Original Article

Efficacy of coconut oil as therapeutic agent with potential anticancer activity in immunosuppressed mice with cryptosporidiosis: Parasitological, histopathological and immunohistochemical studies

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ABSTRACT

Background: Cryptosporidiosis is an important worldwide opportunistic infection. It causes severe life-threatening diarrhea in immunocompromised patients and has a carcinogenic predisposition in chronic cases. Until now, there are no available drugs that can control this serious effect on the ileocecal region. Coconut oil (CO) is rich in many saturated fatty acids like lauric acid (LA) which has many uses in the field of traditional medicine, and also showed anticancer activity although its mechanism of action is not well studied.

Objective: To assess the efficacy of CO in immunosuppressed mice with chronic cryptosporidiosis.

Material and Methods: Forty white Albino mice of CDI strain were immunosuppressed and divided into 4 groups. GI: non-infected (negative control); GII: infected for 60 d, and non-treated (positive control); GIII: infected for 60 d then Nitazoxanide (NTZ) treated; GIV: infected for 60 d then CO treated. Parasitological, histopathological, and immunohistochemical (IHC) studies were conducted at the ileocecal region to estimate the parasite burden, caspase-3 mediator of apoptosis, and CDX2 biomarker of tumorigenesis.

Results: Parasitological examination showed marked reduction of parasite load in GIV compared to GII, and GIII. Histopathological examination showed focal villous tip erosions and mild villous core infiltration by mononuclear inflammatory cells in GIII, while GIV showed a mostly preserved villous pattern with mild villous core inflammation. Immunohistochemical examination showed the best results in GIV in which there was significant positive nuclear staining in acini for CDX2 with nearly negative cytoplasmic staining in acini for caspase-3.

Conclusion: Coconut oil is a natural product with significant anti-*Cryptosporidium* effects and a promising ability to decrease the incidence of dysplastic changes in chronic cryptosporidiosis.

Keywords: anticancer activity; coconut oil; cryptosporidiosis; *in vivo*, nitazoxanide.

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INTRODUCTION

Cryptosporidium spp. are obligate, intracellular protozoan parasites that belong to the Apicomplexa, causing cryptosporidiosis, a disease with global distribution. These species infect the epithelium of the gastrointestinal and respiratory tracts of different vertebrate hosts, including humans[1], and as opportunistic enteric parasites may cause serious life-threatening infection in patients with compromised immune system[2]. Several studies reported coexistence of cryptosporidiosis in patients having different malignancies[3-6]. Other studies concerned with cancer patients proved their susceptibility to severe cryptosporidiosis[7-9]. The link between colorectal adenocarcinoma and cryptosporidiosis was reported in epidemiological studies[8,9]. Nevertheless, the clinical correlation between cryptosporidiosis and alimentary tract malignancies in man remains ambiguous. Questions are still raised about the ability of *Cryptosporidium* to induce cancers in the human gastrointestinal tract (GIT)[8]. However, a study presented a case of a Spanish patient who died from colonic adenocarcinoma with concomitant cryptosporidiosis[10]. Another study described a case of biliary tract cryptosporidiosis that was clinically alike pancreatic cancer in a patient with HIV[11]. Hence, the risk for colon carcinoma in AIDS patients was raised in association with cryptosporidiosis[6]. The correlation between bile duct carcinoma and cryptosporidiosis in children with X-linked hyper-IgM syndrome and in immunocompromised mice was reported by several studies[12-14]. It was suggested that this gene mutation might encourage *Cryptosporidium* colonization of the biliary epithelium leading to chronic infection and inflammation resulting in the dysplastic changes[13,14]. Not only these conditions were diagnosed in children, but also there was a case of cholangiocarcinoma reported in an adult patient with CD40L deficiency and

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a medical history of sclerosing cholangitis in addition to chronic cryptosporidiosis[15]. A Chinese case control study showed that infection rate of cryptosporidiosis in patients with colorectal cancer was 17.24% (20/116) before start of chemotherapy. Besides, the parasite was demonstrated in cancers of the small intestine, esophagus, and liver[16].

It is worth mentioning that CO contains many saturated fatty acids as LA, capric acid and caprylic acid that have many uses in the field of traditional medicine. Notably, LA, a main component in CO, proved to have anticancer effect mediated through oxidative stress-evoked apoptosis, but its cellular signaling mechanism is still ambiguous[17]. However, it was recently reported that lipids play crucial roles in cellular and intracellular signaling through regulation of membrane fluidity and lipid rafts, and the synthesis of lipid-derived mediators regulating important aspects in tumor biology such as immune evasion and cellular invasion[18]. Coconut palm plantations *Cocos nucifera* (*C. nucifera*) of the Arecaceae family was used as a natural medicinal therapy in African countries since its anticancer activity was mediated through oxidative stress-evoked apoptosis; and in South Asia, and America also as a diuretic[19]. It was also used for treating inflammation of joints, diarrhea, fevers, dermatologic infections, bronchial asthma, prevention of abortion and treatment of infertility in females[19]. A leaf extract from the coconut plant was used to reduce amyloid-β1–42 aggregation and paralysis[20]. Catechins and epicatechins that were obtained from the husk of *C. nucifera* were reported to have anti-parasitic activity against *Leishmania* parasites[21]. The *C. nucifera* husk fibers were also found to contain alkaloids, tannins, and flavonoids that account for their activity against *P. falciparum* W2 strain[22]. Besides, they were also effective against *P. berghei* NK65, leading to >50% decrease in parasitemia on the 4th and 6th days after inoculation with different administered doses[22].

