Immunomodulating potential of *Ekebergia capensis* in murine *Schistosomamansoni* juvenile and adult infection

Original Research Article

ABSTRACT

Schistosomiasis is a neglected tropical disease occurring in sub-Saharan Africa and affecting almost 250 million people. The drug of choice for treatment for Schistosomiasis has been Praziquantel that has been used for many years and there is need to develop new drugs.The immunomodulatory potential of *Ekebergia capensis* extract on both juvenile and adult *Schistosoma mansoni* infection in vivo was evaluated in this study.Swiss albino mice were infected individually with 90 *S. mansoni*cercariae and randomized into groups of five each for i) plant extract treated groups ii) positive control groups treated with conventional drugs PZQ or artemether iii) infected but untreated (negative control) groups. The mice were treated orally with aqueous extracts of *E. capensis* at doses of 200 and 400 mg/kg at 2 weeks (juvenile worms) and 7 weeks (adult worms) post infection. Immune enhancing potential of the medicinal plant was determined by analyzing the levels of cytokines in serum samples that were collected before and after treatment. A BD-Cytometric Bead Array (CBA) mouse Th1/Th2/Th17 kit was used to quantitate the levels of cytokines using flow cytometer (FACS Calibur) and analysis of the data was done using FCAP software. Results indicated that the medicinal plant extract had immunomodulatory effect. There was a significant increase ($P<0.05$) in Th1 cytokines (IL-2, IFN- γ and TNF-α), a decrease in Th2 cytokines (IL-4, IL-6 and IL-10) and an increase in Th17 (IL-17). These findings confirm the potential use of medicinal plants in the management of schistosomiasis.

Key words: Schistosomiasis, Praziquantel, *Schistosoma mansoni*, Artemether, *Ekebergia capensis*Immunomodulatory, Cytokines

1. INTRODUCTION

Schistosomiasis (an acute and chronic parasitic disease) is one of the neglected tropical diseases caused by helminthic flatwormsof the genus *Schistosoma* that reside in the blood vasculature and produce eggs that result in pathology. It is endemic in 78 countries, with over 90% of cases occurring in sub-Saharan Africa and an estimated 251.4 million people require preventative treatment (WHO, 2023). The disease remains second to malaria in terms of socio-economic impact in tropical and subtropical areas (Almeer et al., 2018).

There are two forms of the disease; the intestinal schistosomiasis caused by *Schistosoma mansoni, S. japonicum, S. mekongi, S. guineensis and S. intercalatum* and urogenital schistosomiasis caused by *S. haematobium* (WHO, 2023). The dioecious adult schistosome worms reside in blood vessels of vertebrate hosts while the asexual phase multiplies in the snail

intermediate host (*Biompalaria*spps for *S. mansoni*, *Bulinus* spp. for *S. haematobium* and *Oncomelania* spp. for *S. japonicum*) (CDC, 2019).Schistosomiasis is contracted during normal activities like swimming, bathing, fishing, and farming where schistosome cercariae released by infected intermediate (snail) hosts penetrate the skin of the mammalian host transforming into a schistosomula. The schistosomula migrates to the lungs and penetrates the pulmonary capillaries to be carried to the systemic circulation and to the portal system. In the hepatic circulation, they mature into adults, pair up and migrate to the mesenteric veins where they mate and eggs are excreted in feces for intestinal schistosomiasis. The eggs hatch in water into miracidia which penetrate the intermediate snail hosts for the cycle to continue (CDC, 2010).Schistosomiasis is divided into three stages: (i) cercarial dermatitis (Swimmers itch) which occurs 24 h after penetration of the cercariae into the dermis and the affected person develops an itchy maculo-papular rash in the area that was in contact with the infested water (Tracz et al., 2019) and occurs within 1-3 weeks (Leshem et al., 2008), (ii) acute schistosomiasis that takes place 3-8 weeks after infection and is due to cercariae-induced hypersensitivity reaction and the (iii) chronic stage that occurs months or years after infection results from formation of granulomas in the tissues around the trapped eggs resulting in granulomatous inflammation and fibrosis (Salvana& King, 2008). There are varied immune responses that take place during infection with schistosomiasis.

The immune response during acute *S. mansoni* infection (caused by schistosomula and juvenile worms) at around 4-5 weeks is T helper type 1 (Th1) with the expression of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α)interferon-gamma (IFN-γ), and the interleukins (IL) IL-1, IL-2, and IL-12and is characterized by a dry cough, fever, angioedema and blood eosinophilia is experienced during this stage (Jauréguiberry et al., 2010). T helper 2 (Th2) is induced by soluble egg antigens (SEA) from 5-6 weeks with the onset of egg deposition causing a shift from Th1 to Th2 characterized by IL-4, IL-5, IL-10, IL-13 and immunoglobulin E (de Jesus et al., 2002; Fallon, 2000). The Th2 response peaks at 8-10 weeks and is responsible for the coordination of granulomatous inflammation (Fallon et al., 2000; Phythian-Adams et al., 2010) and also dampens the Th 1 component. The Th17 on the other hand is at low levels compared to Th1 and Th2 responses and emerges in CBA mice and not BALB/C or C57BL/6 (Kalantari et al., 2019) and has been reported to promote immune pathology rather than benefit the host (Rutitzky et al., 2009). As the disease progresses there is a immunoregulation of the Th1/Th2 balance by the T regulatory cells (Tregs) and B regulatory (Bregs) that causes a reduction of Th2 inflammation via an IL-10-mediated pathway (van der Vlugt et al., 2014).

