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THE EFFICACY OF THREE MEDICINAL PLANTS: GARLIC, GINGER AND MIRAZID AND A CHEMICAL DRUG METRONIDAZOLE AGAINST *CRYPTOSPORIDIUM PARVUM*. I- IMMUNOLOGICAL RESPONSE

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Abstract

Cryptosporidisis *parvum* is a zoonotic protozoan parasite infects intestinal epithelial cells causing a major health problem for man and animals. Experimentally the immunologic mediated elimination of *C. parvum* requires CD4+ T cells and IFN-γ. 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 shows a concomitant Th1 and Th2 cytokine mRNA expression, with a crucial role for IFN-γ in the resolution of the infection.NK cells and IFN-γ have been shown to be important components in immunity in T and B cell-deficient mice, but IFN-γ-dependent resistance is demonstrated in alymphocytic mice. Epithelial cells may play a vital role in immunity as once infected these cells have increased expression of inflammatory chemokines and cytokines and demonstrate anti-infection killing mechanisms.

C. parvum immunological response was used to evaluate the efficacy of anti-cryptosporidisis agents of Garlic, Ginger, Mirazid and Metronidazole in experimentally infected mice.

Key words: Anti-cryptosporidisis, 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. Some coccidiae can undergo extra-intestinal development as tissue-cyst forms (*Sarcocystis*, *Toxoplasma*), others develop in the gastrointestinal or respiretory tract, without formation of tissue cysts (*Eimeria*, *Isospora* and *Cryptosporidium*). Like others, it was thought that *Cryptosporidium* would be highly host specific and almost 20 species were named after the species of the infected host isolated from (Current *et al,* 1986). Cross-transmission studies with mammalian isolates of *Cryptosporidium* indicated low host specificity, which first prompted (Tzipori *et al,* 1994) to consider *Cryptosporidium* as a single species of the genus and then led (Levine, 1984) to suggest that only four species may be valid. Later the valid number of species was increased to six species with *C. parvum* causing respiratory and intestinal infections whereas *C. muris* causing stomach infections. Cryptosporidiosis in birds was caused by *C. baileyi, C. meleagridis* & *C. serpentis* in reptiles and *C. nasorum* in fish (Fayer *et al,* 1997; Koudela and Modry, 1998; Lindsay *et al,* 2000).

In humans cryptosporidiosis is acquired by ingestion or inhalation (i.e. fecal oral route, foodborne, waterborne…etc.) of the infective stage; oocyst. The incubation period (pre-patent) depends on various factors (host susceptibility, strain virulence, route of infection…etc.), but may be from 5-28 days (Current *et al,* 1983; Højlyng *et al,* 1987).

Cryptosporidium infections are associated with acute and clinical disease characterized by diarrhea in humans and many domestic and wild animals including birds. Infections are most pathogenic in neonatal animals, and in humans from 3 day old infant up to 95 year old (Anderson *et al,* 1982). Disease causes profuse, watery diarrhea, abdominal cramping, nausea, vomiting and low grade fever, particularly in immunocompetent patients infection may last 2-12 days but usually self-limiting. Infections may continue for

two weeks or more and require fluid replacement therapy. In congenital or acquired immune deficiencies or malnourished patients, infection can be prolonged, causing malabsorption, severe dehydration and even fatal (Current *et al,* 1983; Højlyng *et al,* 1987; O'Donoghue, 1995; Fayer *et al,* 2000; Xiao, 2010).

This work evaluated the immune response in *Cryptosporidium parvum* by studying the release and levels of two specific cytokines, IFN-γ & IL-5 representing Th1 and Th2 respectively, and the effect of some natural products on the control and treatment of the infection as compared available chemotherapeutic drug. Study the release of cytokines during the treatment and protection schedule to compare the release pattern among different groups in comparison with the controls.