The present study aimed to investigate the potential efficacy of CO as anti-*Cryptosporidium* drug with anticancer activity in chronically infected immunosuppressed mice, in comparison with the golden standard drug (NTZ).

MATERIAL AND METHODS

This experimental case-control study was performed in the biological unit of Theodor Bilharz Research Institute (TBRI) during the period from December 2020 till September 2021.

Study design: The study included negative and positive control groups (GI, and GII), while mice of GIII, and GIV were treated with NTZ and CO, respectively. Parasitological, histopathological, and IHC parameters were utilized to investigate CO potential efficacy in treatment of chronic cryptosporidiosis and in prevention of dysplastic changes in the ileocecal region.

Experimental animals: The study included forty laboratory bred female, white Albino mice of CD-1 strain (Clostridium difficile infected), in which *Cryptosporidium* spp. have a complete successful cycle. This CD1 strain of mice have a uniform genotype, that allows for easier and more reliable interpretation of results, with a much lower tendency to acquire, and retain genetic abnormalities conferring serious illness than the commonly used inbred strains. Besides, CD1 strain displays minimal evasion, struggling and aggressive behavior when caught and held[23-26]. Mice, ~4-6 weeks old and 20-25 g, were purchased from TBRI animal house, and kept in well-ventilated plastic cages with clean wood-chip bedding. The cages were kept in conditioned rooms (27±2°C) and away from direct sunlight. The animal experiments were conducted in TBRI biological unit.

Study groups: Mice of CD-1 strain were immunosuppressed for 2 w and then divided into 4 groups: GI: non-infected (negative control); GII: infected for 60 d with no treatment (positive control); GIII: infected for 60 d, and received NTZ for 7 d; and GIV: infected for 60 d then treated with CO for 7 d.

Immunosuppression: Dexazone (dexamethasone sodium phosphate) 0.25 ug/g/day was administered to each mouse orally by gavage using an esophageal tube. The drug was given daily for 2 w prior to induction of infection with *Cryptosporidium* oocysts with a maintenance dose given weekly during the whole experiment for all groups[27].

Infection: *Cryptosporidium* spp. oocysts were obtained from the Animal Research Institute in Giza governorate, Egypt. Infection of mice with *Cryptosporidium* spp. oocysts was performed by gavage using an esophageal tube. The infection dose was given once in the form of 10^3 *Cryptosporidium* spp. oocysts dissolved in 200 µL of PBS[28].

Drugs and therapeutic doses: NTZ was purchased from a local pharmacy in the form of powder to prepare 100 mg suspension/5 ml (Nanazoxide®). An oral suspension of NTZ was given to each mouse of GIII in a dose of 65 mg/mouse/day (3.25 ml). The drug was given for 7 consecutive days starting 60 d post infection (dpi). The doses were adjusted by an extrapolation table for the therapeutic doses of man and animal[29]. On the other hand, CO was purchased from a local market in Giza governorate, Egypt. The identity of the plant was authenticated at the Department of Botany, Faculty of Science, Cairo University, Egypt. A dose of pure CO was given to each mouse of GIV in the form of 0.2 ml/20 g body weight for 7 consecutive days starting 60 dpi[30].

Parasitological examination: Starting from the 67th dpi, stool samples were collected daily, stained by modified Zheil Nelsen (MZN) stain[31] and examined microscopically using the oil immersion lens (x100) to ensure establishment of infection and to count oocysts. A counting chamber was used to estimate the *Cryptosporidium* oocysts parasite load in the fecal pellets. One mg stool pellet was weighed, then preserved in 1 ml 10% formalin. Concentration of the stool suspension was by centrifugation at 500 g for 10 min. Oocysts count in 1 ml of stool sample was determined in 100 ul of dried and MZN-stained stool sediment, examined under the oil immersion lens (x100). Oocyst count was accomplished by calculating the average of 3 counts multiplied by 10 to define the count of oocysts in one ml of stool[32]. The number of oocysts was expressed per gram of feces[33].

Histopathological examination: All mice were sacrificed on the 67th dpi. Ileocecal region specimens were fixed in 10% buffered formalin solution, inserted in paraffin wax blocks, sectioned and stained with Hematoxylin and Eosin (H&E) to assess the pathological changes and check for any abnormal proliferation pattern[34].

Immunohistochemical examination: Ileocecal, formalin-fixed paraffin sections were prepared for IHC analysis of CDX2 and caspase-3. Examinations were performed on 4 µm thick sections. Antigen retrieval was achieved with 10 ml sodium citrate buffer (pH 6.0) at 90°C for half an hour. Incubation of the sections in 0.03% H_2O_2 for 10 min at room temperature to eliminate the activity of endogenous peroxidase, was followed by addition of blocking serum: 0.04% bovine serum albumin A2153 (Sigma-Aldrich, Shanghai, China), and 0.5% normal goat serum X0907 (Dako Corporation, Carpinteria, CA, USA), in PBS for half an hour at room temperature. Anti-CDX2 (monoclonal mouse anti-human, Clone: DAK-CDX2, Code Number: IR080) and anti-caspase-3 (Rabbit polyclonal, Novus, NB600-1235) antibodies were used separately at a dilution of 1:200. Incubation of the CDX2 antibody was done at room temperature for 2 h, whereas the caspase-3 antibody was incubated at 4°C overnight. PBS was used to wash sections 3 times for 5 min each. Blocking of non-specific staining was achieved by 0.5% casein and 5% normal serum for half an hour at room temperature. Finally, sections were stained with diaminobenzidine substrate, followed by counterstaining with hematoxylin. Sections were examined by a light microscope (Scope A1, Axio, Zeiss, Germany), provided with a camera for photomicrographs (AxioCam, MRc5, Zeiss, Germany). Semi-quantitative analysis of both CDX2 and caspase-3 expression was made in reference to the percentage of nuclear and cytoplasmic immunostaining respectively. Moderate and strong immunostaining were recorded as positive. Caspase-3 as a marker for cytoplasmic apoptosis increases with tumorigenesis and decreases under the influence of tumor therapy. While CDX2 marker is normally expressed in the nuclei of colonic mucosa and decreases in cases of tumorigenesis[35].