The treatment and control of morbidity of schistosomiasis is dependent on a single drug, Praziquantel (PZQ) which has been the main drug of choice for the treatment of all species of schistosomes because of its efficacy, ease of administration, safety, and cost with mild to moderate side effects that include nausea, dizziness, headache, drowsiness and abdominal pain (Gray et al., 2011). There are however concerns about decreased efficacy and the emergence of resistance with continued use of PZQ (Kabuyaya et al., 2018). The drug is only effective against adult worms but not juvenile worms which go on to establish infection together with the associated morbidity. There is also limited pharmaceutical investment for exploring novel antischistosomal agentsbecause the disease is neglected (de Moraes &Geary, 2020; Ferreira et al., 2022).Development of new drugs that are active against adults, juvenile worms and possibly anti-schistosomula face the challenge of need for international product development partnership and collaboration due to the resources involved (Caldwell et al., 2022). In addition, schistosomiasis elimination requires a multifaceted approach that include health education, snail

control, improved water sanitation and hygiene, accurate diagnosis and surveillance response systems (McManus et al., 2018).The World Health Organization's (WHO) neglected tropical diseases (NTDs) roadmap however targets to eliminate schistosomiasis by 2030 (WHO, 2021).With this challenge there is need to come up with new alternative drugs that effective and potentiallywith immunomodution potential and plants are a good source.

Medicinal plant extracts are now used as medicine and food supplement and WHO listed 21,000 medicinal plants worldwide (Harun et al., 2020) out of the 400,000 plants in the world (Pal & Nayak, 2021). They have been in use for a long time because they are reliable, inexpensive and are available to people who do not have access to conventional drugs. In addition,they have negligible side effects and have multicomponent agents as opposed to synthetic drugs (Fu et al., 2018). They have been used as anti-inflammatory, antioxidant, antibacterial, antifungal, anthelmintic, anticancer, immunomodulators and for treatment of cardiovascular disease.

The plant *Ekebergia capensis*(EC) from the family Meliaceae (The Mahogany family) has been used traditionally to treat many ailments and itis widely distributed in the Central and Nyanza regions of Kenya (Gachathi, 2007),Uganda, Ethiopia, Zimbabwe, Swaziland and South Africa. The plant has been studied and reported widely for its medicinal properties. The in vivo antischistosomal potential of both ECand *Azadiracta indica* (neem) against juvenile and adult infections was evaluated in a study in which ECshowed more potency in reduction of both the worm burden and tissue egg load at both stages (Musili et al., 2015). This study therefore determined theimmunomodulatory potential of EC in Swiss albino mice infected with*S. mansoni* at juvenile and adult stages. The immunomodulating effect of these herbs was assessed by measuring levels of circulating cytokines before and after treatment with the herbal extracts.

2. MATERIAL AND METHODS

This experimental study was carried out in the Animal house facility, Schistosomiasis and Immunology laboratories at the Kenya Medical Research Institute.

2.1 Maintenance of *S. mansoni*parasite

S. mansoni cercariae were obtained from 3 Swiss albino mice which were already infected and on life cycle maintenance at the Institute. Livers from 3 mice were emulsified and filtered in nested sieves to obtain a filtrate containing ova which was then illuminated using a lamp to hatch as described by Wanlop et al (2022). The eggs were hatched into miracidia and were used to infect 30 *B.pfeifferi* snails of 4mm diameter using a routine optimized technique. The infected snails were maintained in freshly prepared aquariums and maintained at an ambient temperature of 25-28^{0}C as described by Thiam et al (2022). The snails were fed with lettuce and supplemented with bone meal for 28 days.

2.2 Infection of mice

Infected snails were collected from the aquaria and placed in 50ml beakers filled to 1/3 with dechlorinated water and exposed to artificial light to enhance cercariae shedding for 2 hours. To avoid unisexual infection, more than 25 snails were used. To enumerate cercariae produced, 5, 50µl subsamples of the cercariae suspension were obtained after gently swirling the beaker and were stained with Lugol's iodine on a Petri dish. 50 male Swiss albino mice aged 6-8 weeks old and weighing 20-22g were infected with 90 cercariae each using the abdominal ring method (Mathew et al.,2001). The mice were maintained with mice pellets and water ad libitum.

