THE EFFICACY OF THREE MEDICINAL PLANTS; GARLIC, GINGER AND MIRAZID AND A CHEMICAL DRUG METRONIDAZOLE AGAINST CRYPTOSPORIDIOsis PARVUM: II- HISTOLOGICAL CHANGES

By

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Abstract

Cryptosporidiosis parvum is a zoonotic protozoan parasite infects intestinal epithelial cells of man and animals causing a major health problem. This study was oriented to evaluate the protective and curative capacity of garlic, ginger and mirazid in comparison with metronidazole drug (commercially known) against Cryptosporidium in experimental mice. Male Swiss Albino mice experimentally infected with C. parvum were treated with medicinal plants extracts (Garlic, Mirazid, and Garlic) as compared to chemical drug Metronidazole. Importantly, C. parvum-infected mice treated with garlic, Mirazid, garlic and metronidazole showed a complete elimination in shedding oocysts by 9th day PI. The reduction and elimination of shedding oocysts in response to the treatments might be attributable to a direct effect on parasite growth in intestines, sexual phases production and/or the formation of oocysts. The results were evaluated histopathological examination of ileum section of control mice (uninfected, untreated) displayed normal architecture of the villi. Examination of infected mice ileum section (infected, untreated) displayed histopathological alterations from uninfected groups. Examination of ileum section prepared from mice treated with garlic, ginger, mirazid, and metronidazole displayed histopathological alterations from that of the control groups, and showed marked histologic correction in the pattern with the four regimes used in comparison to control mice. Garlic successfully eradicated oocysts of infected mice from stool and intestine. Supplementation of ginger to infected mice markedly corrected elevation in the inflammatory risk factors and implied its potential antioxidant, anti-inflammatory and immunomodulatory capabilities. Infected mice treated with garlic, mirazid, garlic and metronidazole showed significant symptomatic improvements during treatment.

Key words: Cryptosporidiosis, Histopathology, Garlic, Ginger, Mirazid, Metronidazole, mice

Introduction

Protozoan parasites of the genus Cryptosporidium belong to the class Sporozoasida, family Cryptosporidiidae and phylum Apicomplexa. They are often referred to as coccidia. While some coccidia can undergo extra-intestinal development and are tissue-cyst forming (Sarcocystis, Toxoplasma) others develop in the gastrointestinal or respiratory tract, without formation of tissue cysts (i.e. Eimeria, Isospora & Cryptosporidium). Like others, it was thought that Cryptosporidium was highly host specific and almost 20 species were named according to species of infected host from which they were isolated (Tyzzer, 1912; Levine, 1984; Current et al. 1986; Fayer and Ungar 1986). Later, cross-transmission studies with mammalian isolates of Cryptosporidium indicated low host specificity, that first prompted (Tzipori et al, 1994) to consider Cryptosporidium as a single species genus and Levine (1984) suggested that only four species may be valid. The valid number of species was increased to six species with C. parvum causes respiratory and intestinal infections but, C. muris causes stomach infections. Respiratory and intestinal cryptosporidiosis in birds was attributed to C. baileyi, C. meleagridis, C. serpents infects reptiles & C. nasorum infected fish (Fayer et al, 1997; Koudela and Modry, 1998; Lindsay et al, 2000).
In man, cryptosporidiosis, infections are acquired by ingestion or inhalation (fecaloral route, foodborne, waterborne…etc.) of the parasite’s transmissive stage known as the oocyst. The time between acquisition of infection and manifestation of symptoms (pre-patent period) is dependent on various factors (i.e. host susceptibility, virulence of the infecting strain of oocysts, oocyst infectivity…etc.) but may be somewhat from 5-28 days mean 7.2 (Anderson et al, 1982; Current et al. 1983; Højlyng et al. 1987).