Immune Response: since there is no effective therapeutic agent with anti-cryptosporidial activity, a better understanding of the immune response to this parasite may facilitate the development of effective therapeutic agent (Ungar *et al,* 1991). No doubt, the initial innate responses in limiting parasite number, but the clearance of infection ultimately require a cell mediated immune response (McDonald and Bancroft, 1994). The nature of an acquired immune response to any infection is mostly determined by the balance between T helper 1 (Th1) and T helper 2 (Th2) phenotypes. Th1 lymphocytes are involved in cellular immune responses, mainly through the production of interferon (IFN)-γ, tumor necrosis factor (TNF-α) and interleukin (IL-2), particularly effective against intracellular infections. Th2 cells are involved in development of humoral immune responses by producing IL-4, IL-5, IL-10 & IL-13. Th2 cells are mainly involved in immune responses to parasites and allergic responses (Melinceanu *et al,* 2009).

The most likely source of IFN-γ is natural killer (NK) and Th1 cells, stimulated by IL-12 and TNF-α, and negatively regulated by IL-10 (Kapel *et al,* 1996). IFN-γ is a proinflamatory cytokine, involved in the synthesis of immunoglobulin (Ig) G2a (B cells) and inhibition of Th2 cell growth. IFN-γ proved to be the key cytokine in both the innate and the adaptive immunity during *C. parvum* infection (Tessema *et al,* 2009). IL-5 is involved in IgA synthesis as well as eosinophils production (Petry *et al,* 2010).

In *C. parvum* infection both Th1 &Th2 cytokines act in a well regulated mechanism for an effective control (Huang *et al,* 1996; Ehigiator *et al.,* 2005; Tessema *et al,* 2009). McDonald (2000) reported a strong early Th1 response and later more balanced response with a Th2 component to facilitate cure. Susceptibility or resistance to infection correlated to produce characteristic cytokines panels and Ig (Singh *et al,* 2005).

Cytokines: Cytokines are proteins play a key role in modulation of innate and adaptive immune responses (Theodos, 1998). Studies in mice showed that IFN-γ is a major player not only in cell-mediated immunity, but also in early innate immune responses (Riggs, 2002). Depending on the nature of the antigens that the immune system encounters, CD4+ T helper (Th) cells may induce a cell-mediated immune response (Th1) or antibody-mediated response (Th2). These diverse Th responses are determined by the spectrum of cytokines produced by the T-cells themselves and by antigen-presenting cells. In a Th1 response, IL-12 produced by dendritic cells and macrophages drives the T-cells to produce IFN-α. This type of response is usually required to control and eliminate intra-cellular infections (Lean *et al,* 2002). A Th2 response is associated with production of IL-4, IL-5, IL-9, and IL-13.

Th1 Cytokines: IFN-γ: Most studies have indicated that the most effective adaptive immune response to *Cryptosporidium* infection involves IFN-γ activity (Urban *et al,* 1996). Infection with *C. parvum* has been shown to induce IFN-γ mRNA and protein expression in the intestine measured by RT-PCR and ELISA, respectively (Kapel *et al,* 1996). In neonatal mice the kinetics of IFN-γ expression reflected the pattern of acute

infection, with the levels of IFN-γ increasing as infection approaches its peak level and declining rapidly as recovery gets under way (Kapel *et al,* 1996; McDonald *et al,* 2004). No doubt, IFN-γ and other pro-inflammatory cytokines activate antimicrobial killing mechanisms including production of toxic nitric oxide derivatives or oxygen radicals, or creating a deficiency of metabolites required for growth of microorganisms as tryptophan or cellular iron (Rottenberg *et al,* 2002). High levels of nitric oxide production can be stimulated by IFN-γ, often acting in concert with other cytokines such as TNF-α (Nacy *et al,* 1991).

Th2 Cytokines: IL-5: IL-5 contributes to a humoral response. This cytokine is produced by Th2 lymphocytes, and takes part in growth induction and differentiation of B- and T-cells. IL-5 stimulates the proliferation and differentiation of eosinophil precursors, stimulates their degranulation and production of reactive oxygen compounds. It exerts a chemotactic effect on eosinophils and induces eosinophilia (Weltman, 2000)

Treatment: Bioactive plants used as non-conventional anti-parasitic treatment received considerable attention due to increasing resistance development to chemical drugs (Hoste *et al,* 2008). So, protective and cure action of garlic, ginger, mirazid and metronidazol were evaluated against cryptosporodiosis.