2.3 Study groups

The infected mice were randomized into groups of 5 mice each and placed in separate cages representing the juvenilestage at 2 weeks post infection (pi) and adult stage at 7 weeks pi using two different concentrations of the medicinal plant extract (200mg/kg and 400mg/kg). The control groups were included in the experiment and were the infected untreated group (negative control) and two positive control groups of juvenile infection (200mg/kg of artemether) and adult infection (200mg/kg of PZQ).

2.4 Extraction of plant and treatment

The bark of the *E. capensis*(EC) had been collected from the Mount Kenya Forest and taken to the East African Herbarium in Nairobi for cataloging and voucher specimens deposition (*E. capensis*: Stem bark (Ec-SB/04) 26). The water extraction of plant bark was done at the KEMRI's Centre for Traditional Medicine and Drug Research.Treatment was done by administering the mice with two doses (200mg/kg and 400mg/kg) of the herbal extracts orally by gastric gavage using stainless steel needles. Two positive control groups were treated with artemether at 200mg/kg administered orally by gastric gavage once a day for 3 consecutive days using an oral volume of 0.2 ml per mouse (Utzinger et al., 2002) for comparison with the juvenile group (Liu et al., 2014) and another one with PZQ at a dose of 200 mg/kg body weight per day using a dose volume of 0.05 ml for 5 consecutive days to a total dosage of 1000 mg/kg (Gönnert, 1977) for comparison with the adult group since PZQ targets adult worms.

2.5 Collection of blood

On the day of treatment, blood samples were collected from the tail ends of all the infected mice on days 14 and 49 post-infection (pi) from juvenile and adult worm groups respectively then treatment was started on the same day (a few hours after bleeding). Blood samples were collected again one day before perfusion that is on days 41 and 69 from juvenile and adult worm groups respectively. Briefly, the tip of the tail was nicked off (2-3mm) using a sterile sharp pair of scissors and blood was collected drop by drop into a sterileEppendorf tubes. This procedure was performed ascetically to avoid infection. To enhance blood flow, the mice were placed near a table lamp or their tails were dipped in warm water to dilate the tail veins. All blood samples were placed on ice to enhance blood clotting and spun in a microfuge at 1500rpm for 30 minutes to separate serum. The serum was pipetted out using a micropipette and sterile tips into 200µl storage tubes, labeled, and stored at -80^{0}C until analysis of cytokine profiles. An approximate volume of 300µl was collected from each mouse during each bleed and adequate drinking water was provided for the mice. For collection of the second blood sample (before perfusion), the wounds at the tips of the tails were reopened by cutting 2-3mm using a sterile pair of scissors and bled the same way as before.

2.6 Cytometric Bead Array (CBA) assay

Cytokine analysis was done using the BD Cytometric Bead Array (CBA) Mouse Th1/Th2/Th17 Cytokine Kit which allowed for the simultaneous detection of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17A. Aliquots of sera that had been frozen at -80^{0}C were thawed and CBA analysis performed as per the manufacturer's instructions.

The samples were acquired on the flow cytometer the same day they were prepared since prolonged storage of the samples once the assay is complete would result to increased

background and reduced sensitivity. To facilitate analysis of samples using FCAP Array software, the manufacturer's recommendations were followed in that the samples were acquired from the lowest (0 pg/ml) to the highest (Top Standard) concentration followed by the test samples. All the Flow Cytometer Standard (FCS) files (standards and samples) were stored in a single folder for analysis using FCAP v3 software.

2.7 Statistical Analysis

The Mouse Th1/Th2/Th17 Cytokine data was analyzed using FCAP v3 Array Software and the results were saved in Excel sheets for statistical analysis to be conducted. The mean level of cytokines in the groups was also subjected to Student's t-test using Microsoft Excel® to determine their statistical significance in comparison with the control groups. The data was considered significant if $P<0.05$, highly significant if $P<0.01$ and very highly significant if $P<0.001$.

3. RESULTS

Effect of treatment with EC at 7 weeks post infection on Th1/Th2/Th17 cytokines

We examined the expression of Th1/Th2/Th17 cytokines which are responsible for immune reaction during acute *S. mansoni* infection inorder to determine the immunomodulatory effect of EC extract at the two different stages of the disease.The CBA assay results showed that EC at a concentration of 400mg/kg and 200mg/kg body weight at adult (7weeks) infection resulted in a varied effect on the levels of the Th1/Th2/Th17 cytokine after treatment and when compared to the infected untreated group.

The results showed that there was a decrease in the level of TNF-α after treatment with EC extract at both 400mg/kg and 200mg/kg while in both the PZQ treated group and the infected untreated group there was an increase. IFN-γ and IL-2 increased in all the treatment groups after treatment as shown in Figure 1a.

On the other hand, there was a slight increase in the level of IL-6 and a decrease in IL-4cytokine after treatment with EC at both concentrations. The level of IL-10 reduced after treatment with EC at 400mg/kg but surprisingly increased in the 200mg/kg group. In the PZQ treatment group IL-6 increased while IL-4 and IL-10 levels dropped after treatment. In the infected untreated group, all the cytokines increased after 7 weeks as shown in Figure 1b.