*Cryptosporidium* infections are associated with acute and clinical disease characterized by diarrhoea in man and various domestic and wild animals including birds. *Cryptosporidium* infections are most pathogenic in neonatal hosts, however in humans infections were reported from a 3 day old infant, from a mother with cryptosporidiosis, to a 95 year old individual. Disease often manifests itself with profuse, watery diarrhea, abdominal cramping, nausea, vomiting and low grade fever. In well nourished, immunocompetent patients, cryptosporidiosis may last 2-12 days but, usually self-limiting. Occasionally, infections may continue for two weeks or more and may require fluid replacement therapy. Nowadays, no effective chemotherapeutic treatment is available and duration of disease is dependent upon the patient’s immune status. In patients with congenital or acquired immune deficiencies or malnourished ones, infection can be considerably prolonged, resulting in malabsorption, severe dehydration and death (Anderson et al, 1982; Current et al, 1983; Højlyng et al, 1987, O’Donoghue, 1995; Fayer et al, 2000; Xiao, 2010).

The work aimed to study the effect of some natural products on the control and treatment of the infection in comparison with a chemotherapeutic drug already used. Study the effect of the infection and the treatment and protection on the histology of the small intestine and if there is any effect on the small intestine by different treatment’s strategies.

Treatment: The search for bioactive plants which can be used as non-conventional antiparasitic treatment has received considerable attention in recent times because of the increasing worldwide development of resistance to chemical drugs in parasitic populations. However, scientific evidence to validate the use of plants remains limited (Hoste et al. 2008) Thus, this study oriented to evaluate the protective and curative capacity of garlic, ginger, mirazid and metronidazole against cryptosporidial infection.

Mirazid: Mirazid is an oleo-resin extract derived from Myrrh which is obtained from the stem of *Commiphora molmol*, a thorny tree that grows in Somalia and Arabian Peninsula (Massoud et al, 2001). The antiseptic and antineoplastic properties of myrrh are thought to be attributed to terpenoids (Nomicus, 2007). Myrrh was approved by US food and Drug Administration (Ford et al., 1992). Mirazid has been reported in several clinical and experimental trials to be a safe and effective natural herbal drug. Evidence anti-trematode activity was demonstrated in schistosomiasis (Massoud, 1999; Badria et al, 2001), in fascioliasis (Massoud et al, 2001; Abo-Madyan et al, 2004), in experimental and human heterophyidiasis (Fathy et al, 2005; Massoud et al, 2007) and as anti-cestode in monisziiasis expansa (El-Shazly et al, 2004) and Bertiella studeri (Al-Mathal et al, 2010) and as anti-nematode in strongyloidiasis stercoralis (Massoud et al, 2006). Anti-protozoal activity of Mirazid has been proved in human against *Cryptosporidium parvum* (Massoud et al., 2008), in hepatic coccidiosis produced by *Eimeria stidae* in rabbits (Baghaddi and Al-Mathal, 2010) and also against *Trichomonas vaginalis* in women with infection resistant to metronidazole. Therapeutic efficacy of mirazid on experimental *Giardia lamblia* infection in Albino rats as indicated by a100% reduction in parasite-load of both intestinal and fecal parasitic count by using tinidazole as control (Fathy, 2011).
Ginger: Zingiber officinale Roscoe (ginger, Zingiberaceae) is a widely used spices and it is a common additive in large number of compounded foods and beverages due to its flavor and pungency. Its rhizome is one of the most commonly used medicinal herbs as well as one of the most commonly used condiments in Chinese cuisine. Several pharmacological effects of Zingiber plant was reported such as antiulcer effect (Yoshikawa et al., 1994), antioxidant effect, potent antibacterial activity (Mahady et al., 2003), potent antifungal activity (Ficker et al., 2003) and anthelmintic activity (Iqbal et al., 2001). *Z. officinale* extracts have been extensively studied for a broad range of biological activities including antibacterial, anti convulsant, analgesic, antiulcer, gastric anti-secretory, antitumor, antifungal, antispasmodic, antiallergenic, and other activities such as the ability to increase digestive fluids, plus absorb and neutralize toxins and stomach acid. *Z. officinale* increased bile secretion, as well as increase the action and tone of the bowels (Bradley, 1992). The anti- giardial activity of *Z. officinale* was reported using experimental infected Balb/c mice with *Giardia lamblia*. The extract of *Z. officinale* was more active specially when mixed with honey the watery extract of *Z. officinale* reduced the *G. lamblia* trophozoites (Al-masoudi, 2011).

