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THE EFFICACY OF THREE MEDICINAL PLANTS; GARLIC, GINGER AND MIRAZID AND A CHEMICAL DRUG METRONIDAZOLE AGAINST *CRYPTOSPORIDIUM 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 (Ginger, Mirazid, and Garlic) as compared to chemical drug Metronidazole. Importantly, *C. parvum*-infected mice treated with ginger, 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 ginger, 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. serpentis* 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 (feco-oral 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 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) Thus, this study oriented to evaluate the protective and curative capacity of garlic, ginger, mirazid and metronidazole against cryptosporodial 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. Evident 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 monisziasis *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 antisecretory, 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 antigiardial 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; Masamha *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; Masamha *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* (Fareed *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 (Hoffer, 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 tissular 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 ventilated cages with perforated covers, supplied with standard pellet food and water. Bedding was changed every day. The mice were allowed to adapt to the laboratory environment 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 penicillin, streptomycin, gentamycin, amphotericin B & 0.01% Tween 20) at 4°C until use.

Experimental design: The experimental animals (male Swiss Albino mice) were divided into the following groups (5 mice /group) as follow: Control group (uninfected- untreated) Mice of this group did not receive any treatments. Infected group (infected-untreated) Mice were inoculated orally with the isolated *Cryptosporidium* oocysts at a dose of 10^4oocysts/mouse (Gaafar, 2007). Ingestion was performed by gastric gavage, using a 23-gauge needle tipped with plastic tubing (Riad *et al,* 2009). Experimental 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 continued to receive mirazid daily for 12 days post-infection. d- Subgroup IV - Prophylactic 4(P4): received metronidazole two days before infection then 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- Subgroup 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 isolated *Cryptosporidium* oocysts at a dose of 10^4 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 aqueous solution. Raw garlic juice was aliquoted

and was stored at -20^{0}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 1: Stool analysis of different groups.

Data collections	Control negative	Control infected	Prophylactic				Experimental			
			P1	P2	P3	P4	Ex T1	Ex T2	Ex T3	Ex T4
1 week before infection	- ve	- ve	- ve	-ve	- ve	- ve	- ve	- ve	- ve	- ve
2 days before infection	- ve	- ve	- ve	-ve	- ve	- ve	- ve	- ve	- ve	- ve
2 days post infection	- ve	+ ve	+ve	+ve	+ ve	+ve	+ ve	+ ve	+ ve	+ ve
6 days (PI)	- ve	+ ve	+ve	+ve	+ ve	+ve	+ ve	+ ve	+ ve	+ ve
9 days (PI)	- ve	+ ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
11 days (PI)	- ve	+ ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
15 days (PI)	- ve	+ ve	xxx	xxx	xxx	xxx	- ve	- ve	- ve	- ve

– 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 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).

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 shed- ding oocyst by 9^{th} 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-cryptosporidisis 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 Thl & 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 alymphocytic 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 cryptoaporidiosis, 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 diarrhoeic patients. Stool samples were collected from 865 diarrhoeic 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 allover 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 in diarrheic Egyptians was of distinct endemicity, with the bimodel mostly influenced by population dynamics, with a clear high prevalence in preschool children and predominating anthroponotic (*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 immnunocompromised 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 proparia (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 denucleated 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).

Fig. 8: An ileum of garlic protected mice showing disturbed villous architecture (V), ulceration at villi top (UL), with loss of columnar epithelium (AC), loss of brush border (BB), (H&E, X100).

Fig. 9: Ileum C.S. of ginger protected mice showing near normal villi (V) apart from mild atrophy (AT), (H&E, X100).