The effect of treatment with EC at both 400mg/kg and 200mg/kg as well as PZQ resulted in an increase in the levels of IL-17 cytokines but a reduction was observed in the infected untreated group as shown in Figure 1c.

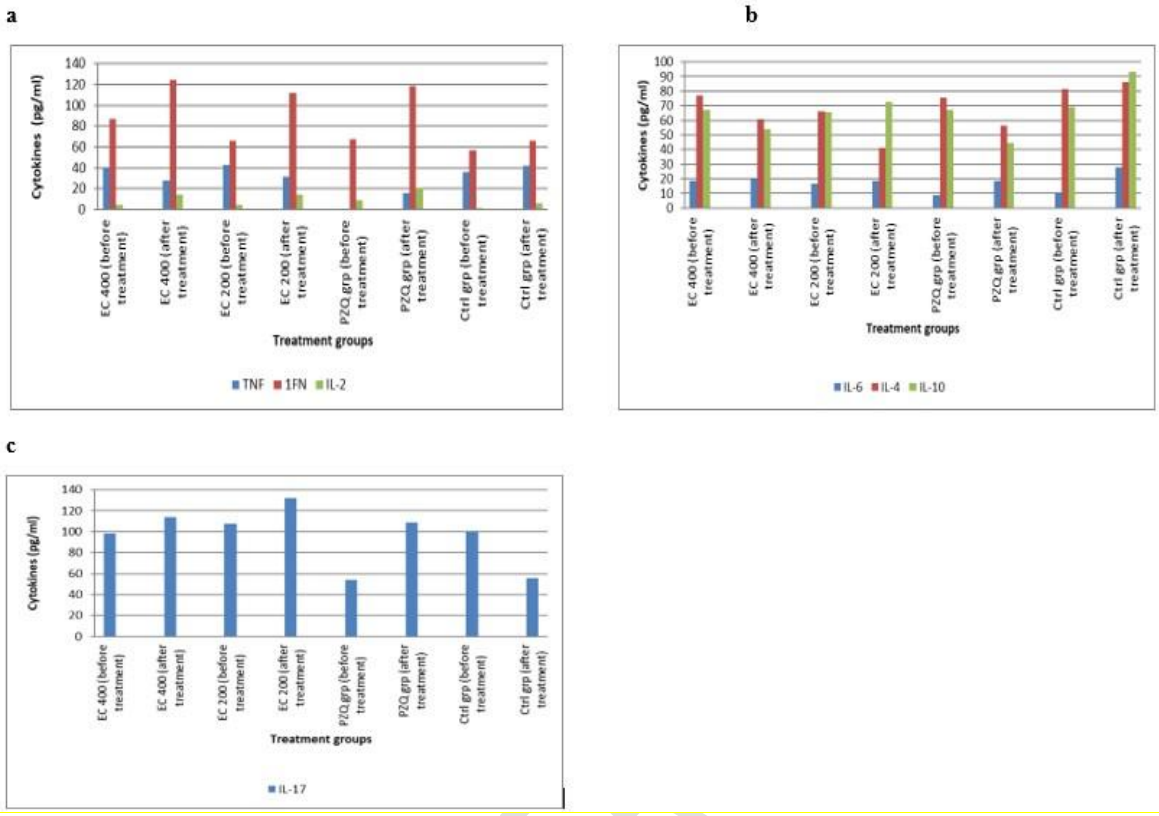

Figure1.Mean serum levels of Th1/Th2/Th17 cytokines after treatment with aqueous extracts of EC 7 weeks post infection. **(a)** Graph showing the mean serum levels of Th1 cytokines before and after treatment. **(b)** Graph showing the mean serum levels of Th2 cytokines before and after treatment. **(c)** Graph showing the mean serum levels of Th17 before and after treatment. n=5. PZQ was used as a control drug while the Ctrl group in infected untreated group. A representative of 2 similar experiments.

At 400mg/kg, ECcaused an increase in the mean serum levels of IFN-γ (124.7pg/ml, $P<0.001$), IL-2 (14.1pg/ml, $P<0.5$) at and Th17(113.8pg/ml, $P<0.01$) whereas, a decrease in TNF-α (27.4pg/ml, $P<0.001$), IL-4 (60.8pg/ml, $P<0.01$), IL-6 (20.5pg/ml, $P<0.01$) and IL-10 (53.8pg/ml, $P<0.001$) was noticed.

The extract at 200mg/kg showed a reduction in TNF-α, IL-4, IL-6 and IL-10 at 31.6pg/ml ($P<0.001$), 41.3pg/ml ($P<0.001$), 18.5pg/ml ($P<0.01$) and 72.8pg/ml ($P<0.001$) respectively when compared to the infected untreated group and an increase in IFN-γ at 111.5pg/ml ($P<0.001$), IL-2 at 14.04pg/ml ($P<0.05$) and a dramatic increase in the level of IL-17 at 131.9pg/ml $P<0.001$). This was comparable to the PZQ treated group as shown in Table 1.