Garlic: Allium sativum (*A. sativum*) or garlic is used as food and medicine in many cultures for thousands of years, dating at least as far back as the time that the Giza pyramids were built. It has been recognized not only as a spice but also as a substance exerted a control on microorganisms (Soffar and Mokhtar, 1991; Ayaz et al., 2008; Masmaha et al., 2010). *A. sativum* is remarkable for a number of potentially active chemical constituents. It contains seventeen amino acids as arginine, at least 33 organosulphate compounds as allin and allicin, eight minerals (germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc), enzymes as allinase, and the vitamins A, B1 & C. The physiological activity of dietary *A. sativum* is attributed to allicin (diallyl thiosulphinate) is one of the organosulphate compounds found in the bulb. It is responsible for the anti-microbial properties and the characteristic flavor of fresh garlic (Ayaz et al., 2008; Masmaha et al., 2010; Thompson and Ali, 2003). Ancient Egyptians realized the benefits of garlic; its medical and magical powers were described on the walls of ancient temples and on papyri dating to 1500 BC. Garlic proved to have antimicrobial, antithrombotic, hypolipidemic, hypoglycemic and antitumor activities (Thompson and Ali, 2003). Lately, garlic has widely been used to treat intestinal parasites. Antihelminthic effect of garlic is a matter of interest of researchers. Their results showed that treatment with garlic evoked a significant reduction in the worm load (Soffar and Mokhtar, 1991; Abdel-Rahman et al., 1998; Sutton and Haik, 1999; Ayaz et al., 2008; Riad et al., 2009). Also, garlic was used successfully in a single uncontrolled study in China applied on 20 AIDS patients to treat *Cryptosporidium* (Faried et al., 1996) Besides, garlic compounds were purified and tried as complementary medicine in the leishmaniasis management (Wabwoba et al., 2010). Many microorganisms were significantly susceptible to garlic extract and gave good results broad-spectrum therapeutic agent (Adetumbi and Lau, 1983).

Metronidazole: Also known as: Flagyl, Metronidazol, Gineflavir, Meronidal, Metronidaz, Trichazol, Trichopol, Danizol, Trivazol. Metronidazole 250mg and 500mg tablets are for oral administration. The discovery of metronidazole and its long acting derivative, secnidazole first synthesized in the early 1960s by Jacobs et al. completely changed the treatment of some protozoan infections such as urogenital trichomoniasis, amebiasis and giardiasis. Second generation derivatives, generally long acting compounds, quickly appeared with tinidazole (Miller et al., 1970) and ornidazole synthesized (Hoffner, 1969). They were highly ef-
fective in vitro and in vivo against these protozoa and quickly underwent clinical trials worldwide. Metronidazole received regulatory approvals in a large number of countries in the developed and in the developing world and became the treatment of choice for trichomoniasis and amebiasis, both tissue-
lar such as in amebic liver abscess and intestinal. Darbon et al. (1962) showed that metronidazole could be used in giardiasis. Metronidazole is completely absorbed after oral administration and penetrates body tissues and fluids such as saliva, breast milk, semen, and vaginal secretions. The drug is metabolized mainly in the liver and is excreted in the urine (Lau et al., 1992).

Materials and Methods

Animals used were male Swiss Albino mice, aged three to five weeks, weighing 25-30 grams. They were housed in well venti-
lated cages with perforated covers, supplied with standard pellet food and water. Bed-
ing was changed every day. The mice were allowed to adapt to the laboratory environ-
ment for one week before the experiment (El-Fakhry et al., 1998) and their stools were examined by direct wet saline smear, iodine and Sheather’s sugar flotation method to exclude the presence of parasites (Garcia and Brucker, 1997).