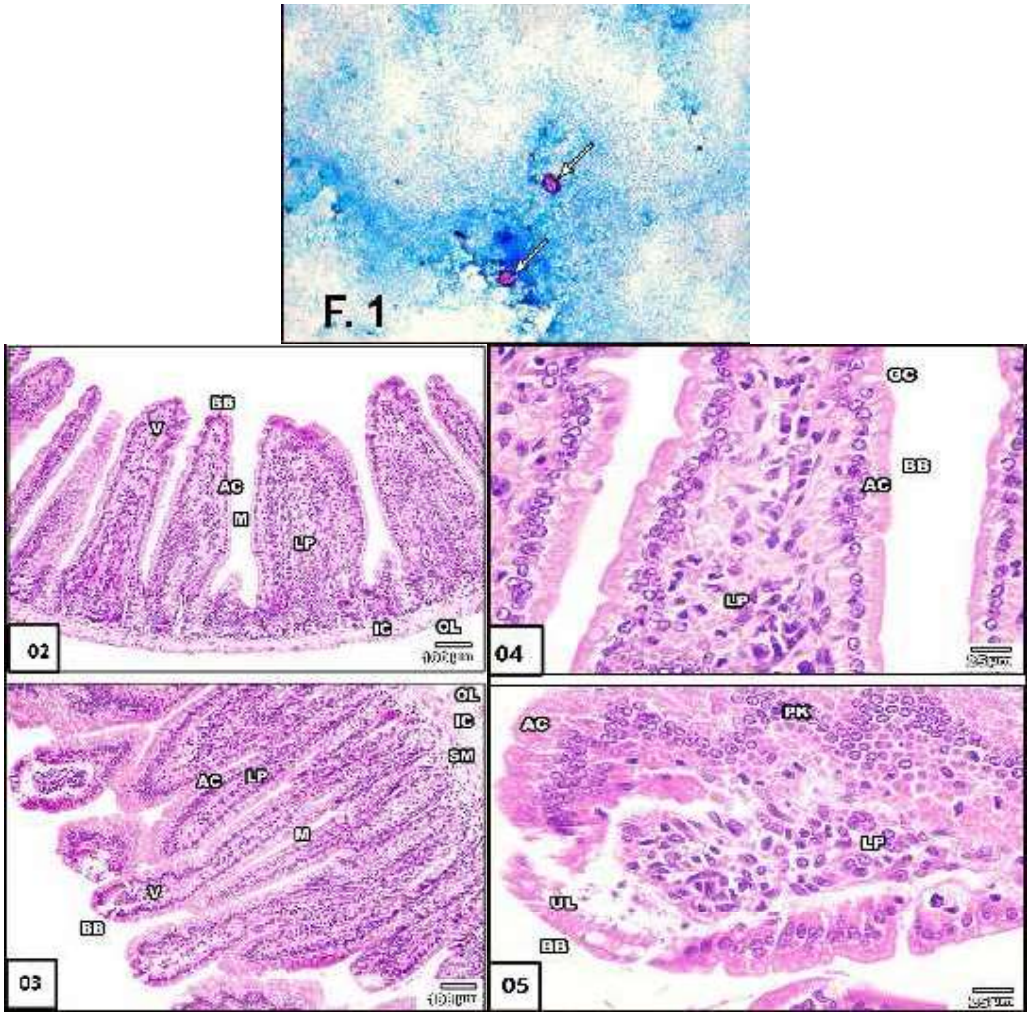
Fig. 10: Villi of garlic protected mice showing degeneration of columnar cells (AC), no brush border (BB) with areas of ulceration (UL), atrophy at villi top (AT), (H&E, X400).

