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Dina El-Shewehy [1*], Gehad Elshobaky[2], Amira Ismail[3], Shimaa Hassan[4], Amany Ramez[1]

[1]Zoology Department faculty of Science Mansoura university

[2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University

[3]Department of Parasitology, Faculty of Medicine, Mansoura University

[4]Zoology Department, Faculty of Science, Zagazig University

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Original Article

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[1]Zoology Department faculty of Science Mansoura university

[2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University

[3]Department of Parasitology, Faculty of Medicine, Mansoura University

[4]Zoology Department, Faculty of Science, Zagazig University

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Abstract

Humans frequently contract the diarrheal disease known as cryptosporidiosis, which is brought on by the protozoan parasite Cryptosporidium. The current study looked at how pomegranate (Punica granatum) peel extracts, garlic (Allium sativum), and ginger (Zingiber officinale) affected the biochemical and antioxidant parameters of a mouse model of experimental cryptosporidiosis. 82 mice were allocated into 6 groups; Control group, Infected non-treated group (experimentally infected by 104 C. oocysts/mouse), Ginger, garlic, and pomegranate groups experimentally infected with C. parvum and treated with the plants' extracts, as compared to the reference drug, metronidazole (MTZ).We observed that all therapies promoted liver function by reducing serum levels of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes and raising levels of total protein, albumin, and globulin. Furthermore, the levels of serum malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) were likewise restored to nearly their normal values in the treated groups. Garlic extract was noticed to have a noteworthy impact on the infected mice than the other treatments and ameliorated health benefits, performing a natural efficient therapeutic alternative, without drug resistance and with the fewest side effects possible for treating cryptosporidiosis.

1. Introduction

A parasitic illness, cryptosporidiosis is brought on by the apicomplexan parasite Cryptosporidium (Ryan et al., 2014). This parasite infects several hosts, including humans and other mammals (Kotloff et al., 2013). It inhabits gastrointestinal and respiratory tracts, causing significant morbidity and mortality (Kotloff et al., 2013). The small intestine is the main site of infection, although infection might be found in extraintestinal sites such as the lung, stomach, and spleen (Dubey et al., 2002; Matsubayashi et al., 2011; Sponseller et al., 2014; Wang et al., 2009). The biology and treatment of gastric cryptosporidiosis have not been well evaluated compared with that of intestinal cryptosporidiosis. Water diarrhea, severe dehydration, electrolyte disturbances, malnutrition, and weight loss are all indications of Cryptosporidiosis (Xiao et al., 2004). Although infection causes self-limited diarrhea in healthy individuals, it can be lethal in immunocompromised ones (Shirley et al., 2012).

Since Cryptosporidium parvum is highly resistant to current drug treatments, control of Cryptosporidiosis is still considered a serious challenge in both veterinary and human medicine (Armson et al., 2003; Ryan et al., 2014). Several endeavors have been made by pharmaceutical industries to provide efficient treatment for this disease; yet it remains a serious health problem that requires the creation of a reliable and safe treatment (Del Coco et al., 2009).

Current treatments for Cryptosporidiosis are inadequate. Several antiparasitic drugs are used such as paromomycin, nitazoxanide, and metronidazole (MTZ). These drugs show only temporary effects and usually relapses happened (Gargala, 2008;

Smith and Corcoran, 2004). Recently, the most effective way to cure parasite infections is to boost individuals' immune systems by employing plants and their bioactive substances as an alternative (Jamil et al., 2022). The application of plant-derived compounds seems to be logical due to their low toxicity and high therapeutic efficacy (Braga et al., 2005; Perrucci et al., 2006; Razavi et al., 2015; Wabwoba et al., 2010). Numerous studies looked at how plant extracts, including garlic, could be used to treat Cryptosporidiosis (Abdel Megeed et al., 2015; Abouel-Nour et al., 2016; Gaafar, 2012), onion and cinnamon (Abu El Ezz et al., 2011; Hazaa et al., 2016), curcumin (Asadpour et al., 2018), ginger (Abouel-Nour et al., 2016; Abouelsoued et al., 2020), and pomegranate (Aboelsoued et al., 2019; Al-Mathal and Alsalem, 2012).

Since ancient times, numerous societies have used garlic (Allium sativum) as food and medicine. It has been used not only as a spice but also as a substance showing many beneficial effects such as antioxidant, anti-inflammatory, antimicrobial (Myneni et al., 2016), antihelminthic (Riad et al., 2009), antiprotozoal (Al-Azzawi, 2003), antithrombotic, hypoglycemic (Lopez-Jaramillo, 2016; Ried, 2016) and antitumor activities (Thomson and Ali, 2003). Because many of the medically important parasites are susceptible to garlic extract, it holds a promising position as a therapeutic agent.