Cryptosporidium parvum oocysts were purchased from Waterborne™, Inc. (New Orleans, Louisiana) and stored in shipping medium (Phosphate-buffered saline with pen-
icillin, streptomycin, gentamycin, amphotericin B & 0.01% Tween 20) at 4°C until use.

Experimental design: The experimental animals (male Swiss Albino mice) were di-
vided into the following groups (5 mice /group) as follow: Control group (uninfect-
ed- untreated) Mice of this group did not receive any treatments. Infected group (infect ed-untreated) Mice were inoculated orally with the isolated Cryptosporidium oocysts at a dose of 10⁶ oocysts/mouse (Gaafar, 2007). Ingestion was performed by gastric gavage, using a 23-gauge needle tipped with plastic tubing (Riad et al., 2009). Experim-
tental Prophylactic group, which was subdivided as following: a- Subgroup I- Prophylactic 1 (P1): received garlic two days before infection then these mice continued to receive garlic daily for 12 days post-infection. b- Subgroup II - Prophylactic 2 (P2): received ginger two days before infection then these mice continued to receive ginger daily for 12 days post-infection. c- Subgroup III - Prophylactic 3 (P3): received mirazid two days before infection then these mice con-
tinued to receive mirazid daily for 12 days post-infection. d- Subgroup IV - Prophylactic 4(P4): received metronidazole two days before infection then these mice continued to receive metronidazole daily for 12 days post-
infection. Experimental Therapeutic group, was subdivided as following: a- Subgroup I-
Treatment 1 (T1): received garlic one day after the infection then these mice continued to receive garlic daily for 2 weeks. b- Sub-
group II- Treatment 2 (T2): received ginger one day after the infection then these mice continued to receive ginger daily for 2 weeks. c- Subgroup III - Treatment 3 (T3): received mirazid one day after the infection then these mice continued to receive mirazid daily for 2 weeks. d- Subgroup IV- Treatment 4 (T4): received metronidazole one day after infection then these mice continued to receive metronidazole daily for 2 weeks. Each mouse of the infected, experimental Prophylactic, and experimental therapeutic groups was inoculated orally with the isolat-
ed Cryptosporidium oocysts at a dose of 10⁶ oocysts/ mouse (Gaafar, 2007) Ingestion was performed by gastric gavage, using a 23-gauge needle tipped with plastic tubing (Riad et al, 2009).

Garlic dose was 50mg/kg body-weight/day an hr before breakfast. Preparation: Fresh garlic bulbs were separated, peeled, and washed with distilled water. After drying; about 500g of garlic bulbs were crushed in a blender until a uniform consistency was achieved. The resulting paste was diluted with distilled Water to obtain a 1 g/ml aque-
ous solution. Raw garlic juice was aliquoted
and was stored at -20 C until use (Burke et al, 2009). Working solution was made from stock one by dilution with distilled water. The selected dose for the present work was 50 mg/kg body weight (Masamha et al, 2010). Ginger dose was 50mg/kg body weight/ day, an hr before breakfast. Mirazid dose was 10 mg/kg body weight/day, two hr before breakfast. Metronidazole dose was 50mg/kg body weight /day an hr before breakfast.

Stool analysis: Undiluted stool samples from all mice were stained by MZN (Modified Ziel-Nelseen) and examined by light microscope.

Histological investigations: Using over dose of ether, the sacrifice of animals was achieved 12 days post infection for the subgroups (P1), (P2), (P3), and (P4), while sacrifice of mice of the subgroups, (T1), (T2), (T3) & (T4) was achieved 15 days post infection. For light microscopy, autopsy samples were taken from the intestine of mice in different groups and fixed in 10% neutral formalin for 24 h. Then washing was done in tap water then serial dilutions of alcohol (absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 hr. Paraffin bees wax tissue blocks were prepared for sectioning at a thickness of 4μm by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for routine examination then examination was done through the light electric microscope (Banchroft et al, 1996).