Table 1: Mean serum levels of cytokines following treatment with EC 7 weeks post infection

	Th1			Th2			Th17
	TNF	**1FN**	**IL-2**	**IL-4**	**IL-6**	**IL-10**	**IL-17**
Infected untreated (Control)	41.6±1.0	65.8±0.9	5.8±0.3	86.1±1.8	28.01±1.7	93.1±2.6	55.9±1.2
PZQ, 200mg/kg	15.5±0.8 (¤¤¤, ***)	118.0±1.9 (¤¤¤,***)	21.1±1.4 (¤¤, **)	56.2±1.1 (¤¤¤, ***)	18.5±0.9 (¤¤¤, **)	44.8±1.3 (¤¤¤, ***)	108.6±3.6 (¤¤¤,**)
EC,400mg/kg	27.4±0.9 (◊◊◊, ***)	124.7±3.1 (¤ ¤, ◊, * * *)	14.1±2.0 (¤, ◊◊, *)	60.8±2.2 (¤¤,◊, ***)	20.5±0.6 (¤, ◊, **)	53.8±1.3 (¤¤¤,◊◊◊,***)	113.8±4.8 (¤,◊,**)
EC,200mg/kg	31.6±0.9 (¤¤¤,◊◊◊,***)	111.5±2.1 (¤¤¤,◊,***)	14.04±2.2 (¤,◊,*)	41.3±1.4 (¤¤¤,◊◊◊,***)	18.5±1 (**)	72.8±1.4 (¤¤,◊◊◊,***)	131.9±1.2 (¤¤¤,◊◊,***)

Data were expressed as mean±SD, n=5. Data analyzed by t-test: two sample assuming unequal variance. Values with superscript: ¤comparison between before treatment and after treatment, ◊comparison with PZQ, *comparison with infected untreated (control) group. ¤ or ◊ or *significant (P<0.05), ¤¤ or ◊◊ or **highly significant (P<0.01) and ¤¤¤ or ◊◊◊ or ***very highly significant (P<0.001). A representative of 2 similar experiment.

Effect of treatment with EC at 2 weeks p.i on Th1/Th2/Th17 cytokines

Treatment was also done using the plant extract to target juvenile worms (two weeks pi) and using artemether (ART) as a positive control drug for comparison. At 2 weeks pi the results showed that all the Th1 cytokines increased in all the treatment groups but IL-2 was very low in the infected untreated group. The effect of treatment with EC extract at both concentrations of 400mg/kg and 200mg/kg was similar to the results of the ART group as shown in Figure 2a.

Levels of Th2 cytokines reduced in all the treatment groups after treatment but increased after the 2 weeks in the infected untreated group as shown in Figure 2b. The level of Th 17 also increased in all the treatment groups and also in the infected untreated group at 2 weeks (Figure 2c).