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 any parasites, also smears were stained with mo-dified Ziel-Nelseen (MZN) to exclude *Cryptosporidium* species as well as post-treatment to evaluate the cure rate(Garcia and Brucker 1997).

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

Experimental design: Experimental animals were divided into groups of 5 mice each: G1: Control negative group (neither infected nor treated). G2: Infected group (infected-untreated) inoculated orally with *Cryptosporidium* oocysts at a dose of 10^4 oocysts/mouse (Gaafar, 2007), by gastric gavage, using a 23-gauge needle tipped with plastic tubing (Riad *et al,* 2009). G3: Prophylactic group, was subdivided as follows: G3a: Prophylactic 1 (P1): received garlic two days before infection and then continued to receive garlic daily for 12 days post-infection (P.I.). G3b: Prophylactic 2 (P2): received ginger two days before infection and then continued to receive ginger daily for 12 days P.I. G3c: Prophylactic 3 (P3): received Mirazid two days before infection and then continued to receive mirazid daily for 12 days P.I. G3d: Prophylactic 4(P4): received Metronidazole two days before infection and then continued to receive Metronidazole daily for 12 days P.I.. G4: Treated group, subdivided into: G4a*:* Treatment 1 (T1): received garlic one day P.I. and then continued to receive garlic daily for 2 weeks. G4b: Treatment 2 (T2): received ginger one day P.I. and then continued to receive ginger daily for 2 weeks. G4c: Treatment 3 (T3): received Mirazid one day P.I. and then continued to receive Mirazid daily for 2 weeks. G4d: Treatment 4 (T4): received Metronidazole one day P.I. and then continued to receive Metronidazole daily for 2 weeks.

Experimental infection: Each mouse was orally infected with oocysts10^4 oocysts/ mouse (Gaafar, 2007), by using a 23-gauge needle tipped with plastic tube (Riad *et al,* 2009).

Treatment: Garlic as 50mg/kg body weight /day an hr. before breakfast. Fresh garlic bulbs were separated, peeled, washed with distilled water, and dried, 500g were crushed in a blender to a uniform consistency, and diluted with distilled water to obtain a 1g/ml solution. Aliquot of raw garlic juice was stored at -20^{o}C (Burke *et al*, 2009). Work-solution was prepared from the stock diluted with distilled water (Masamha *et al*, 2010).

Ginger dose was 50mg/kg body weight/ day, Mirazid dose was 10mg/kg/body weight/day and Metronidazole dose was 50 mg/ kg body weight/day, All were given an hr. before breakfast.

Determination of IFN-γ concentration in all experimented groups: IFN-γ was determined by Quantikine-MIF00 mouse IFN-γ ELISA immunoassay Kit (R&D, Minneapolis, MN, USA) according to the manufacturer's instructions. IL-5 concentration in all the experimented groups was determined by mouse IL-5 ELISA Kit from RayBio® Company.

Statistical analysis: Data were computerized and statistically analyzed between control, infected and each group using t- test. Results were expressed as mean ±S.E, where a=significant as compared with control group ($P < 0.05$). b = significant as compared with infected group ($P < 0.05$), while means superscripts with same letters= no significant difference ($P > 0.05$).

Results

The results are shown in tables (1, 2, 3, 4, 5, 6 & 7) and figures (1, 2, 3, 4, 5 & 6).

Table 1: Stool analysis for different groups.

Date collected	Groups									
	G1	G2	Prophylactic				Experimental			
			P1	P2	P3	P4	T1	T2	T3	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 (PI)	- 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, + ve = positive *Cryptosporidium* oocysts but no other parasites.

Determination of IFN-γ level in mice sera: Administration of *Cryptosporidium* oocysts caused a significant increase ($P < 0.05$) in IFN-γ level in serum of infected untreated group of mice as compared to normal value in controls uninfected untreated ones. In contrast, in ginger (P2, T2), Metronidazole (P4, T4), Mirazid (P3, T3) and garlic (P1, T1) protected and treated groups show-ed significant decrease ($P<0.05$) in IFN-γ level in serum of mice compared to infected group. In garlic protected and treated groups (P1, T1) with a significant increase in IFN-γ level compared to uninfected untreated one.