### Results

<table>
<thead>
<tr>
<th>Data collections</th>
<th>Control negative</th>
<th>Control infected</th>
<th>Prophylactic</th>
<th>Experimental</th>
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<tbody>
<tr>
<td>1 week before infection</td>
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<td>2 days before infection</td>
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<td>2 days post infection</td>
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<td>6 days (P1)</td>
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<tr>
<td>9 days (P1)</td>
<td>- ve</td>
<td>+ ve</td>
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<tr>
<td>11 days (P1)</td>
<td>- ve</td>
<td>+ ve</td>
<td>- ve</td>
<td>- ve</td>
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<td>15 days (P1)</td>
<td>- ve</td>
<td>+ ve</td>
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- ve = no *Cryptosporidium* oocysts or any other parasites. + ve = presence of *Cryptosporidium* oocysts without other parasite.

Control G. (uninfected- untreated): Examination of ileum section of control mice displayed normal architecture of the villi. Infected G. (Infected, untreated): Examination of ileum section of the infected mice displayed histopathological alterations from that of the uninfected groups. Experimental Prophylactic G was subdivided into 4 subgroups. Examination of ileum section prepared from mice treated with subgroup I, garlic (P1), subgroup II, ginger (P2), subgroup III, mirazid (P3), and subgroup IV, metronidazole (P4) displayed histopathological alterations from that of control groups. Experimental Therapeutic G was subdivided into 4 subgroups. Examination of ileum section prepared from mice treated with garlic, subgroup I (T1), ginger, subgroup II (T2), mirazid, subgroup III (T3), and metronidazole, subgroup IV (T4) displayed histopathological alterations from that of the control groups

### Discussion

In the present study, all mice used were parasitic-free, their infectivity was successfully achieved and prophylactic and experimental treated groups were all negative by 9th day post infection (PI) indicated that all drugs used were effective in clearing the parasite from the body without signs of infection in stool in comparison with control infected group which still showed oocysts in the stool until the end of the experiment at 15th day P. I.
In the present study, explosive, chronic, fatal, non-bloody diarrhea is considered a very serious management problem in immunosuppressed patients as well as in normal persons in both developed and developing countries. Pathogenic intestinal protozoa represent the main causes of this diarrhea, among which *Cryptosporidium* produces regularly occurred outbreaks throughout the world (Ma, 1989; Farthing, 2006). Most of the immunodeficient patients got failure of the available drugs used for the treatment, besides the multiple adverse effects that they produced (Miao et al, 2000). Thus, new effective drug became consequently urgently needed. The search for bioactive plants which can be used as nonconventional anti-parasitic treatment has received considerable attention in recent times because of the increasing worldwide development of resistance to chemical drugs in parasitic populations. However, scientific evidence to validate the use of plants remains limited (Hoste et al, 2008).

In the present study, examination of ileum section of control mice (uninfected, untreated) displayed normal architecture of the villi and examination of ileum section of infected mice (infected, untreated) were displayed histopathological alterations.

In the present study, experimental treatment group was subdivided into 4 subgroups. Examination of ileum section prepared from mice treated with garlic, subgroup I (T1), ginger, subgroup II (T2), mirazid, subgroup III (T3), and metronidazole, subgroup IV (T4) displayed histopathological alterations from that of control groups.

The allicin was obtained from crushed fresh garlic bulbs (Ankri and Mirelman 1999; Sasaki et al, 1999; Lemar et al, 2002). The dose selected for the present work was 50 mg/kg body weight. Riad et al. (2009) suggested that this dose is equivalent to the daily amount of garlic recommended for an average human to maintain good health (4g). Garlic successfully eradicated *Cryptosporidium* oocysts from stool and intestinal sections of infected mice. Abouel-Nour et al. (2015) proved this immunologically.