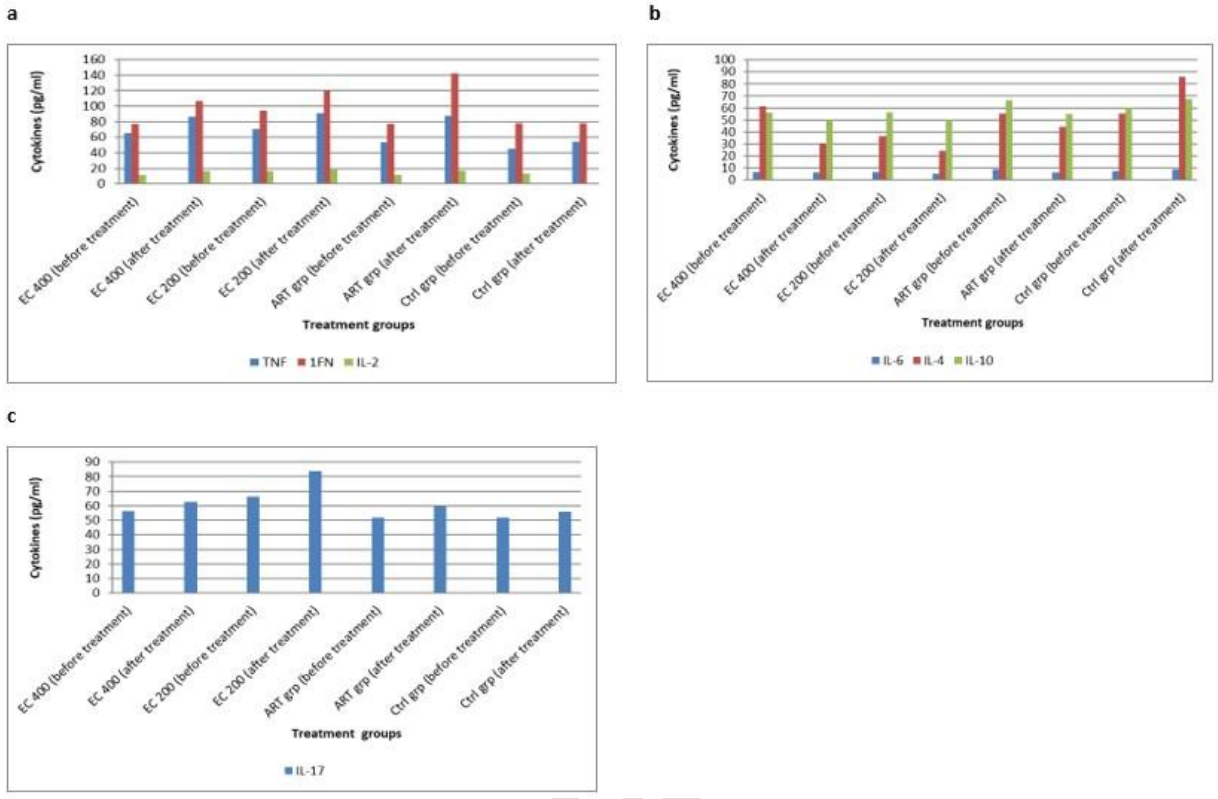

Figure 2.Mean serum levels of Th1/Th2/Th17 cytokines after treatment with aqueous extracts of EC2 weeks post infection. **(a)**Graph showing the mean serum levels of Th1 cytokines before and after treatment. **(b)**Graph showing the mean serum levels of Th2 cytokines before and after treatment. **(c)**Graph showing the mean serum levels of Th17 before and after treatment. n=5. ART (artemether) was used as a control drug while the Ctrl group in infected untreated group. A representative of 2 similar experiment.

At 2 weeks post infection,a comparison was made with infected untreated group and showed a general increase in Th1 cytokines, a reduction in Th2 cytokines and a moderate increase in Th17 cytokine when the mice were treated with *E. capensis* at both concentrations of 400mg/kg and 200mg/kgas shown in Table 2. Again, the effect of the extract at both concentrations on the cytokines gave a pattern that was comparable to the artemether treated group.

There was an increase in Th1 cytokines after treatment with EC at 400mg/kg whereby TNF-α was recorded at a level of 86.7pg/ml ($P<0.001$), IFN-γ at 106.9pg/ml ($P<0.5$), IL-2 at 15.9pg/ml ($P<0.001$), and an increase in the level of IL-17 to 62.4pg/ml ($P<0.01$). The Th2 cytokines reduced to 30.6pg/ml ($P<0.001$), 6.1pg/ml and 50.3pg/ml ($P<0.001$) for IL-4, IL-6 and IL-10 cytokines respectively when compared to the infected untreated group.

At 200mg/kg body the level of IL-17 increased to 83.9pg/ml while the Th1 cytokines also increased to 90.6pg/ml for TNF- α, 119.7pg/ml for IFN- γ and 17.8pg/ml for IL-2. On the other hand, the Th2 cytokines reduced to 24.7pg/ml, 5.1pg/ml and 50.3pg/ml for IL-4, IL-6 and IL-10 respectively when compared to the infected untreated control group.

Table 2. Mean serum levels of cytokines following treatment with EC 2 weeks post infection.

	Th1			Th2			Th17
	TNF	**1FN**	**IL-2**	**IL-4**	**IL-6**	**IL-10**	**IL-17**
Infected untreated (Control)	54.7±0.7	77.9±1.4	1.02±0.1	85.7±1.6	8.9±0.2	67.4±1.5	55.9±1.9
ART,200mg/kg	87.5±1.1 (¤¤¤,***)	142.5±2.5 (¤¤¤,***)	16.9±0.3 (¤¤¤,***)	44.2±0.8 (¤¤¤,***)	6.2±0.4 (¤¤,**)	54.7±0.4 (¤¤¤,**)	59.4±1.0 (¤¤¤)
E.C,400mg/kg	86.7±0.9 (¤¤¤, ***)	106.9±5.6 (¤, ◊◊, *)	15.9±0.1 (¤¤¤,◊,***)	30.6±0.7 (¤¤¤,◊◊◊,***)	6.1±0.2 (***)	50.3±0.8 (¤¤, ◊◊, ***)	62.4±0.9 (¤¤¤, ◊, **)
E.C,200mg/kg	90.6±0.8 (¤¤¤, ◊, ***)	119.7±0.9 (¤¤¤,◊◊◊, ***)	17.8±0.5 (¤, ***)	24.7±1.6 (¤¤¤,◊◊◊,***)	5.08±1.8 (¤¤¤,◊,***)	50.3±1.0 (¤¤¤,◊,***)	83.9±0.5 (¤¤¤,◊◊◊,**)

Data were expressed as mean±SD, n=5, and analyzed by t-test: two sample assuming unequal variance. Values with superscript: ¤comparison between before treatment and after treatment, ◊comparison with ART (Artemisinin),*comparison with infected untreated (control) group. ¤ or ◊ or *significant (P<0.05), ¤¤ or ◊◊ or **highly significant (P<0.01) and ¤¤¤ or ◊◊◊ or ***very highly significant (P<0.001).A representative of 2 similar experiment.