Ginger (Zingiber officinale) has a long history of medical application extending back 2500 years in China and India (Grant and Lutz, 2000). Its pharmaceutical properties include antioxidant, anti-inflammatory, anticancer (Vinothkumar et al., 2014), antihelminthic (Iqbal et al., 2001), antiprotozoal (Al-Masoudi, 2011; Kobo et al., 2014), and anti-leech effects (El-Sayed and El-Saka, 2015).

Pomegranate (Punica granatum) is a centuries-old fruit that has been utilized in traditional medicine to cure a variety of illnesses including diarrhea, urinary tract infections, kidney stones and parasite infections (Navarro et al., 1996; Sudheesh and Vijayalakshmi, 2005). Several publications explored the therapeutic potentials of P. granatum peel, fruit, and juice as a potent antioxidant and anti-inflammatory agent containing tannins and polyphenols (Afaq et al., 2005; Aviram et al., 2002; Aviram et al., 2000; Cerd et al., 2003; Ghasemian et al., 2006; Kim et al., 2002; Suzuki et al., 2004). P. granatum is also considered an anticancer agent (Syed et al., 2007), and its juice is helpful in avoiding Alzheimer's disease (Wang et al., 2009). Although its effect on parasitic infections is still much unknown, some studies have proved that P. granatum has an antiprotozoal substance (Calzada et al., 2006; Dell'Agli et al., 2009; El-Sherbini et al., 2009).

There is a shortage of data about using natural products and medicinal plants for Cryptosporidiosis control in humans and animals. Further, there is a critical need for effective treatments to eradicate this disease. The present study was, therefore, conducted to test the anticryptosporidial action of 3 plant extracts (garlic, ginger, and pomegranate) on C. parvum experimentally infected mice compared with the commonly used chemical drug MTZ and to evaluate the curative capacity of these extracts.

2. Materials and Methods

2.1. Ethics Statement

The Animal Administration and Ethics Committee of the Faculty of Science at Mansoura University reviewed and authorized all animal experiments. The investigation was carried out strictly in accordance with the guidelines and protocol for animal ethics at Mansoura University. Every attempt was made to decrease the number of animals utilized in the experiment and to minimize their suffering. Code number: MU-ACUC (SC.R.22.11.2).

2.2. Experimental Animals

Weighing 25–30 g, male Swiss Albino mice (3-5 weeks old) were purchased from the National Research Center's animal house in Cairo, Egypt. Before the experiment began, the mice were housed in neat, ventilated cages with fresh bedding being refreshed every day for a week to help them get used to the laboratory environment. Using a direct wet salinity approach, the mice faeces were inspected to rule out the presence of parasites (Garcia and Bruckner, 1997). The animals received a proper meal and limitless water during the acclimation phase.

Parasite

C. parvum oocysts were provided by the Department of Parasitology at Theodor Bilharz Research Institute. The oocysts were maintained at 4 °C in a solution of 2.5% potassium dichromate (wt/vol) (Khalifa et al., 2001). In order to eliminate potassium dichromate, before infection, distilled water was used to wash the cryptosporidium oocysts 3 times. Following a 10-minutes at 1500 g centrifugation, the oocysts were then counted using a hemocytometer. By dilution of the oocyst in the required volume of distilled water, about 104 oocysts/mL were prepared for infection of each mouse (Gaafar, 2007).

2.3. Plant Extract Preparation

Ginger

The ginger rhizomes were obtained from the Botany Department, Faculty of Science, Mansoura University, Egypt. Using a Moulinex® grinder, they were mechanically ground after being cut into slices and dried for seven days. A convenient container was filled with 100 g of ground ginger, 400 mL of methanol, and the mixture was stirred for an hour with a magnetic stirrer before being left at room temperature for 24 hours. It was then mixed once

more, filtered through grade one Whatman cellulose filter papers (Bastone, UK), and the solvent was then evaporated in a rotary evaporator.Finally, a sterile container was used to keep the concentrated residue (4 g) in the refrigerator until use (Moazeni and Nazer 2010). 100 mg/kg body weight was the dosage determined for this study.