Statistical analysis: The results were calculated, tabulated, and statistically analyzed using statistical computer program SPSS version 24 under windows 10. Data were expressed as mean ± standard deviation (SD). Differences between groups were determined using a one-way ANOVA to compare one variable between different groups, and post hoc Tukey`s to compare multiple variables between two groups. The level of significance was accepted at *P* value<0.05.

Ethical consideration: This study was approved by the Scientific Research Ethics Committee of TBRI. The internationally valid guidelines were applied during the animal experiments.

RESULTS

Parasitological studies: There was a statistically significant difference (*P*<0.001) between studied groups as regard *Cryptosporidium* spp. oocysts counts. While GIII and GIV showed marked reduction of oocyst count in the stool of the treated mice compared to the non-treated GII, only GIV showed the best results with the lowest parasite load and significant statistical difference from GII (*P*<0.001) and GIII (*P*=0.003) (Fig. 1, and Table 1).

Histopathological and immunohistochemical studies

Negative control (GI): Histopathological examination of ileocecal specimens showed preserved normal villous pattern (Fig. 2a). Immunohistochemical examination revealed positive nuclear staining for CDX2 in most of

Table 1. Post-Hoc test for multiple comparisons between study groups regarding *Cryptosporidium* oocyst load in the stool.

		Least significance difference (LSD)	*P* value
GII (n = 10)	**GIII**	115.8	**< 0.001 S**
	GIV	162.2	**< 0.001 S**
GIII (n = 10)	**GIV**	46.4	**0.003 S**

S: Significant (*P* < 0.05).

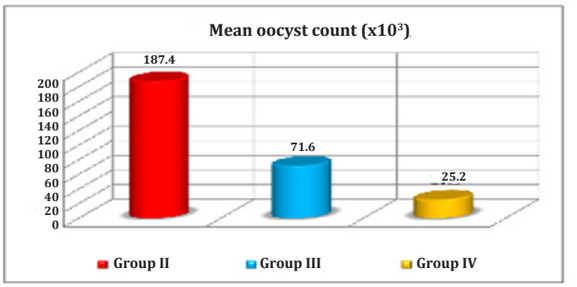

Fig. 1. Mean oocyst count in the stool of the study groups.

the crypt acinar nuclei with mild brownish cytoplasmic staining in acini for caspase-3 (Fig. 2 b, and c).

Infected non-treated (GII): Histopathological examination of ileocecal specimens showed shortening, broadening, and ulceration of villi with mild inflammation and focal low-grade dysplasia at the crypts of the ileocecal region (Fig. 3a). Immunohistochemical examination showed scanty nuclear CDX2 staining in acini, and heavy infiltration by mononuclear inflammatory cells with marked brownish cytoplasmic staining in acini for caspase-3 (Fig. 3 b, and c).

Infected NTZ treated (GIII): Histopathological examination of ileocecal specimens showed focal villous tip erosions and mild villous core infiltration by mononuclear inflammatory cells (Fig. 4a). Immunohistochemical examination showed marked nuclear staining in acini for CDX2, and mild infiltration by mononuclear inflammatory cells in the intervening stroma with mild brownish cytoplasmic staining in acini for caspase-3 (Fig. 4 b, and c).

Infected CO treated (GIV): Histopathological examination of ileocecal specimens showed mostly preserved villous pattern with mild villous core inflammation (Fig. 5a). Immunohistochemical examination showed highly positive nuclear staining in acini for CDX2 and mild infiltration by mononuclear inflammatory cells within the intervening stroma with nearly negative cytoplasmic staining in acini for caspase-3 (Fig. 5b, and c).

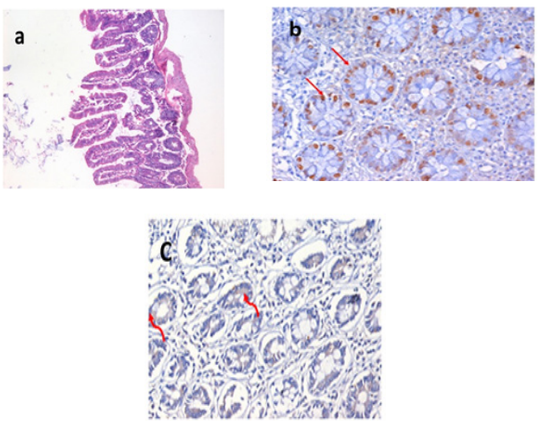

Fig. 2. Sections in the ileocecal region of GI showing **(a)** conventional villous pattern (H&E stain, X200); **(b)** positive nuclear staining for CDX2 in most of the crypt acinar nuclei (red arrows) (IHC for CDX2, X400); **(c)** mild brownish cytoplasmic staining in acini for caspase-3 (red arrows) (IHC for caspase-3, X250).