Table 2: Mean levels of IFN-γ in protected groups compared with infected and uninfected groups.

	CN	CI	P1	P2	P3	P4
Mean	147.49	825	312.49	153.88	179.16	155.33
SE±	6.33 ^{b}	15 ^{a}	14.16^{a,b}	13.88^{b}	1.1^{b}	1.47^{b}

Table 3: Mean levels of IFN-γ in treated groups compared with infected and uninfected groups.

	CN	CI	T1	T2	T3	T4
Mean	147.49	825	461.71	175.94	194.5	181.38
SE±	6.33 ^{b}	15 ^{a}	15.32^{a,b}	2.94^{b}	10.96^{b}	0.27^{b}

Table 4: Mean levels of IFN-γ in all groups.

	CN	CI	P1	P2	P3	P4	T1	T2	T3	T4
Mean	147.49	825	312.49	153.88	179.16	155.33	461.71	175.94	194.5	181.38
SE±	6.33 ^{b}	15 ^{a}	14.16^{a,b}	13.88^{b}	1.1^{b}	1.47^{b}	15.32^{a,b}	2.94^{b}	10.96^{b}	0.27^{b}

Determination of IL-5 Level in mice sera: *Cryptosporidium* oocysts caused a significant decrease ($P<0.05$) in IL-5 serum level of infected untreated group of mice compared with normal ones. In ginger (P2), metronidazole (P4), mirazid (P3) and garlic (P1) protected groups showed significant increase as compared to infected group. Garlic, metronidazole, and mirazid protected group as compared to uninfected group showed a significant decrease. Metronidazole (T4) and mirazid (T3) treated groups, showed significant increase in IL-5 level as compared to infected group, but a significant decrease in IL-5 level as compared to uninfected group. Garlic (T1) treated group showed significant decrease in IL-5 level toward uninfected one. Ginger (T2) treated group showed significant increase toward infected group.

Table 5: Mean levels of IL-5 in protected groups compared with infected and uninfected groups

	CN	CI	P1	P2	P3	P4
Mean	201.33	87.33	136.61	174.33	142.61	159.83
SE±	11.48^{b}	1.1^{a}	$1.05^{a,b}$	6.71^{b}	$0.82^{a,b}$	$2.02^{a,b}$

Table 6: Mean levels of IL-5 in treated groups compared with infected and uninfected groups**.**

	CN	CI	T1	T2	T3	T4
Mean	201.33	87.33	111	169.67	128.83	131.41
SE±	11.48^{b}	1.1^{a}	0.89^{a}	7.25^{b}	$2.24^{a,b}$	$1.45^{a,b}$

Table 7: Mean levels of IL-5 in all groups.

	CN	CI	P1	P2	P3	P4	T1	T2	T3	T4
Mean	201.33	87.33	136.61	174.33	142.61	159.83	111	169.67	128.83	131.41
SE±	11.48^{b}	1.1^{a}	$1.05^{a,b}$	6.71^{b}	$0.82^{a,b}$	$2.02^{a,b}$	0.89^{a}	7.25^{b}	$2.24^{a,b}$	$1.45^{a,b}$

Discussion

Generally speaking, *Cryptosporidium parvum* is mainly an intestinal parasite that infection caused changes in the immune system in order to overcome the infection, and that may be reflected in the production of the immune mediators. Although the cytokines and immunoglobulins are produced in small quantities, the variations in their levels might allow the establishment of a characteristic profile related to *C. parvum* 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 is approved by (FAD) US Food and Drug Administration (Ford *et al,* 1992). Mirazid was reported in several clinical and experimental trials to be a safe and effective natural herbal drug. Evident anti-trematode activity has been demonstrated in schistosomiasis (Massoud, 1999; Badria *et al,* 2001; Massoud *et al,* 2004), in fascioliasis (Massoud *et al,* 2001; Abo-Madyan *et al,* 2004), in experimental and human heterophyidiasis (Fathy *et al,* 2005; Massoud *et al,* 2007), dicrocoeliasis (Massoud *et al,* 2003) 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). Mirazid anti-protozoa activity was proved in zoonotic *C. parvum* (Massoud *et al,* 2008), in hepatic coccidiosis due to *Eimeria stidae* in rabbits (Baghaddi and Al-Mathal, 2010) and also against *Tricomonas vaginalis* in infection resistant to metronidasol. Experimentally *Giardia lamblia* infection in Albino rats was complete curried (Fathy, 2011).