Ginger is one of the most commonly used fresh herbs and spices. Ginger is among the 20 top-selling herbal supplements in the United States (Blumenthal, 1998), pharmacopoeias from different countries list that ginger extract for various digestive diseases (Blumenthal et al, 2000). In this study, the first time that, in addition to an antiemetic effect, ginger extracts exhibit antidiarrheal activities. Administration of ginger extract modulated the harmful effect in liver hydroxyproline and serum AFP induced under the effect of *S. mansoni* infection, indicating their strong anti-fibrotic effect in reducing granuloma formation associated with liver fibrosis. The present results are in harmony with previous data revealing ginger anthelmintic activity against *S. mansoni* (Adewunmi et al, 1990; Sanderson et al, 2002) and gastrointestinal nematodes (Iqbal et al, 2006) by killing parasites through its binding to parasite beta-tubulin and inhibiting glucose uptake. This effect of ginger may be related to the significant anthelmintic activity of its constituents, shogaol (Ali et al, 2008) and gingerol (Lin et al, 2010). These active components of ginger completely abolished the infectivity of *S. mansoni* miracidia and cercariae of *Biomphalaria glabrata* and mice respectively indicating its’ molluscicidal and schistosomicidal activities.

Supplementation of ginger to the infected mice markedly corrected elevation in the inflammatory risk factors in the present study, implying its potential antioxidant, anti-inflammatory and immunomodulatory capabilities. The beneficial effects of ginger were previously reported due to its active constituents such as zingerone, paradol, gingerols and shogaols (Adewusi et al, 1996). Also, Abouel-Nour et al. (2015) proved this immunologically.

Metronidazole, a heterocyclic compound with a nitro group on the fifth position of an imidazole ring, derived from the *Streptomy-
ces antibiotic azomycin. Developed in 1959, metronidazole was approved for the treatment of trichomoniasis in the early 1960s and was the first drug to have a cure rate approaching 100% with systemic treatment (Cosar and Julou, 1959). Metronidazole is a small molecule, does not bind to serum proteins, and is well distributed through bodily tissue and fluids. Therapeutic levels of the drug have been found in blood, cerebrospinal fluid, pulmonary exudates, bile, and seminal fluid, as well as bone, brain, and pelvic tissue. Metronidazole is a small molecule that enters T. vaginalis via passive diffusion. The drug itself is inactive, but anaerobic reduction results in the formation of a cytotoxic nitro radical anion. This nitro radical is hypothesized to bind transiently to DNA, disrupting or breaking the strands and leading to cell death (Lloyd and Kristensen, 1985; Edwards, 1993).

Mirazid (Oleo-resin extract from Myrrh of Commiphora molmol tree, family: Burseraceae) is a product by Pharco Pharmaceutical Company, Alexandria. Administered doses were two equal 600mg oral doses of purified Commiphora extract for 3 consecutive days (Haridy et al., 2003) on empty stomach, at least two hours before eating, oral doses of 10μg/mL (Nogata et al., 2001; Manthey and Guthrie, 2002).

Haridy et al. (2003), Hegab and Hassan (2003) and Massoud et al. (2008) reported that Mirazid proved as an effective fasciolicidal drug, without clinical side effect. Also, Hassan et al. (2003) stated that Mirazid contracted S. mansoni worms muscle and affected its surface by ultra-structure causing tegument disruption and tubercles collapse El-Baz et al. (2003) proved that Mirazid was very effective and safe in treatment of Schistosoma haematobium.

In the present study, infected mice that were treated with ginger, mirazid, garlic and metronidazole showed significant symptomatic improvements during the treatment period. So, the treatments may have a direct and powerful impact on the parasite that consequently minimizes the pathology associated with C. parvum infection.