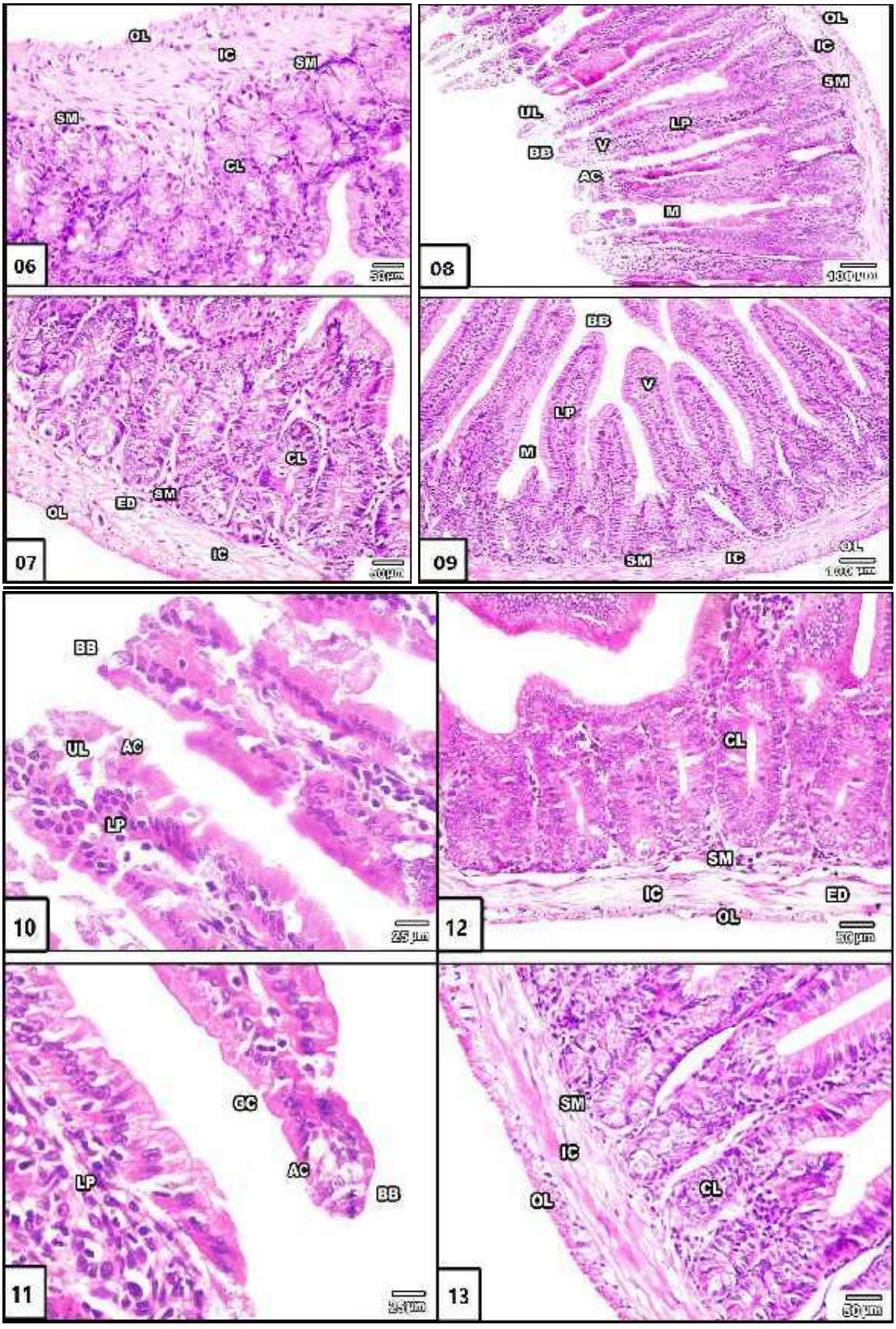
Fig. 11: Villi of ginger protected mice showing very mild degeneration of columnar epithelium (AC) with preserved brush border (BB), (H&E, X400).

Fig. 12: Ileum of garlic protected mice showing mild edema (ED) and inflammation of crypts of Lieberkühn (CL), (H&E, X200).

Fig. 13: Ileum of ginger protected mice showing normal crypts of Lieberkühn (CL) without evidence of inflammation, (H&E, X200).

Fig. 14: Ileum C.S. of Mirazid protected mice showing near villi normal structure (V) with mild atrophy (AT), mild degeneration of columnar epithelium (AC),with regular brush border (BB), (H&E, X100).
Fig. 15: Ileum C.S. of Metronidazole protected mice showing near normal villi structure (V) with very mild ulceration at top (UL), normal connective tissue core (LP), normal columnar epithelium (AC), (H&E, X100).
Fig. 16: Villi of Mirazid protected mice showing mild degeneration of columnar cells (AC), loss of brush border (BB), (H&E, X400).
Fig. 17: Villi of metronidazole protected mice showing degeneration of columnar cells (AC), ulcerated brush border (BB), (H&E, X400).
Fig. 18: Ileum of Mirazid protected mice showing no significant changes apart from mild inflammation in crypts of Lieberkühn (CL), (H&E, X200).
Fig. 19: Ileum of Metronidazole protected mice without significant changes in crypts of Lieberkühn (CL), (H&E, X200).
Fig. 20: Ileum C.S. of garlic treated mice showing disturbed villous architecture (V), ulceration at villi top (UL), with loss of columnar epithelium (AC), loss of brush border (BB) and atrophy (AT), (H&E, X100).
Fig. 21: Ileum C.S. of ginger treated mice showing near normal villi structure (V), regular and continuous Brush border (BB), normal Absorptive columnar cells (AC) and lamina proparia (LP), (H&E, X100).
Fig. 22: Villi of garlic treated mice showing degeneration of columnar cells (AC), loss of brush border with areas of ulceration (UL), presence of subepithelial edema space (*), (H&E, X400).
Fig. 23: Villi of ginger treated mice showing near normal villi (V), normal absorptive columnar cells (AC), regular and continuous brush border (BB), normal connective tissue core (LP), (H&E, o X400).
Fig. 24: Ileum C.S. of garlic treated mice showing mild edema and inflammation in the crypts of Lieberkühn (CL), (H&E, X200).
Fig. 25: Ileum C.S. of ginger treated mice showing normal crypts of Lieberkühn (CL), with no evidence of inflammation, (H&E, X200).
Fig. 26: Ileum C.S. of Mirazid treated mice showing near normal villi (V), normal connective tissue core (LP), regular and continuous brush border (BB), (H&E, X100).
Fig. 27: Ileum C.S. of Metronidazole treated mice showing near normal villi (V), normal connective tissue core (LP), regular and continuous brush border (BB), (H&E, X100).
Fig. 28: Villi of Mirazid treated mice showing normal Absorptive columnar cells (AC), regular and continuous brush border (BB), normal connective tissue core, (H&E, X400).
Fig. 29: Villi of Metronidazole treated mice showing normal Absorptive columnar cells (AC), regular and continuous brush border (BB), normal connective tissue core, (H&E, X400).
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).