Ginger: *Zingiber officinale* Roscoe (ginger, Zingiberaceae) is one of the most widely used spices and it is a common additive in large number of compounded foods and beverages due to its flavor and pungency. The rhizome of this plant 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 had been 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). Also, *Z. officinale* extracts have been extensively studied for a broad range of biological activities including antibacterial, anticonvulsant, analgesic, antiulcer, gastric antisecretory, antitumor, antifungal, antispasmodic, antiallergenic, and other activities such as ability to increase digestive fluids, plus absorb and neutralize toxins and stomach acid. *Z. officinale* has been shown to increase bile secretion, as well as increase the action and tone of the bowels (Bradley, 1992). The antigiardial activity of *Z. officinale* was demonstrated using experimental infections of *Giardia lamblia* in balb/c mice. The extract of *Z. officinale* was more active especially when mixed with honey the watery extract of *Z. officinale* reduced number of *G. lamblia* trophozoite (Al-Masoudi, 2011).

Garlic: *Allium sativum* (*A. sativum*) or garlic has been used as both 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 which exerts a control on microorganisms (Soffar and Mokhtar 1991; 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, B_1 & C. Physiological activity of dietary *A. sativum* is attributed to allicin (diallyl thiosulphinate), which is one of the organosulphate compounds found in the bulb. It has antimicrobial properties with characteristic fresh garlic flavor (Ayaz *et al,* 2008). Ancient Egyptians realized the benefits of garlic; its medical and magical powers were described on walls of ancient temples and on Egyptian Papyri dated 1500 BC. Garlic has multiple beneficial effects as antimicrobial, antithrombotic, hypolipidemic, hypoglycemic & antitumor activities (Thompson and Ali, 2003). Also, it is widely used to treat intestinal parasites with a significant reduction in worm-load (Soffar and Mokhtar, 1991; Abdel-Rahman *et al,* 1998; Sutton and Haik 1999; Riad *et al,* 2009). Also, it was successfully used to treat cryptosporidiosis in 20 AIDS Chinese patients (Fareed *et al*, 1996) Besides, garlic compounds were purified and used in the management of leishmaniasis (Wabwoba *et al*, 2010) Thus, because many of the microorganisms susceptible to garlic extract are medically significant, garlic holds a promising position as a broad-spectrum therapeutic agent (Adetumbi and Lau, 1983).

Metronidazole: Also known as: Flagyl, Metronidazol, Gineflavir, Meronidal, Metronidaz, Trichazol, Trichopol, Danizol or Trivazol. The discovery of metronidazole and its long acting derivative, secnidazole first synthesized in the early 1960s 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 prepared (Miller *et al,* 1970) and ornidazole synthesized (Hoffer, 1969). These compounds were found highly effective *in vitro* and *in vivo* against these three protozoa and quickly underwent clinical trials around the world. Metronidazole received regulatory approvals in a large number of countries in the developed and 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 as saliva, breast milk, semen, and vaginal secretions. Drug is metabolized in liver and excreted in urine (Lau *et al,* 1992).

In the present study, IFN-γ level in serum of mice gave a significant increase ($P<0.05$) in infected untreated group as compared to normal value in control uninfected untreated group. In ginger (P2, T2), metronidazole (P4, T4), mirazid (P3, T3) and garlic (P1, T1) protected and treated groups gave a significant decrease ($P<0.05$) in IFN-γ level of mice as compared to infected group. Garlic protected and treated groups (P1, T1) showed a significant increase in IFN-γ level as compared to uninfected untreated group.