Garlic

After drying, an electric grinder was used to crush around 500 g of clean garlic bulbs. Diluting the paste with distilled water (1 g/mL) produced the working solution, which was stored at -20 °C until it was needed (Masamha et al., 2010). 50 mg/kg body weight was the dose that was selected for this study (Riad et al., 2009).

Pomegranate

P. granatum fruit was bought at a local store. The Botany Department, Faculty of Science, Mansoura University confirmed the P. granatum samples. Percolating with 70% methanol was performed to extract the air-dried powder (100 g) of pomegranate peels. and then kept at 4 °C for 48 h. The extracted substance was then filtered, the solvent evaporated at 40 to 50 °C, and it was stored at 4 °C until it was required (Abdel Moneim, 2012).

2.4. Phytochemical Analysis

According to prior studies, total amounts of flavonoids, cardiac glycoside, total phenols, saponins, tannins, alkaloids, and reducing sugars were quantified (El-Olemy et al., 1994; Higuchi and Bodin, 1961; Lindsay, 1973; Okada et al., 2010; Peng et al., 2001; Sadasivam and Manickam, 2008; Wolfe et al., 2003).

2.5. Experimental Design and Sampling

Six groups of 82 male Swiss Albino mice were created at random (12 mice/group) as follows: The untreated group served as the control. The non-treated infected group was infected experimentally by 104 C. parvum oocysts/mouse via a stomach tube. 104 C. parvum oocysts had used to infect the MTZ group. MTZ was then administered (50 mg/kg BW/day). The daily plant extract doses for the ginger, garlic, and pomegranate groups were 100 mg/kg BW, 50 mg/kg BW, and 3 gm/kg BW, respectively, followed by C. parvum oocysts infection. For 30 days, all therapies were administered intragastrical started the day after the infection. At the end of the experiment, the mice were anesthetized with ketamine/xylazine (0.1 mL/100 g body weight intraperitoneally) after an overnight fast. Anticoagulant-free blood samples were drawn, centrifuged at 3000 rpm for 15 minutes, and the serum was stored at 20 °C for biochemical and antioxidant assays.

2.6. Serum Biochemical Parameters

Alanine (ALT), aspartate aminotransferases (AST; Randox Co., UK), total protein, and albumin (Stanbio Co., USA) were determined in serum samples using a spectrophotometer (5010, Photometer, BM Co., Germany) in accordance with the recommended procedure in each booklet.

2.7. Antioxidant/oxidative stress markers

Spectrophotometric analysis (5010, Photometer, BM Co. Germany) was used to find out the concentrations of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and total antioxidant capacity (TAC) in serum samples with the aid of commercial kits (Biodiagnostic, Egypt).

2.8. Statistical analysis

The SPSS software program, version 20, USA, was used to analyze the data using the Tukey/posthoc test, which is expressed as mean ± standard deviation (SD). The differences were statistically significant at $P < 0.05$.

3. Results

3.1. Phytochemical Components of Different Plant Extracts:

The phytochemical screenings of plant extracts are shown in Table 1, which reveals that carbohydrates were the most active elements, followed by cardiac glycosides, saponins, phenol, reducing sugar, and flavonoids. The levels of tannins and alkaloids were the lowest.

Table 1: Quantitative phytochemical analysis (mean ± SD) of ginger (Zingiber officinale), garlic (Allium Sativum), and pomegranate (Punica granatum) extracts.

Phytochemicalconstituent (mg/g)	Ginger	Garlic	Pomegranate
Flavonoids	7.21± 0.73	17.44± 0.73	5.21± 1.54
Glycosides	11.54±2.36	9.76±1.46	5.33±1.46
Phenols	18.47±1.52	33.83±3.75	9.44±2.51
Saponins	12.31 ±2.77	14.56 ±3.21	6.32 ±1.22
Tannins	1.27 ± 0.86	5.44 ± 0.52	3.21 ± 0.97
Alkaloids	2.52 ±0.09	4.32±0.17	0.24±0.05
Reducing Sugar	-	0.94±0.25	1.53±0.34

3.2. Levels of Serum Hepatic Biomarkers:

As illustrated in Figure 1, the infected non-treated group showed significant hepatic damage represented by higher ALT and AST activities ($p < 0.05$) compared with the control group. Moreover, when compared to the control group, the proteinogram revealed a substantial (p 0.05) decrease in serum total protein, albumin, and globulin in the infected non-treated group. Conversely, the MTZ, ginger, garlic, and pomegranate treated groups exhibited reduced hepatic damage represented by decreased ALT and AST levels ($p < 0.05$) with restoring proteinogram unlike that of the infected group. Interestingly, as compared to the control group, ginger and garlic intake significantly (p 0.05) restored ALT, AST,

albumin, globulin and total protein to their normal levels.