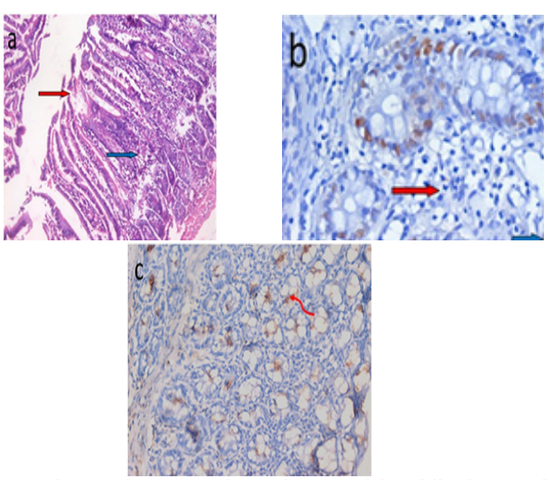

Fig. 4. Ileocecal section of GIII showing **(a)** mildly distorted villous pattern with focal villous tip erosions (red arrow) and mild villous core infiltration by mononuclear inflammatory cells (blue arrow) (H&E stain, X200); **(b)** apparent positive nuclear staining in acini for CDX2 (blue arrow), and mild infiltration by mononuclear inflammatory cells in the intervening stroma (red arrow) (IHC for CDX2, X400); **(c)** mild brownish cytoplasmic staining in acini for caspase-3 (arrow) (IHC for caspase-3, X250).

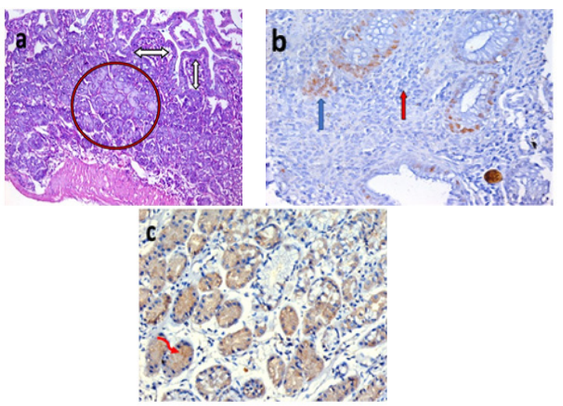

Fig. 3. Sections in the ileocecal region of GII showing **(a)** villous shortening (vertical white double headed arrow), broadening (horizontal white double headed arrow), ulceration, mild inflammation, and focal low-grade dysplasia (inside the red circle) at the crypts of the ileocecal region (H&E stain, X200); **(b)** less nuclear staining in acini for CDX2 (blue arrow), and more infiltration by mononuclear inflammatory cells (red arrow) (IHC for CDX2, X400); **(c)** obvious brownish cytoplasmic staining in acini for caspase-3 (arrow) (IHC for caspase-3, X250).

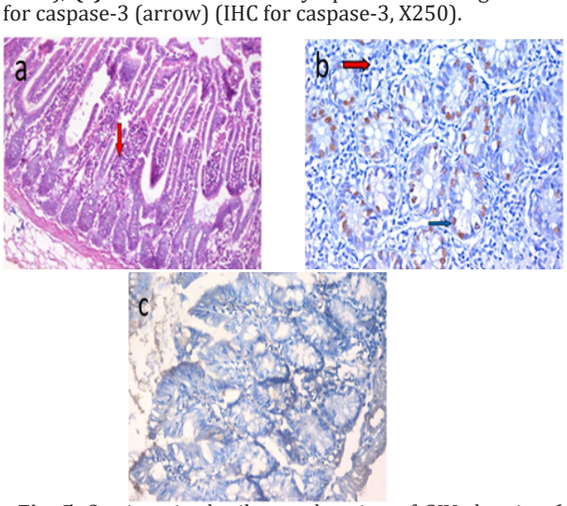

Fig. 5. Sections in the ileocecal region of GIV showing **(a)** mostly preserved villous pattern with mild villous core inflammation (H&E stain, X200); **(b)** apparent positive nuclear staining in acini for CDX2 (blue arrow), and mild infiltration by mononuclear inflammatory cells within the intervening stroma (red arrow) (IHC for CDX2, X400); **(c)** nearly negative cytoplasmic staining in acini for caspase-3 (IHC for caspase-3, X250).

DISCUSSION

Infection could be considered as one of the most significant predisposing causes of cancer and numbers of infection-associated cancers are elevating at a serious rate all over the world[36]. The estimated total number of cancer cases due to infections in the year 2002 was 1.9 million, equivalent to 17.8% of the total cases of cancer[37]. The most significant known pathogens are *Helicobacter pylori* (5.5%), human papilloma viruses (HPV) (5.2%), hepatitis B and C viruses (4.9%), Epstein-Barr virus (EBV) (1%), human immunodeficiency virus (HIV), human herpes virus 8 (HHV-8) rated (0.9%), and human T-lymphotropic virus (HTLV-I) (0.03%) [37]. There are, however, other pathogens that could also stimulate the tumorigenesis, such as parasites. Among helminths, the trematode *S. haematobium* may stimulate the development of urinary bladder cancer. Other trematodes like *O. viverrini* and *C. sinensis* were sometimes accompanied with cholangiocarcinoma in widespread regions of the Far East[36,38]. Among the parasitic protists, there was an expected relation between some apicomplexan and flagellate species on one hand and neoplastic changes in the host tissues on the other hand. It is noteworthy that many intracellular protists (*Leishmania* spp., *T. cruzi, C. parvum, T. gondii, Plasmodium* spp., *Theileria* spp.) suppress apoptosis[39], predisposing to malignancy[40]. Nevertheless, the induced transformation of host cells was only reported experimentally in association with the apicomplexans *Cryptosporidium* and *Theileria*. Besides, it was reported that *C. parvum* induced invasive cancer in the GIT and biliary epithelia of mice suffering from severe combined immunodeficiency[16,41-43].