4. DISCUSSION

Schistosomiasis remains a major public health problem of great socioeconomic impact (Hajissa et al., 2018). The antimonialsthat were in use for treatment of schistosomiasis in the 1918 were reported to havenumerous side effects (Cioli et al., 1995). This resulted in the search for metal-free drugs as alternatives (Katz & Coelho, 2008). In the 1960s there was a breakthrough in the treatment of schistosomiasis with the advent of metrifonate, nitrofurans, lucanthone, niridazole, hyacanthone and finally oxamniquine. In 1970s, several antischistosomal drugs emerged such as tubercidin, amoscanate, PZQ and its benzodiazepine derivative Ro11-3128 and oltipraz. However, the therapeutic doses of most of these drugs were found to cause major side effects (Fenwich& Webster, 2006).The anthelminthic drug PZQ has been used for treating schistosomes for more than 40 years since its discovery by German pharmaceutical companies Bayer AG, Leverkusen and E. Merck, Darmstadt in 1972 (Fukushige et al., 2021) and remains the only available drug for the treatment of all human schistosomiasis species because it is effective, safe and affordable (WHO, 2023). However, since PZQ has been used to treat individuals and in mass drug administration (MDA) programs (Lago et al., 2018) for a long period, there are fears of emergence of reduced efficacy or emergence of resistance with continued use (Kabuyaya et al., 2018). There have been reports of reduced treatment success in some *S. mansoni* endemic communities (Crellen et al., 2016; Melman et al., 2009; Lamberton et al., 2010; Wang et al., 2012, Doenhoff et al., 2002). However, this reduced efficacy might also be caused by high infection intensities, low drug absorption, high rates of reinfections and minimal immunological response to previous infections (Danso-Appiah & De Vlas, 2002).Evidences of parasite resistance to PZQhave been reported based on low cure rates but widespread clinical resistance has not occurred, leaving PZQstill the drug of choice for treatment of schistosomiasis (CDC,

2020).Treatment with PZQ is successful in controlling disease morbidity and prevalence but it is ineffective against juvenile parasites making repeat dosing necessary (WHO, 2022). The need for a drug that has activity on schistosomula and juvenile stages in addition to adults would result in reduction in egg production and prevent granuloma formation before fibrosis is established.There are no documented alternative treatments to PZQ and there is a need to develop other drugs for schistosomiasis and medicinal plants are a preferred alternative.

Natural products have for many years been used as a foundation of drug leads in the pharmaceutical industry and many of them have shown potency on the schistosome parasite even with application of computer aided drug discovery methods but none has reached the market as new drugs (Mtemeli et al., 2022).Exploring plants for development of complementary or alternative drugs to be used with PZQ will be of value to reduce morbidity and mortality of schistosomiasis.The water extract of the bark of EC was tested for immunomodulation activity on *S. mansoni* in vivo.

At 7 weeks p.i, treatment with *E. capensis* at 200mg/kg and 400mg/kg resulted in a reduction in TNF-α but an increase in IFN-γ and IL-2 (Th1 cytokines). Effect on Th2 cytokines resulted in a reduction in IL-4, an increase in IL-6 and a reduction in IL-10 levels. There was however an increase in IL-17 at both doses, and this was comparable to the observation in the PZQ treatment group. The effect of the plant extract on juvenile *S. mansoni* infection (2 weeks p.i) resulted in an increase in TNF-α, IFN-γ and IL-2 cytokines. There was a reduction in Th2 cytokines IL-4, IL-6 and IL-10 and an increase in IL-17 (Th17) cytokine. At 2 weeks p.i, a Th1 immune response was maintained due to death of larval worms after treatment and therefore fewer eggs being laid by the surviving worms. An interesting observation was that Th1 and Th17 cytokines increased in most of the treatment groups while Th2 cytokines decreased. A decrease in IL-10 also seemed to result in an increase in IL-17.

The plant was found to modulate the course of schistosome infection at 7 weeks and 2 weeks p.i with an interesting trend that showed an increase in Th1, a decrease in Th2 and an increase in Th17 cytokines. The predominance of Th1 cytokines that was observed in this study could be as a result of helminthotoxic effect of these medicinal plant extracts to schistosomulae thus preventing/ reducing the development of egg laying adult worm pairs (2 weeks p.i). A reduction in worm burden in the mice could have resulted in down regulation of egg induced Th2 response and maintenance of Th1 predominant cytokine profile characterized by high IFN-γ and low IL-4. IFN-γ has been shown to be involved in protective immunity to schistosomiasis in murine models (Hewitson et al., 2005). Maintenance of high levels of Th1 and Th17 cytokines and low levels of Th2 cytokines is also indicative of a failure of a switch from Th1 to Th2 due to the death of worms after treatment and therefore fewer eggs being laid by the surviving worms.