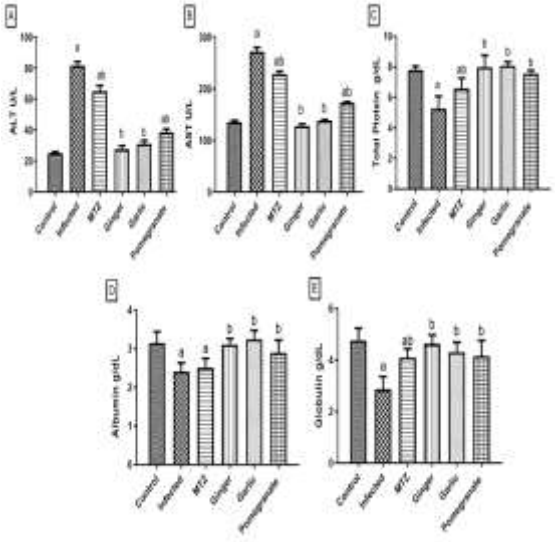

Figure 1: Effect of MTZ, ginger, garlic, and pomegranate peel on serum protein profile and liver enzymes in Cryptosporidium experimentally infected mice

3.3. Variations in Oxidative Stress/Antioxidant Markers:

To find out how garlic, ginger, pomegranate, and MTZ affected the enzymatic oxidative stress markers in the serum of Cryptosporidium-infected non-treated mice, the MDA, catalase, TAC, and SOD activities were measured (Figure 2). Significant elevation ($p < 0.05$) in MDA content as well as significantly lower ($p < 0.05$) TAC, catalase, and SOD activities were found in the infected non-treated group induced compared with the control one. These findings were significantly ameliorated ($p < 0.05$) by pomegranate administration. MTZ–treated mice also showed oxidative stress demonstrated by significantly ($p < 0.05$) higher MDA levels and lower TAC and SOD activity when compared with the control group. Interestingly, the treatment of mice with ginger and garlic extracts significantly ($p < 0.05$) restored the levels of pro-oxidants and antioxidants proteins close to their normal values, demonstrating their potent antioxidant role against Cryptosporidium infection.

4. Discussion

C. parvum is a parasite with vast distribution and potential relevance. Finding an authentic treatment for cryptosporidiosis without suffering from undesirable side effects is a major medical issue. Many drugs, such as MTZ, are used for cryptosporidiosis treatment, yet the growing drug resistance by the parasite is a rising problem, and thus there is an increasing need for developing effective and alternative remedies against Cryptosporidium.

Numerous research focused on the anti-protozoal activity of garlic, ginger, and pomegranate extracts as well as their phytochemicals against different protozoal diseases, including cryptosporidium (e.g., El-Saber Batiha et al., 2020). Yet, this study is regarded as the earliest attempt to ascertain the preservation benefits brought about by pomegranate peels, ginger, and garlic in relation to stomach and splenic damage caused by the C. parvum infection.

In mice, cryptosporidiosis may initiate a chronic disease state that spreads to extraintestinal sites and results in hepatic impairment (Mead et al., 1994). In this study, the changes in liver function were described by testing the serum albumin, globulin, and total protein levels of the studied groups. Compared to healthy mice, experimentally infected non-treated animals with Cryptosporidium had lower levels of serum total protein, albumin, and globulin. These data matched similar findings reported by Abdel Megeed et al. (2015).

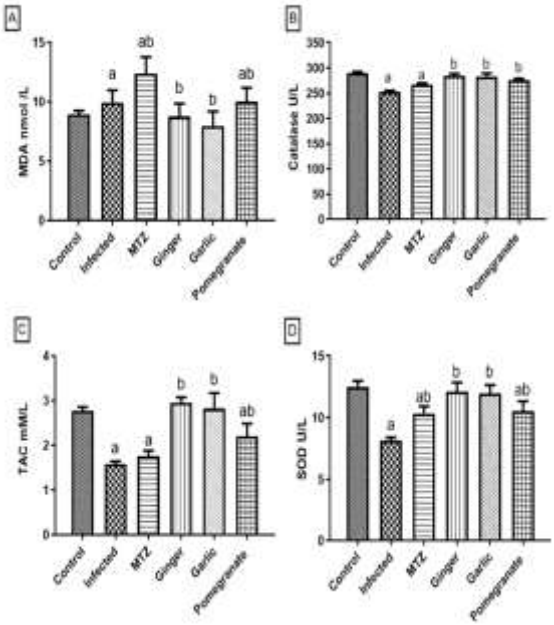

Figure 2: Effect of MTZ, ginger, garlic, and pomegranate peel on MDA, CAT, SOD, and TAC in Cryptosporidium experimentally infected mice