The natural CO collected from fresh, mature coconut kernels, was found to have significant anti-parasitic properties[21,22]; and good anti-inflammatory activity[44]. It is noteworthy that CO was also proved to have important anticancer activity especially against liver and oral cancers[45]. In the present study, we observed that immunosuppressed-infected-CO treated mice (GIV) showed the best results with the lowest *Cryptosporidium* spp. parasite load between the study groups with a significant statistical difference from the positive control group (P<0.001) and significant statistical difference with GIII, treated by NTZ (P=0.003) (Fig. 1, Table 1). This concurs with studies that proved anti-parasitic activity of CO against *Leishmania* spp., *P. falciparum* and *P. berghei*[21,22].

In our study histopathological examination of specimens from the ileocecal regions of treated groups revealed dramatic improvement of the pathological changes with preserved villous pattern, and very mild villous core inflammation in CO treated group that apparently exceeded the healing effect induced by NTZ. This is in congruence with a study that proved important anti-inflammatory healing properties of Coconut palm (*C. nucifera*)[46].

We used IHC staining of ileocaecal region to reveal dysplastic changes using CDX-2 and caspase-3 staining. This agrees with Saller *et al*.[47] who emphasized the use of CDX-2 as an important predictive marker for the existence of dysplasia. This also concurs with a study that proved caspase-3 as a crucial mediator of apoptosis by inducing condensation of chromatin, apoptotic bodies formation, and DNA fragmentation[48]. So, detection of caspase-3 in cells could be considered as a significant method for apoptosis stimulated by different apoptotic signals[49].

Immunohistochemical examination of specimens from the ileocecal regions of all groups showed apparent positive nuclear staining in acini for CDX2, and mild infiltration by mononuclear inflammatory cells within the intervening stroma with nearly negative cytoplasmic staining in acini for caspase-3 in GIV. This agrees with Craig-Schmidt *et al*.[50] who observed an important decline in dimethyl benz[a]anthracene (DMBA)-induced mammary tumorigenesis in Balb/c mice that had received a therapeutic combination of CO and menhaden oil. Analysis of the individual fatty acid components of oil revealed the potential role of medium chain saturated fatty acids (MCFAs), mainly LA. Lauric acid was proved to have various pharmacological effects such as anti-diabetic[51], lowering of blood pressure in spontaneously hypertensive rats[52], and protection of neuronal cells by enhancing ketone body production[53]. Anticancer activity of LA was also reported in colon and breast cancer cells[54-55]. Various pathways were associated with cancer advancement and cell survival. Among these, cyclin-dependent kinase (CDK)[35], the epidermal growth factor receptor (EGFR)[56], dihydrofolate reductase (DHFR)[57], thymidylate synthase (TS)[58], vascular endothelial growth factor receptor-2 kinase (VEGFR)[59], estrogen receptor (ER)[60] and B-cell lymphoma-extra-large (Bcl-xl)[61] proved important. Of these various pathways, the EGFR axis is the most significant[62-64]. Many natural products are known to interfere with such pathways and start apoptosis in cancer cells[65-67]. Significantly, the EGFR pathway suppression stimulates cytotoxic and anti-metastatic effects[67-69] in different cancers and showed a significant amelioration in the survival rate of cancer patients[70]. Treatment of colon cancer by LA at a dose of 0.5 mM (about 100 mg/ml), could induce apoptotic changes and cell cycle arrest in G0/G1 and G2/M interphase and mitotic phase respectively; the treatment also elevated the intracellular reactive oxygen species with a concomitant reduction in the intracellular reduced glutathione levels[52,71]. Treatment of breast cancer using LA at a dose of 100 mM (about 20 mg/ml) was reported to induce apoptosis mediated through the phosphorylation of EGFR and Rho-associated kinase pathway[72].

It was concluded that CO has an effective anti-*Cryptosporidium* activity with promising ability to decrease the incidence of dysplastic changes in

chronic cases. Further studies should focus on deep understanding of its mechanism of action, optimum dose, and the probable side effects.

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Author contribution: Abdelmaksoud HF designed the plan of work and performed the parasitological studies. Aboushousha T performed histopathological and immunohistochemical studies of the ileocecal region of the small intestine and liver with the portal tracts. El-Ashkar AM shared in designing the plan of work, analyzing the data, 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.

Conflicts of interest: None.