C. parvum-infected mice treated with ginger, mirazid, garlic and metronidazole showed a complete elimination of shedding oocyst by 9th day PI. Reduction and elimination of shedding oocyst in response to the treatments might be attributable to a direct effect on parasite growth in the intestines, the production of the sexual phases, and/or the formation of oocysts. The present histopathological changes in the infected/untreated group ileum tissues were reported (Tzipori et al., 1994; Capet et al., 1999; Leitch and He, 1999; Motta et al., 2002; Guitard et al., 2006; Maruyama et al., 2007; Robinson and Smyth, 2008). Pathological changes were attributed to C. parvum displacing brush borders causing an asymmetrical loss of epithelial cells resulting in shortening and fusing of villi. Cryptosporidiosis of villi atrophy might be due to secreted toxins that directly damage epithelial cells (Heine et al., 1984; Tzipori, 2002). C. parvum infection also resulted in T-cell migration to lamina propria. Abouel-Nour et al. (2015) used C. parvum immunological response to evaluate the efficacy of anti-cryptosporidiosis agents of Garlic, Ginger, Mirazid and Metronidazole in experimentally infected mice. Experimentally the immunologic mediated elimination of C. parvum required CD4+ T cells and IFN-gamma. But, the innate immune responses also have a significant protective role in both man and animals. The mucosal immune response to C. parvum in C57BL/6 neonatal and GKO mice showed a concomitant Th1 & Th2 cytokine mRNA expression, with a crucial role for IFN-gamma in infection resolution. NK cells & IFN-gamma proved important components in immunity in T & B cell-deficient mice, but the IFN-gamma-dependent resistance was detected in aliphymocytic mice. Epithelial cells played a vital role in immunity as once infected cells increased expression of inflammatory chemokines and cytokines and demonstrated anti-infection killing mechanisms.
In Egypt, many authors dealt with zoonotic cryptosporidiosis, the selected ones are of interest from epidemiological point of view. Shalaby and Shalaby (2015) determined cryptosporidiosis among 120 randomly chosen school children aged 4-16 years. Medical sheets were filled out on each child. They found that watery and loose diarrhea was more significant among infected children. Positive stool samples were among 37 (30.8%), while ELISA and IFA detected 30 (25%) and 33 (27.5%) respectively. The validity test of ELISA declared sensitivity and specificity with 93.3% and 90% while IFA declared 90.9% and 91.1% respectively. El-Shazly et al. (2015) in Egypt examined 50 children with the chronic liver diseases (CLD) of different etiology and 50 non-CLD children with gastrointestinal complaints served as controls. They found the commonest intestinal protozoa in CLD patients were: Entamoeba histolytica/E. dispar (16%), Giardia lamblia (14%), Blastocystis hominis (14%), C. parvum (10%), E. histolytica and G. lamblia (2%), E. histolytica and B. hominis (2%), G. lamblia and B. hominis (2%), B. hominis and E. coli (2%) and Microsporidium (2%). As compared to controls, incidence of these organisms in CLD patients was significantly higher (p<0.045) as regards stool examination by unstained techniques and no significant difference between both groups as regards stool examination by stained techniques (p<0.478). They concluded that CLD affect the immunity of the patients showed significant increase in the incidence of intestinal parasites in cases compared to controls. El-Badry et al. (2015) studied the molecular prevalence and seasonality of Cryptosporidium over a period of one year in a cohort of Egyptian diarrheic patients. Stool samples were collected from 865 diarrheic patients attending outpatient clinics of Cairo University Hospitals, from all age groups over one year, examined microscopically for oocysts by the acid-fast staining method and for copro-DNA detection using nPCR. Cryptosporidium copro-DNA was detected in 19.5% of all over year, with a major peak in summer (August) and a small rise in spring (April). Infection was mainly C. hominis (95.8%) followed by C. parvum (3.0%), affecting all age groups, with predominance in the pre-school age group, and decrease with age. They concluded that infection of diarrheic Egyptians was of distinct endemicity, with the bimodal mostly influenced by population dynamics, with a clear high prevalence in preschool children and predominating anthropoctic (C. hominis) transmission throughout the year and that Cryptosporidium was a water contaminant and an important cause of health problems in Egypt, necessitating further studies of the risk factors. El-Shabrawi et al. (2015) stated that diarrhea continues to cause significant morbidity in Egypt. They detected Rotavirus in 11% of patients. Enterotoxigenic Escherichia coli (ETEC), Campylobacter, Shigella, and Salmonella in 7%, 3.7%, 1.1% &1.4% of patients, respectively; and in 11.1%, 3.1%, 0.6% & 0.6% of controls, respectively. Cryptosporidium was detected in 3.9% of cases. Mixed infection was detected in 5.9% of cases and 0.9% of controls, with a significant difference (p<0.001). Ibrahim et al. (2016) investigated the epidemiology and public health significance of Cryptosporidium species and genotypes were in Beni-Suef Governorate. By microscopic examination, the overall estimated prevalence in cattle, buffaloes, and humans was 10.2, 12.3, and 19%, respectively. The highest rates were in the calves less than 2 months of age (17.1%) and diarrheic animals (13.0%). In man, the highest prevalence was in infants (31.3%) and diarrheic individuals (21.1%), infection was commonest in males (21.7%) than females (14.5%). Based on molecular characterization, Cryptosporidium oocyst wall protein (COWP) and gp60 genes were successfully amplified in 36/50 samples subjected to genotyping. The restriction fragment length polymorphism (RFLP) analysis of COWP fragments revealed that C. parvum was de-
tected only in cattle (12 isolates) and buffaloes (4 isolates), while in man, species were C. hominis (15 isolates) and C. parvum (5 isolates). Sequence analysis of the gp60 gene identified the subtype IIdA20G1 within C. parvum isolated from both animals and humans. The common occurrence of zoonotic subtypes of C. parvum in cattle and buffaloes highlights the potential role of these animals as significant reservoirs of infection to humans.