In the present study, increase in IFN-γ cytokine secretion was found in infected mice as compared to non-infected control ones, showed trials to overcome their infection.

In the present study, immune response appeared to be mainly a Th1 response, as increased expression of the immune mediator IFN- γ during the infection was observed, which agreed with McDonald (2000). These cytokines might allow Th1 cells to be mainly effective in protection against intracellular infections by *C. parvum* (Petry *et al,* 2010). Although IFN-γ proved to play an important role in both the innate and adaptive immune responses to *C. parvum*, yet resistance mechanisms mediated by this cytokine alone were not well understood (Aliberti *et al,* 1996). The crucial role of IFN-γ in host resistance to infection with the American trypanosome *Trypanosoma cruzi* was reported (Cardillo *et al,* 1996). Post infection, the level of IFN-γ was significantly increased, as a primary response against *C. parvum*. The delicate balance between Th1 (to control parasitic growth) and Th2 cytokines (limit pathogenesis) agreed with others (Lean *et al,* 2002; Tessema *et al,* 2009).

In the present study, the administration of *Cryptosporidium* oocysts caused a significant decrease ($P<0.05$) in IL-5 serum level of infected untreated group of mice compared to normal value. Ginger (P2), metronidazole (P4), mirazid (P3) & garlic (P1) protected groups showed significant increase as compared to infected group. Garlic, metronidazole & mirazid protected group with uninfected group showed a significant decrease.

In the present study, metronidazole (T4) & mirazid (T3) treated groups showed a significant increase in IL-5 level as compared to infected group, and a significant decrease in IL-5 level as compared to uninfected group. Garlic (T1) treated group showed a significant decrease in IL-5 level toward uninfected one. Ginger (T2) caused significant increase toward infected group. Changes in immunological indices levels of parasitic invasions, as increase in IL-5 characteristic of parasitosis was reported (Faccioli *et al,* 1997). The early stage of *Onchocerca volvulus* infection (Cooper *et al,* 2001) increased production of IL-5 and IFN-γ. Brattig *et al.* (2002) reported an increase in IL-5 & IL-13 in response to administration of extract of soluble antigen of *Onchocerca volvulus*. In form of *Schistosoma mansoni*, both the level of IL-5 and IFN-γ showed a marked increase (de Jesus *et al,* 2002). Patients infected with *Giardia lamblia* showed a significant increase in IL-5 concentration (Matowicka-Karna *et al,* 2009). Ajdary *et al.* (2009) in chronically infected untreated patients with *Leishmania* found a significant increase in IL-5, IFN-γ & IL-13 levels produced by peripheral blood mononuclear cells. They suggested occurrence of mixed type Th1/ Th2 response. Turner *et al.* (2011) stated that the eggs of *Schistosoma mansoni* accumulate in the colon following infection and generate Th2-biassed inflammatory granulomas that became down-modulated in size as infection led to chronicity. They added that CD4+ CD25+FoxP3+ regulatory T cells (T(regs)) are known to suppress Th1-mediated colitis, but, it was not clear whether they control Th2-associated pathologies of the large intestine which characterize several helminthes infections. They used a novel 3D-multi-photon confocal microscopy approach to visualize and quantify changes in the size and composition of colonic granulomas at