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REFERENCES

1. Ryan U, Zahedi A, Paparini A. *Cryptosporidium* in humans and animals: A one health approach to prophylaxis. Parasite Immunol 2016;38(9):535-47.
2. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. *Cryptosporidium* pathogenicity and virulence. Clin Microbiol Rev 2013; 26(1):115–134.
3. Berahmat R, Mahami-Oskouei M, Rezamand A, Spotin A, Aminisani N, Ghoyounchi R, *et al*. *Cryptosporidium* infection in children with cancer undergoing chemotherapy: How important is the prevention of opportunistic parasitic infections in patients with malignancies? Parasitol Res 2017; 116(9):2507–2515.
4. Hunter PR, Nichols G. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. Clin Microbiol Rev 2002; 15:145–154.
5. García-Elorriaga G, Corona-de Los Santos JC, Méndez-Tovar S, del Rey-Pineda G, Pérez-Casillas R. Opportunistic bacteria and microbial flora in children with leukemia and neutropenic enterocolitis. Rev Med Inst Mex Seguro Soc 2013; 51(4):424–427.
6. Shebl FM, Engels EA, Goedert JJ. Opportunistic intestinal infections and risk of colorectal cancer among people with AIDS. AIDS Res Hum Retroviruses 2012; 28(9):794–799.
7. Sulzyc-Bielicka V, Kołodziejczyk L, Jaczewska S, Bielicki D, Kładny J, Safranow K. Prevalence of *Cryptosporidium* spp. in patients with colorectal cancer. Pol Prz Chir Polish J Surg 2012; 84(7):348–351.
8. Osman M, Benamrouz S, Guyot K, Baydoun M, Frealle E, Chabe M, *et al*. High association of *Cryptosporidium* spp. infection with colon adenocarcinoma in Lebanese patients. PLoS One 2017; 12(12): e0189422.
9. Sulżyc-Bielicka V, Kołodziejczyk L, Jaczewska S, Bielicki D, Safranow K, Bielicki P, *et al*. Colorectal cancer and *Cryptosporidium* spp. infection. PLoS One 2018; 13(4): e0195834.
10. Izquierdo J, Antúnez I, Calderón M., Pérez Giraldo C, Muñoz Sanz A. Diarrhea caused by *Cryptosporidium* and colonic neoplasia. Rev Clínica Española 1988; 182:393–394.
11. de Souza Ldo R, Rodrigues M, Morceli J, Kemp R, Mendes R. Cryptosporidiosis of the biliary tract mimicking pancreatic cancer in an AIDS patient. Rev Soc Bras Med Trop 2004; 37(2):182–185.
12. Hayward A, Levy J, Facchetti F, Notarangelo L, Ochs H, Etzioni A, *et al*. Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. J Immunol 1997; 158(2):977-983.
13. Stephens J, Cosyns M, Jones M, Hayward A. Liver and bile duct pathology following *Cryptosporidium parvum* infection of immunodeficient mice. Hepatology 1999; 30(1):27–35.
14. Leven E, Maffucci P, Ochs H, Scholl P, Buckley R, Fuleihan R, *et al*. Hyper IgM syndrome: A report from the USIDNET registry. J Clin Immunol 2016; 36(5):490–501.
15. Rahman M, Chapel H, Chapman R, Collier J. Cholangiocarcinoma complicating secondary sclerosing cholangitis from cryptosporidiosis in an adult patient with CD40 ligand deficiency: Case report and review of the literature. Int Arch Allergy Immunol 2012; 159(2):204–208.
16. Zhang N, Yu X, Zhang H, Cui L, Li X, Zhang X, *et al*. Prevalence and genotyping of *Cryptosporidium parvum* in gastrointestinal cancer patients. J Cancer 2020; 11(11):3334–3339.
17. Sheela DL, Narayanankutty A, Nazeem PA, Raghavamenon AC, Muthangaparambil SR. Lauric acid induce cell death in colon cancer cells mediated by the epidermal growth factor receptor downregulation: An *in silico* and *in vitro* study. Hum Exp Toxicol 2019; 38(7):753–761.
18. Butler L, Perone Y, Dehairs J, Leslie EL, Vincent dL, Ali T *et al*. Lipids and cancer: Emerging roles in pathogenesis, diagnosis, and therapeutic intervention. Adv Drug Deliv Rev 2020; 159:245–293.
19. Ross IA. Chemical Constituents, traditional and modern medicinal uses. Medicinal plants of the World. Humana Press. Totowa, New Jersey 2005; Volume 3, pp. 155-184.
20. Manalo RV, Silvestre MA, Barbosa ALA, Medina PM. Coconut (*Cocos nucifera*) ethanolic leaf extract reduces amyloid-β (1-42) aggregation and paralysis prevalence in transgenic *Caenorhabditis elegans* independently of free radical scavenging and acetylcholinesterase inhibition. Biomedicines 2017; 5(2):17.
21. Mendonça-Filho RR, Rodrigues IA, Alviano DS, Santos ALS, Soares RMA, Alviano CS, *et al*. Leishmanicidal activity of polyphenolic-rich extract from husk fiber of *Cocos nucifera* Linn. (Palmae). Res Microbiol 2004; 155(3):136–143.
22. Adebayo JO, Balogun EA, Malomo SO, Soladoye AO, Olatunji LA, Kolawole OM, *et al*. Antimalarial activity of *Cocos nucifera* husk fibre: Further studies. Evid Based Complement Alternat Med 2013; 2013:742476.
23. Aldinger KA, Sokoloff G, Rosenberg DM, Palmer AA, Millen KJ. Genetic variation and population substructure