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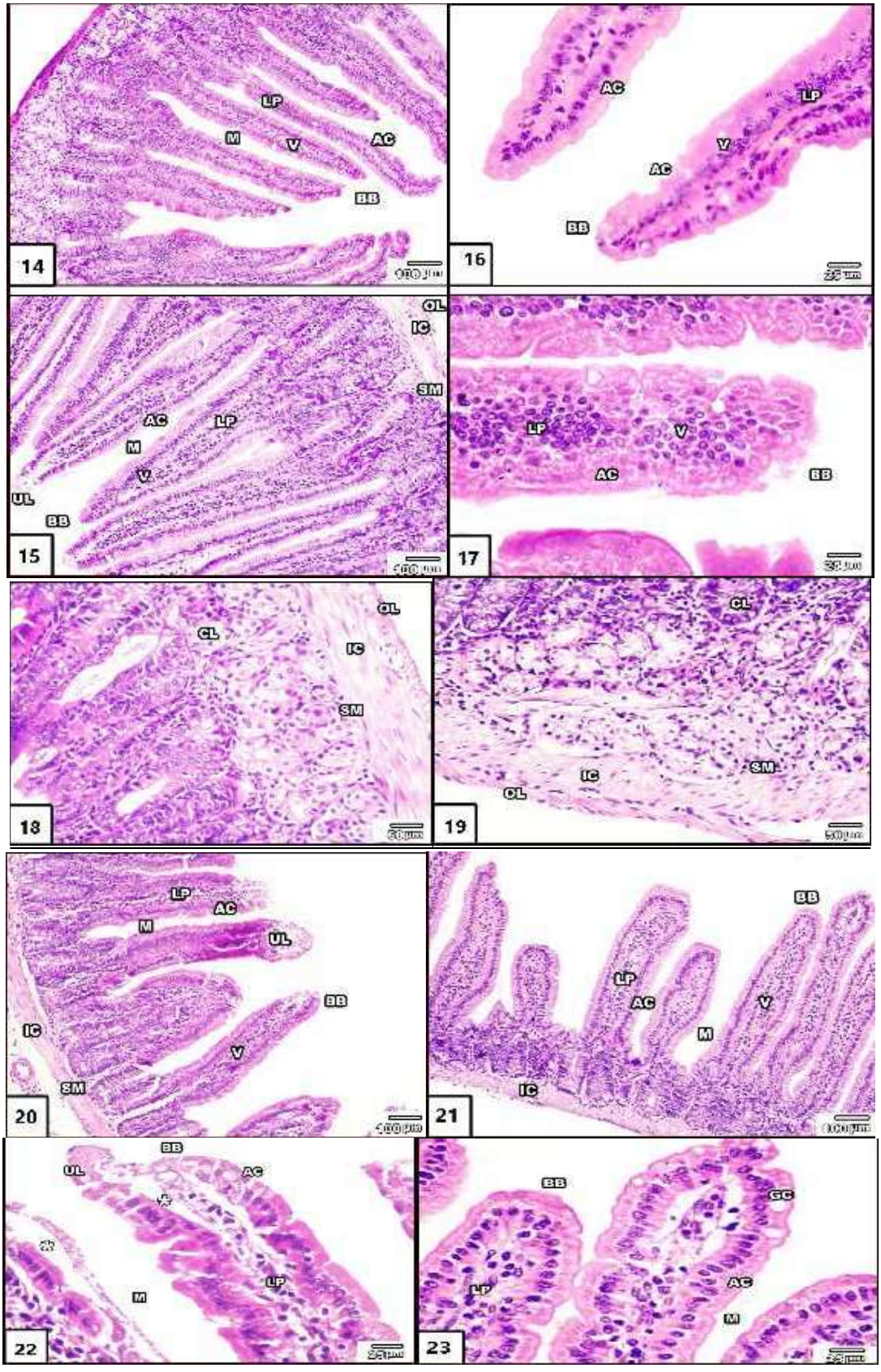