the acute and chronic phases of *S. mansoni* infection and reported decreased granuloma size, as well as reductions in the abundance of DsRed+T cells and collagen deposition at 14 weeks (chronic) compared to 8 weeks (acute) post-infection. They added that proportion of CD4+CD25+FoxP3+T (regs) in the mLN that were CD103+ and CCR5+ also increased indicating an enhanc-ed potential to home to intestinal sites. The CD4+CD25+cells suppressed antigen-specific Th2 mLN cell proliferation in vitro, while their removal during chronic disease resulted in significantly larger granulomas, partial reversal of Th2 hyporesponsiveness and an increase in the eosinophils in colonic granulomas. They concluded that CD4+ CD25+FoxP3+T(regs) appeared to control Th2 colonic granulomas during chronic infection, and likely had a role in pathogenesis of intestinal schistosomiasis. Aihara *et al*. (2015) stated that chronic HCV infection induced monoclonal or oligoclonal proliferation of B cells that produced IgM rheumatoid factor, led to the development of mixed cryoglobulinemia (MC). The antigen-driven lymphoproliferation was essential to the onset of MC. They found that type II MC was induced by *Cap-illaria hepatica* infection by a mechanism in which splenic B-1a cells reacted to *C. hepatica*-specific antigen selectively proliferate, producing IgM rheumatoid factor under co-stimulation of specific worm antigen and IL-5. In vitro assays using B-1a cells from infected mice showed that stimulation by *C. hepatica* soluble fraction promoted the proliferation of B-1a cells and IgM secretion that reacted with the 75-kDa antigen in the soluble fraction and MC severity correlated with increase in serum IL-5 levels in the infected mice They concluded that selective proliferation of IgM rheumatoid factor-secreting B-1a cells was induced by co-stimulation by the specific pathogen antigen and IL-5 in the development of MC in *C. hepatica*-infected mice.

On the other hand, patients with acute disease showed Th1 response, which was indicated by increased concentration of IFN-γ and low levels of IL-5 and IL-13. IFN- γ produced by activated Th1 cells gave a suppressive action on the synthesis and release of IgE. By inducing the production of reactive oxygen and secretion of hydrogen peroxide, IFN-γ stimulates intracellular killing of parasites by macrophages (Lucey *et al,* 1996). Touil-Boukoffa *et al*. (1997) reported that the defense mechanisms in the course of echinococcosis involve, apart from IFN- γ, also IL-6. Ishikawa *et al*. (1998) found mice experimentally infected with *Trichinella spiralis* or *Nippostrongylus brasiliensis*, in response to which cytokines (among others IL-5 & IFN-γ) released from Th1 & Th2 cells were involved. Ajami and Rafiei (2007) in *Hymenolepis nana* infected patients reported an increase in levels of IL-5, IL-12, IL-13 and IFN-γ. Wu *et al.* (2015) stated that inflammatory cytokines produced at the early stages of the malaria infection contribute to shaping protective immunity and pathophysiology. They determined the cytokine responses by monocytes, macrophages, and dendritic cells (DCs) to purified *Plasmodium falciparum* and *P. berghei* ANKA, and by spleen macrophages and DCs from *P. yoelii* 17NXL-infected & *P. berghei* ANKA-infected mice. They found that monocytes and macrophages did not produce inflammatory cytokines to malaria parasites and that DCs were primary source early in infection, and DC subsets differentially produce cytokines and blocking of phagosomal acidification by inhibiting the vacuolar-type H(+)-ATPase enabled macrophages to elicit cytokine responses. They concluded that important implications for enhancing the efficacy of a whole parasite-based malaria vaccine and for designing strategies for the development of protective immunity to pathogens that induce immune responses primarily through endosomal receptors.

The present study, showed elevated levels of IL-5 & IFN-γ in *C. parvum* infected mice. Th1 lymphocytes produce IFN-γ, which inhibits proliferation and the action of Th2

cells, and in cellular type response. Th2 lymphocytes generate IL-4, IL-5, IL-6, IL-10 & IL-13, and promote humoral response. Anti-parasitic used to treat *C. parvum* infected mice gave a significant increase in IL-5 level but a decrease in IFN-γ levels.

Conclusion

The results proved that occurrence of immune and inflammatory responses in *C. parvum* invasion, when IFN- γ, TNF, IL-1b, IL-5 & IL-13 were released. Immune response elevation clarified infection post-treatment which elevated the pattern of cytokine release levels in comparison with the uninfected and infected controls. The best result in descending was ginger, metronidazole, mirazid and garlic respectively.

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Fig. 1: Mean levels of IFN-γ in protected groups.
Fig. 2: Mean levels of IFN-γ in treated groups.
Fig. 3: Mean levels of IFN-γ in all groups.
Fig. 4: Mean levels of IL-5 in protected groups
Fig.5: Mean levels of IL-5 in treated groups
Fig. 6: Mean levels of IL-5 in all groups.