in outbred CD-1 Mice: Implicatins for genome-wide association studies. PLoS ONE 2009; 4(3), e4729.
24. Arabo A, Potier C, Ollivier G, Lorivel T, Roy V. Temporal analysis of free exploration of an elevated plus-maze in mice. J Exp Psychol Anim Learn Cogn 2014; 40(4):457-466.
25. Patil SS, Sunyer B, Höger H, Lubec G. Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the multiple T-maze and in the Morris water maze. Behav Brain Res 2009;198(1):58-68.
26. Hsieh LS, Wen JH, Miyares L, Lombroso PJ, Bordey A. Outbred CD1 mice are as suitable as inbred C57BL/6J mice in performing social tasks. Neuroscience Letters 2017; 637:142–147.
27. Rehquel T, David A, Belwett N, S M, Carmona P. *C. parvum* infection in experimentally infected mice: Infection dynamics and effect of immunosuppression. Folk Parasitol 1998; 45:101-107.
28. Abdelmaksoud HF, El-Ashkar AM, Elgohary SA, El-Wakil ES. Potential therapeutic and prophylactic effects of Asafoetida in murine cryptosporidiosis. J Parasit Dis 2020; 44(3):646–653.
29. Barnes J, Paget G. Mechanisms of toxic action. Prog Med Chem 1965; 4:18–38.
30. Silalahi J, Pertiwi D, Dalimunthe A, Silalahi YCE. Effect of acute consumption of coconut and palm oil on swimming capacity endurance of mice (*Mus musculus*). Int J Pharm Tech Res 2015; 8(9):55-59.
31. Henricksen S, Pohlenz J. Staining of *Cryptosporidium* by a modified Ziehl-Neelsen technique. Acta Vet Scand 1981; 22:594–596.
32. Garcia LS (Editor). Clinically important human parasites: Intestinal protozoa: *Cryptosporidium* spp. In: Diagnostic Medical Parasitology. 5th edition ASM Press Washington DC; 2007, pp. 761–818.
33. Benamrouz S, Guyot K, Gazzola S, Mouray A, Chassat T, Delaire B, *et al*. *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 2012; 7(12):e51232.
34. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. Methods Mol Biol 2014; 1180:31–43.
35. Aboushousha T, Hammam O, Aref A, Kamel A, Badawy M, Abdel Hamid A. Tissue profile of CDK4 and STAT3 as possible innovative therapeutic targets in urinary bladder cancer. Asian Pac J Cancer Prev 2020; 21(2):547–554.
36. de Martel C, Franceschi S. Infections and cancer: Established associations and new hypotheses. Crit Rev Oncol Hematol 2009; 70(3):183–194.
37. Parkin D. The global health burden of infection-associated cancers in the year 2002. Int J cancer 2006; 118(12):3030–3044.
38. World Health Organization. Monograph on the evaluation of carcinogenic risks to humans. Part B: Biological agents. International Agency for Research on Cancer (IARC) 2011; 1–487.
39. Carmen J, Sinai A. Suicide prevention: Disruption of apoptotic pathways by protozoan parasites. Mol Microbiol 2007; 64(4):904–916.
40. Lowe S, Lin AW. Apoptosis in cancer. Carcinogenesis 2000; 21:485–495.
41. Certad G, Ngouanesavanh T, Guyot K, Gantois N, Chassat T, Mouray A, *et al*. *Cryptosporidium parvum*, a potential cause of colic adenocarcinoma. Infect Agent Cancer 2007; 2:22.
42. Certad G, Creusy C, Guyot K, Mouray A, Chassat T, Delaire B, *et al*. Fulminant cryptosporidiosis associated with digestive adenocarcinoma in SCID mice infected with *Cryptosporidium parvum* TUM1 strain. Int J Parasitol 2010; 40(13):1469–1475.
43. Certad G, Creusy C, Ngouanesavanh T, Guyot K, Gantois N, Mouray A, *et al*. Development of *Cryptosporidium parvum*-induced gastrointestinal neoplasia in severe combined immunodeficiency (SCID) mice: Severity of lesions is correlated with infection intensity. Am J Trop Med Hyg 2010; 82(2):257–265.
44. Lin T-K, Zhong L, Santiago JL. Molecular sciences anti-inflammatory and skin barrier repair effects of topical application of some plant oils. Int J Mol Sci 2017; 19(1):70.
45. Verma P, Naik S, Nanda P, Banerjee S, Naik S, Ghosh A. *In vitro* anticancer activity of virgin coconut oil and its fractions in liver and oral cancer cells. Anticancer Agents Med Chem 2019; 19(18):2223–2230.
46. Roopan S. An overview of phytoconstituents, biotechnological applications, and nutritive aspects of Coconut (*Cocos nucifera*). Appl Biochem Biotechnol 2016; 179(8):1309–1324.
47. Saller J, Al Diffalha S, Neill K, Bhaskar RA, Oliveri C, Boulware D, *et al*. CDX-2 Expression in esophageal biopsies without goblet cell intestinal metaplasia may be predictive of Barrett's esophagus. Dig Dis Sci 2020; 65(7):1992-1998.
48. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol 2013; 5:a008656.
49. Choudhary GS, Al-Harbi S, Almasan A. Caspase-3 activation is acritical determinant of genotoxic stress-induced apoptosis. Methods Mol Biol 2015; 1219:1–9.
50. Craig-Schmidt M, White M, Teer P, Johnson J, Lane H. Menhaden, coconut, and corn oils and mammary tumor incidence in BALB/c virgin female mice treated with DMBA. Nutr Cancer 1993; 20(2):99–106.
51. Sheela DL, Nazeem PA, Narayanankutty A, Shylaja RM, Davis SP, James P, *et al*. Coconut phytocompounds inhibits polyol pathway enzymes: Implication in prevention of microvascular diabetic complications. Prostaglandins Leukot Essent Fat Acids 2017; 127:20–24.
52. Alves NFB, de Queiroz TM, de Almeida Travassos RT, Magnani M, de Andrade Braga VB. Acute treatment with lauric acid reduces blood pressure and oxidative stress in spontaneously hypertensive rats. Basic Clin Pharmacol Toxicol 2017;120(4):348–353.
53. Nonaka Y, Takagi T, Inai M, Nishimura S, Urashima S, Honda K, *et al*. Lauric acid stimulates ketone body production in the KT-5 astrocyte cell line. J Oleo Sci 2016; 65(8):693–699.
54. Fauser JK, Matthews GM, Cummins AG, Howarth GS. Induction of apoptosis by the medium-chain length fatty