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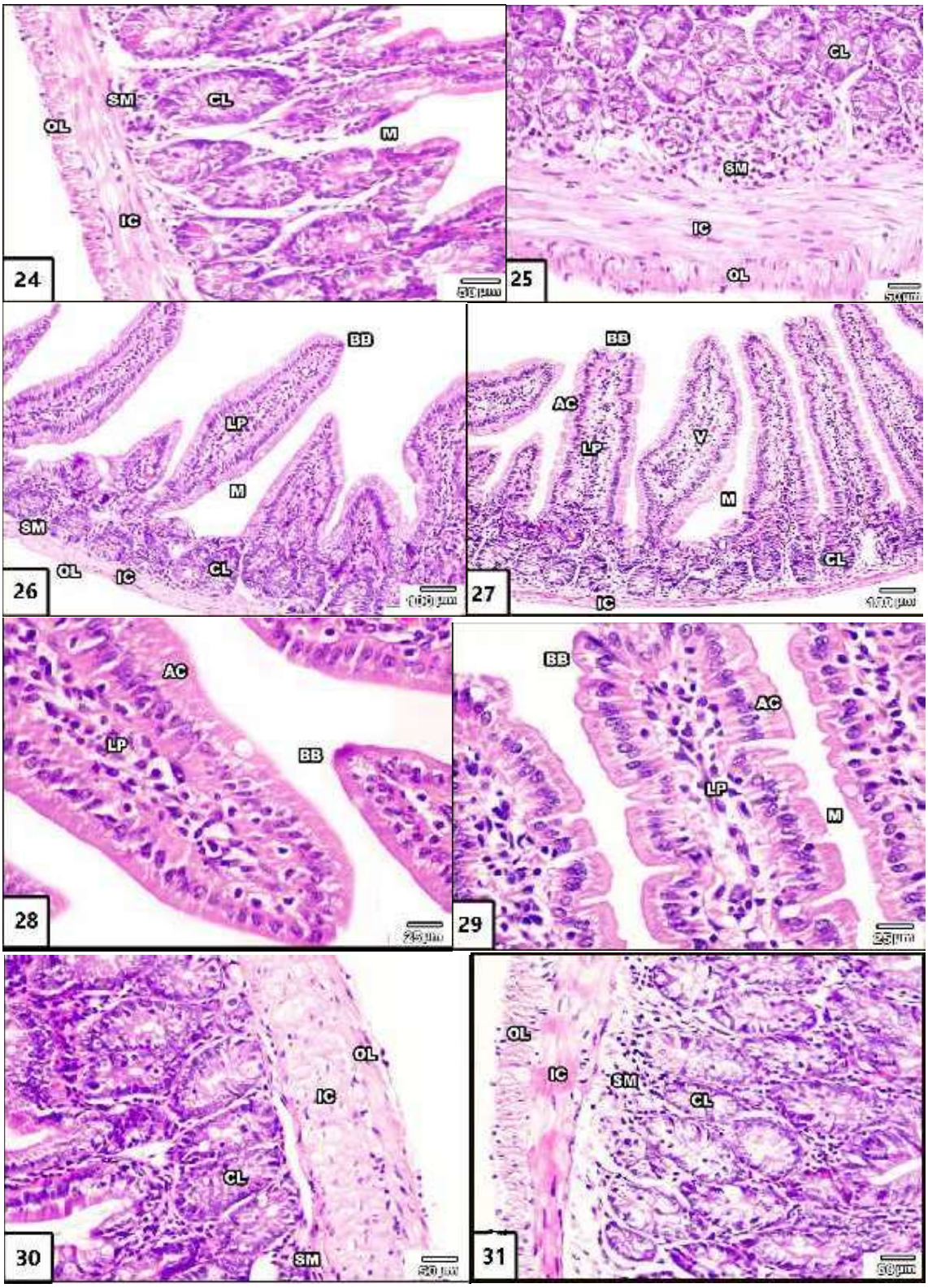
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