef, FG, Adib, I, Riddle, MS, Schlett, C D, 2008: A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol. 38, 1:9-28**.**
Zaki, WM, El-Amir, YO, **2018:** Therapeutic efficacy of a cysteine proteases inhibitor (phenyl vinyl sulfone) either alone or combined with *Nigella sativa* on experimental cryptosporidiosis. JESP 48:165-74.
Zhang, Y, Lee, B, Thompson, M, *et al,* 2000: Lactulosemannitol intestinal permeability test in children with diarrhea caused by *Rotavirus* and *Cryptosporidium*. J. Pediatr. Gastroenterol. Nutr. 31:16-21.
Xiao, L, Fayer, R, Ryan, U, *et al,* 2004: *Cryptosporidium* taxonomy: Advances and implications for public health. Clin. Microbiol. Rev. 17:72-9.

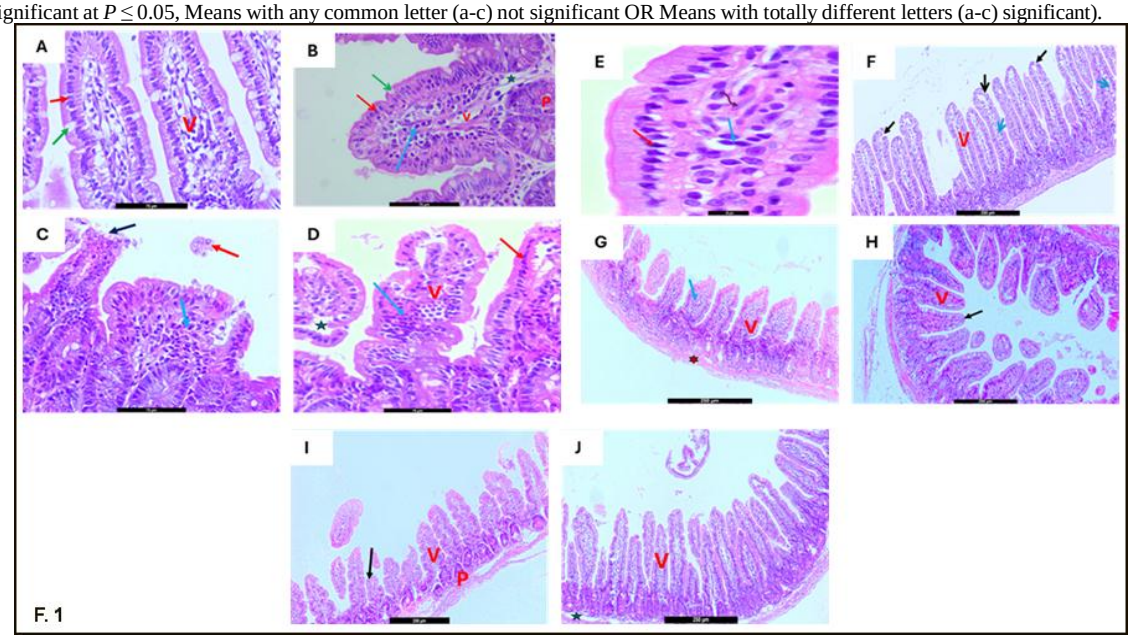
Explanation of figures

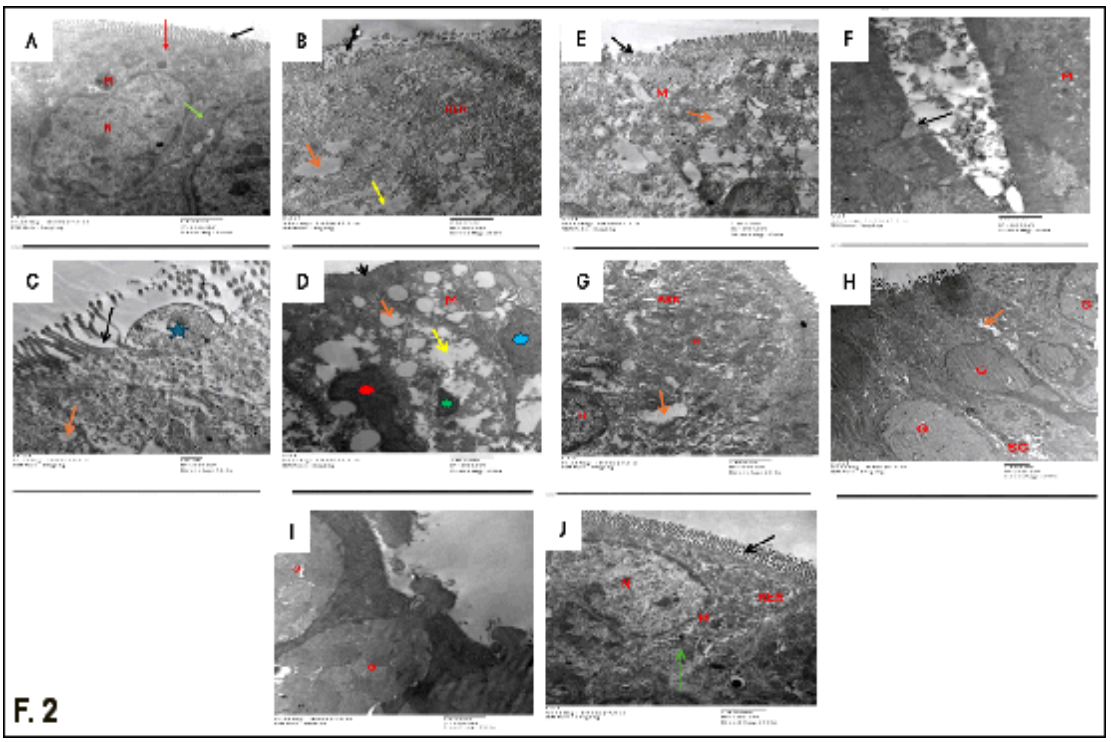
Fig. 1: Mice small intestine (H&E, ×400). A- Immunocompetent negative control with normal intestinal villi (V) covered by simple epithelial columnar (red arrow) with goblet cells (green arrow). B- Immunocompromised negative control with normal intestinal villi (V) covered by simple epithelial columnar (red arrow) with goblet cells (green arrow), part of gland showed paneth cells (P), mild edema (star) and few inflammatory cells in villi core (blue arrow). C- Positive control a week after treatment start with interruption of brush border (black arrow), detachment of surface epithelial cells (red arrow) and inflammatory cellular infiltration in lamina propria (blue arrow). D- Positive control after 2 weeks after treatment start with irregular villi (V), deeply acidophilic cytoplasm and abnormal aggregation of nuclei in epithelial surface (red arrow), inflammatory cellular infiltration (blue arrow) and edema (star). E- Infected NTZ-treated, one week post treatment with abnormal shaped dark nuclei of epithelial cells with dysplastic changes (red arrow) and inflammatory cellular infiltration (blue arrow) with proliferation of fibroblasts (zigzag arrow) (H&E ×100). F- Infected NTZ-treated two weeks post treatment with normal regular few villi (V), discontinuity of epithelial surface in some villi (black arrow) and mild inflammatory cellular infiltration (blue arrow). G- Infected NF-