acid lauric acid in colon cancer cells due to induction of oxidative stress. Chemotherapy 2013; 59: 214–224.
55. Kadochi Y, Mori S, Fujiwara-Tani R, Luo Y, Nishiguchi Y, Kishi S, *et al*. Remodeling of energy metabolism by a ketone body and medium-chain fatty acid suppressed the proliferation of CT26 mouse colon cancer cells. Oncol Lett 2017; 14: 673–680.
56. Chan DLH, Segelov E, Wong RSH, Smith A, Herbertson RA, Li BT, *et al*. Epidermal growth factor receptor (EGFR) inhibitors for metastatic colorectal cancer. In: Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2017.
57. Morales C, García MJ, Ribas M, Miró R, Muñoz M, Caldas C, *et al*. Dihydrofolate reductase amplification and sensitization to methotrexate of methotrexate-resistant colon cancer cells. Mol Cancer Ther 2009; 8(2):424–432.
58. Niedzwiecki D, Hasson RM, Lenz H, Ye C, Redston M, Ogino S, *et al*. A study of thymidylate synthase expression as a biomarker for resectable colon cancer: Alliance (Cancer and Leukemia Group B) 9581 and 89803. Oncologist 2017; 22(1):107–114.
59. Saif MW. Anti-VEGF agents in metastatic colorectal cancer (mCRC): Are they all alike? Cancer Manag Res 2013; 5:103–115.
60. Caiazza F, Ryan EJ, Doherty G, Winter DC, Sheahan K. Estrogen receptors and their implications in colorectal carcinogenesis. Front Oncol 2015; 5:19.
61. Scherr AL, Gdynia G, Salou M, Radhakrishnan P, Duglova K, Heller A, *et al*. Bcl-xL is an oncogenic driver in colorectal cancer. Cell Death Dis 2016; 7(8):e2342.
62. Weihua Z, Tsan R, Huang WC, Wu Q, Chiu CH, Fidler IJ, *et al*. Survival of cancer cells is maintained by EGFR independent of its kinase activity. Cancer Cell 2008; 13(5):385–393.
63. Crook M, Upadhyay A, Ido LJ, Hanna-Rose W. Epidermal growth factor receptor cell survival signaling requires phosphatidylcholine biosynthesis. G3 (Bethesda) 2016; 6(11):3533–3540.
64. Chen Y, Henson ES, Xiao W, Huang D, McMillan-Ward EM, Israels SJ, *et al*. Tyrosine kinase receptor EGFR regulates the switch in cancer cells between cell survival and cell death induced by autophagy in hypoxia. Autophagy 2016; 12(6):1029–1046.
65. Angulo P, Kaushik G, Subramaniam D, Dandawate P, Neville K, Chastain K, *et al*. Natural compounds targeting major cell signaling pathways: A novel paradigm for osteosarcoma therapy. J Hematol Oncol 2017;10(1):10.
66. Narayanankutty V, Narayanankutty A, Nair A. Heat Shock Proteins (HSPs): A novel target for cancer metastasis prevention. Curr Drug Targets 2018; 20(7):727–737.
67. Illam SP, Narayanankutty A, Mathew SE, Valsalakumari R, Jacob RM, Raghavamenon AC. Epithelial mesenchymal transition in cancer progression: Preventive Phytochemicals. Recent Pat Anticancer Drug Discov 2017; 12(3):234-246.
68. Nabarun R, Arunaksharan N, Nazeem P, Valsalan R, Babu T, Mathew D. Plant phenolics ferulic acid and P-coumaric acid inhibit colorectal cancer cell proliferation through EGFR down-regulation. Asian Pac J Cancer Prev 2016; 17(8):4019-4023.
69. Roy N, Nazeem PA, Babu TD, Abida PS, Narayanankutty A, Valsalan R, *et al*. EGFR gene regulation in colorectal cancer cells by garlic phytocompounds with special emphasis on S-allyl-L-cysteine sulfoxide. Interdiscip Sci Comput Life Sci 2018; 10(4):686–893.
70. Kuan FC, Kuo LT, Chen MC, Yang CT, Shi CS, Teng D, *et al*. Overall survival benefits of first-line EGFR tyrosine kinase inhibitors in EGFR-mutated non-small-cell lung cancers: A systematic review and meta-analysis. Br J Cancer 2015; 113(10):1519–1528.
71. Fauser JK, Matthews GM, Cummins AG, Howarth GS. Induction of apoptosis by the medium-chain length fatty acid lauric acid in colon cancer cells due to induction of oxidative stress. Chemotherapy 2014; 59(3):214–224.
72. Lappano R, Sebastiani A, Cirillo F, Rigiracciolo DC, Galli GR, Curcio R, *et al*. The lauric acid-activated signaling prompts apoptosis in cancer cells. Cell Death Discov 2017; 3(1): 17063.