Murine schistosomiasis is characterized by Th1 reaction (with a predominant secretion of IFN-γ, minimal level of IL-4 and IL-5) occurring during prepatency and then shifting to a Th2-based profile which develops after the onset of oviposition and persists throughout the acute phase of infection (with high IL-4 and IL-5, but low IFN-γ) (Davies et al, 2004). Ironically, egg induced Th2 responses are an immunologic double-edged sword, participating in protection of host tissues from egg-induced injury (Brunet et al.,1997) and in the development of the egg-induced

pathology and fibrosis associated with chronic schistosome infection (Wynn and MacDonald, 2004). The natural and induced forms of severe schistosomiasis correlates with high levels of pro-inflammatory cytokines IFN-γ and IL-17 (Rutitzky et al., 2008). This is indicative of the Th1 and Th17 subpopulations of CD4 T lymphocytes. This can be related to the observation in this study. The shift from Th2 to Th1-like immune response (as observed in this study) is essential for the down modulation of granuloma reaction and disease control. Th1 cytokine profile results in the development of smaller granulomas (Brunet et al., 1998). IFN-γ is involved in protective immunity to schistosomiasis in murine models. The results from this study is supported by a study that was carried out on *A. indica* (extracts of neem leaves) where it was shown to have immunomodulatory response to live Newcastle disease vaccine (Garbaa et al., 2013). Neem leaf preparation enhanced Th1 immune response and anti-tumour immunity against breast tumour associated antigen (Mandal-Ghosh et al., 2007). There is no evidence of previous studies on the immunomodulatory effect of *E. capensis*, thus this is the first one.

The efficacy of PZQ has been shown to be dependent on host immune responses for example in a study that showed activity of PZQ to be dependent on T cell mediated immunity (Ammann et al., 2004) and many experimental studies in immunosuppressed *S. mansoni* infected mice that were T-cell deprived or B-cell depleted (Brindley & Sher, 1987; Doenhoff, 1989; Doenhoff et al., 1988). Another study showed that following PZQ exposure in vivo, sexually mature worms had damaged tegument surfaces that resulted in exposure of parasite antigens to the host humoral immune system (Reimers et al., 2015) and also triggered recruitment of innate immune cells most likely causedparasite elimination (Panic et al., 2017). PZQ's lack of efficacy to immature worms maybe as a result of the need of involvement of the hosts' immune system since eggs are released by sexually mature worms and the host responds to their antigens with a wave of macrophage recruitment to the liver and an acute Th2 response (Nacsimento et al., 2014; Barron & Wynn, 2011). A study by McCusker et al., reported that in vivo exposure to PZQ resulted in the alteration of the transcription gene products of *S. mansoni* (2021).

The immunomodulatory potential of EC was reported in this study since the levels of cytokines changed variedly during the course of both juvenile and adult infection. The results of this study are relatable to a study on Curcumin (a polyphenol derived from the dietary spice turmeric) which was reported to modulate granuloma formation through regulation of cytokines expression like TNF-α and inhibition of the release of IL-2 and IL-4 that play a role in granuloma formation (Allam, 2009). Another study showed that ginger played an immunomodulatory role through its effect on IgE, IgG and IgM and IL-4, IL-10 and IL-12 (Aly &Mantawy, 2013). A study on combination of ginger and PZQ showed potency due to the different impact of both drugs on cytokines since PZQ increased TNF-α, IL-6, IL-8, IFN-γ, IL-12 and IL-23 levels, while Turmeric inhibited TNF-α, IL-6 and IL-8 (Hussein et al., 2017).

5. CONCLUSION

In conclusion, the results from this study showed that the water extract of EChada significant immune-enhancing abilityin the experimental schistosomiasis on both adult and juvenile infection. This was comparable to the effect PZQ on adult infection and also of ART on juvenile infection and means that EC extract is a potential source of a lead immunomodulatory drug.

Therefore, further studies should be carried out on this extract to determine the exact mechanism of action, and also to determine if the extracts can be used singly or in combination with PZQ in the management of schistosomiasis.This could lead to effective and targeted therapies and also become a strategy for transmission control which can reduce the morbidity and mortality of schistosomiasis in endemic regions. The isolation and characterization of the bioactive compounds of the plant that were responsible for immunomodulatory activity that was observed in this study are recommended. More in depth studies that include toxicity studies are required to understand the underlying mechanisms of action and safety levels of this plant as a potent and safe immunomodulatory agent. Immunomodulatory agents from plants origin that are safe can be used to treat many illnesses alternatively to conventional drugs and help also to reduce the side effects and high costs of synthetic compounds.

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