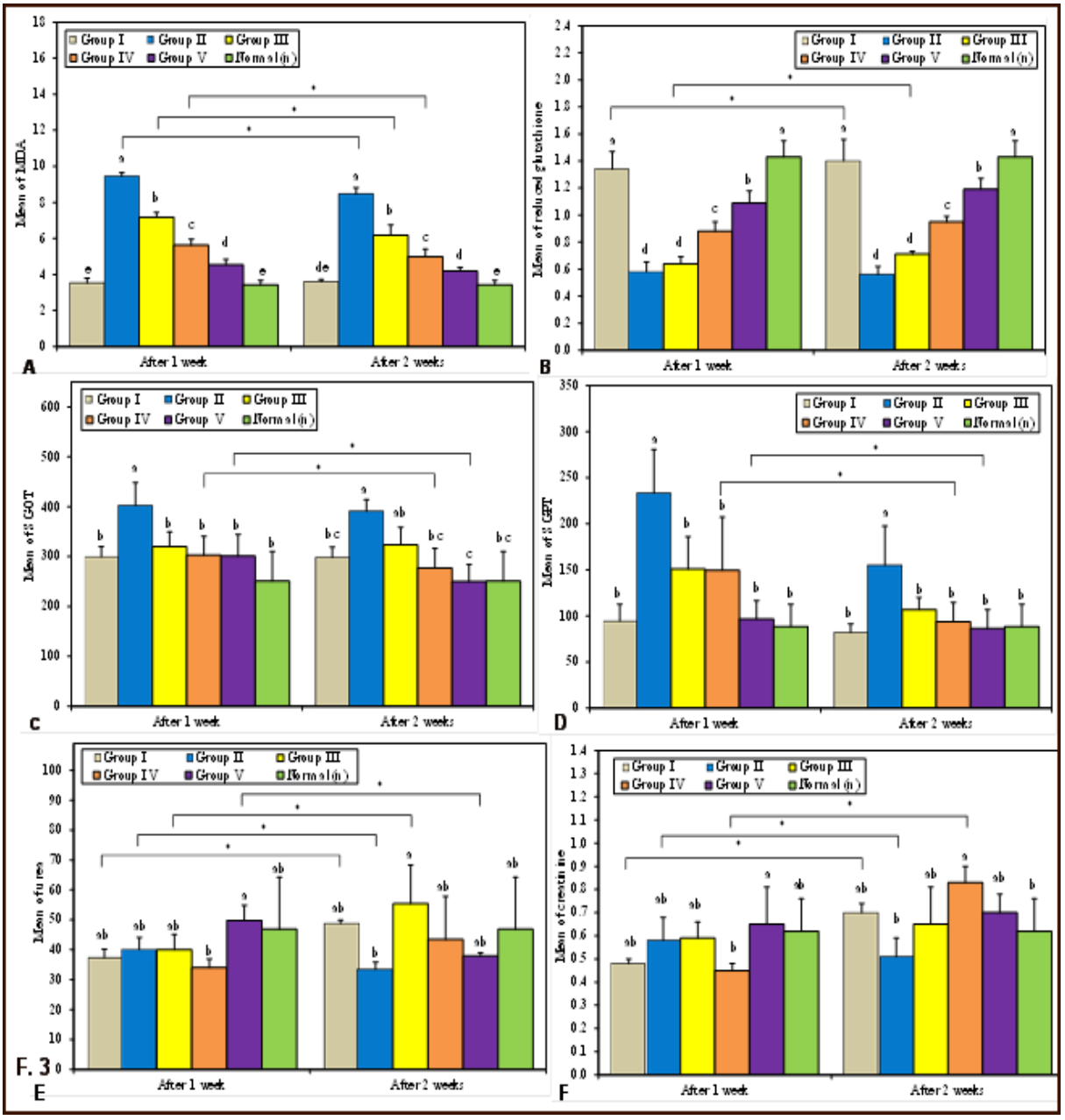
treated, one week post treatment with normal villus pattern (V), mild inflammatory infiltrate (blue arrow) and continuous muscle layer (red star(. H- Infected NF-treated, two weeks post treatment with intact villi (v) and continuous brush border (black arrow). I- Infected NTZ+NF treated a week po-st treatment with normal villi pattern (V), intact paneth cells (P) and minimal surface erosion (black arrow). J- Infected NTZ+NF treated two weeks post treatment with intact finger- like villi (V) and some edema (star).

Fig. 2: TEM (×2500) of mice ileum showed: A: GI with normal columnar epithelium (red arrow), mitochondria (M), normal nuclear shape (N), well-developed apical microvilli (black arrow) and intact tight junction (green arrow). B: GII one week post treatment with loss of brush border (black arrow), multiple vacuoles (orange arrow), cytoplasmic loss (yellow arrow) and dilated RER). C: GII one week post tre-atment with multiple vacuoles (orange arrow), trophozoite attached to brush border (star), with discontinuity of brush border and decrease in number of adjacent microvilli (black arrow). D: GII two weeks post treatment with low brush border (black arrow), cytoplasmic loss (yellow arrow), degenerated mitochondria (M), multiple large vacuoles (orange arrow), degenerated nuclei different forms [dark with irregular shape (red star), pyknotic (green star) and dark mostly heterochromatin (blue star). E: GIII a week post treatment with loss microvilli (black arrow), large vacuoles (orange arrow) and some degenerated mitochondria (M). F: GIII two weeks post treatment with loss microvilli (black arrow) and partial of mitochondria degeneration (M). G: GIV a week post treatment with intact nucleus (N), normal mitochondria (M), dilated RER and some vacuoles (orange arrow). H: GIV two weeks post treatment with intact paneth cell contained secretory granules (SG), goblet cells (G) with few vacuoles (orange arrow). I: GV a week post treatment with multiple goblet cells with abundant secretions (G). J: GV two weeks post treatment with normal brush border (black arrow), nucleus (N), mitochondria (M), RER and intact tight junction (green arrow).

Fig. 3: Different groups comparison as mean levels of (A) MDA, (B) GSH, (C) SGOT, (D) SGPT, (E) urea and (F) creatinine level. *Significant at $P \leq 0.05$, Means with any common letter (a-c) not significant OR Means with totally different letters (a-c) significant).






Group I
Group II
Group III
Group IV
Group V
Normal(n)
Mean of MDA
Mean of reduced glutathione
Mean of GOT
Mean of GPT
Mean of urea
Mean of creatinine
After 1 week
After 2 weeks
A
B
C
D
E
F

F. 3