The drop in albumin levels might be due to hepatocellular injury (Ruot et al., 2000), poor nutrient absorption, and excessive breakdown of protein (Malina, 1994). Regarding the determined liver enzymes in this study, experimentally infected mice with Cryptosporidium had higher levels of ALT and AST. ALT serum increase implies cell membrane injury, whereas AST shows hepatic mitochondrial damage. (AbouGabal et al., 2015). These results confirmed that cryptosporidiosis has extraintestinal impacts (Chalmers and Davies, 2010). Mousa et al. (2014) identified cryptosporidiosis as a major potential cause for hepatic encephalopathy. In this study, albumin and globulin concentrations increased in MTZ, garlic, ginger, and pomegranate peel-treated mice

compared with the infected non-treated mice. After therapy, mice treated with MTZ, garlic, ginger, and pomegranate peel showed significant reduction in liver enzymes ALT and AST. However, when compared to the MTZ and pomegranate peel groups, the levels of the measured biochemical markers were improved towards the healthy normal state after administration with ginger and garlic. This means that these treatments benefit the animals' health. The effectiveness of garlic had been documented in different attempts, including studies using various animal models (Abouel-Nour et al., 2016), ginger (Dyab et al., 2016; Fawzy et al., 2019), and pomegranate (Aboelsoued et al., 2019; Al-Megrin, 2017) as antiprotozoal natural products.

Estimation of serum antioxidant capacity (TAC) and measurement of enzymatic antioxidant activity like SOD and CAT is a dependable method of assessing oxidative stress (Saleh et al., 2011). The current research revealed that MDA serum values increased in infected mice while CAT, SOD, and TAC levels declined after Cryptosporidium infection. The significant increase in the MDA level in the infected group may suggest increased lipid peroxidation after Cryptosporidium infection, which reflects free radical-mediated hepatic cell membrane damage. The treatments applied in this research reduced the oxidative damage caused by Cryptosporidium by boosting antioxidant capacity (SOD, CAT, and TAC) and lowering MDA levels. An overload of oxidative stress while dealing with an infection could exaggerate the negative impacts of this infection on health (Abo-Aziza et al., 2017).

These findings support earlier studies that showed that treatment with pomegranate peel extract boosted the free radical scavenging activity of liver enzymes such CAT, peroxidases, and SOD. (Chidambara Murthy et al., 2002) as it was a possible source of natural antioxidants (Anahita et al., 2015). Moreover, pomegranate peels contain major phenolic compounds such as organic acids (Choi et al., 2011) that can directly inhibit C. parvum infections. Additionally, A prior investigation showed a rise in SOD activity and other antioxidants due to the presence of various flavonoids and Sulphur compounds such as S-methyl-cysteine (SMC) in garlic, which have considerable radical scavenging capabilities (El-Saber Batiha et al., 2020). Furthermore, ginger acts as an anti-inflammatory agent by attenuating serum protein levels and as an antioxidant agent by reducing MDA levels and increasing SOD activity (Mahmoud et al., 2012).

Conclusion

It has been observed that the extracts of garlic, ginger, and pomegranate have anti-cryptosporidial activity and can shield healthy people against cryptosporidiosis. These findings could be applied to similar infections that affect both humans and animals.

Data availability statement

The article/supplementary material includes the original contribution described in the study. Any further questions could be directed to the corresponding author.

Author's contributions

D.M.M.E. and A.M.R. collaborated in research design and methodology, supervision, as well as the analysis and interpretation of the results. G.E.E., A.M.R., S.H., and A.I. participated in methodology and data analysis. The manuscript was drafted and ready for publication by D.M.M.E., G.E.E., A.I., S.H., and A.M.R. The final manuscript was read and approved by all contributors.

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Conflicts of Interest

The authors confirm that they have no conflicts of interest.

List of Abbreviations

Cryptosporidium parvum: C. parvum, PI: Post-infection, AST: Aspartate transferase, ALT: Alanine transferase, MDA: Malondialdehyde, CAT: Catalase, SOD: Superoxide dismutase, TAC: Total antioxidant capacity, MTZ: metronidazole.

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