**Conclusion**

No doubt, cryptosporidiosis is a worldwide problem particularly among children and immunocompromised individuals.

The histopathological patterns were corrected with the drugs used and the best result in descending order was as follow ginger, mirazid and garlic respectively. These three plant extracts proved to have a direct and powerful impact on C. parvum and minimized its pathology complications as compared to Metronidazole.

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Explanation of figures

Fig. 1: Cryptosporidial oocysts in stool of infected mice stained with MZN acid fast stain (x400)
Fig. 2: A cross section (C.S.) of ileum of control uninfected mice showing normal structure of villi (V), regular and continuous Brush border (BB), Absorptive columnar cells (AC) and lamina propria (LP), (H&E, X100).
Fig. 3: Ileum C.S. of control infected mice showing disturbed villous architecture, hyperplasia of villi (V) with local ulceration (UL), (H&E, X100).
Fig. 4: Villi of control uninfected mice showing normal structure of villi (V), regular and continuous brush border (BB), presence of goblet cells between enterocytes (GC), normal connective tissue core of villi (LP), (H&E, X400).
Fig. 5: Villi of control infected mice showing degeneration of columnar epithelium (AC) with many pyknotic nuclei (PK) and appearance of oocyst (OO) at denuded brush border (BB), (H&E, X400).
Fig. 6: Ileum of control uninfected mice showing normal structure of crypts of Lieberkühn (CL), (H&E, X200).
Fig. 7: Ileum C.S. of control infected mice showing mild edema with very mild inflammatory infiltrate in crypts of Lieberkühn (CL), (H&E, X200).
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Fig. 17: Villi of metronidazole protected mice showing degeneration of columnar cells (AC), ulcerated brush border (BB), (H&E, X400).
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Fig. 30: Ileum C.S. of Mirazid treated mice showing normal crypts of Lieberkühn (CL), (H&E, X200).
Fig. 31: Ileum C.S. of Metronidazole treated mice showing normal crypts of Lieberkühn (CL), (H&E, X200).