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Estimation of Immunoglobulin A, Immunoglobulin G, and Immunoglobulin M Antibody Levels in Laboratory Mice Balb/c Infected with Entamoeba histolytica and Treatment with Aqueous Extracts of Cyperus rotundus and Thymus serpyllum

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Abstract

Sixty Balb/c mice (Mus musculus) were used in the present study. It was conducted out in the animal house of the Department of Biology/College of Education for Women. The mice were infected with Entamoeba histolytica isolated from positive stool samples obtained from children in pediatric hospital in Kirkuk city governorate followed by treatment with aqueous extract of Cyperus rotundus and Thymus serpyllum. Results revealed a significant increase of $P < 0.05$ in immunoglobulin A, immunoglobulin G,, and immunoglobulin M antibody levels among infected mice when it was compared with the control and treated groups of aqueous extracts of studied plant. This indicates the efficacy of these extracts as treatment.

Keywords

Antibody levels, Entamoeba histolytica, Thymus serpyllum, Cyperus rotundus, Dysentery

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ORIGINAL ARTICLE

Estimation of Immunoglobulin A, Immunoglobulin G, and Immunoglobulin M Antibody Levels in Laboratory Mice Balb/c Infected with *Entamoeba histolytica* and Treatment with Aqueous Extracts of *Cyperus rotundus* and *Thymus serpyllum*

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ABSTRACT

Sixty Balb/c mice (*Mus musculus*) were used in the present study. It was conducted out in the animal house of the Department of Biology/College of Education for Women. The mice were infected with *Entamoeba histolytica* isolated from positive stool samples obtained from children in pediatric hospital in Kirkuk city governorate followed by treatment with aqueous extract of *Cyperus rotundus* and *Thymus serpyllum*. Results revealed a significant increase of $P < 0.05$ in immunoglobulin A, immunoglobulin G,, and immunoglobulin M antibody levels among infected mice when it was compared with the control and treated groups of aqueous extracts of studied plant. This indicates the efficacy of these extracts as treatment.

Keywords: Antibody levels; Entamoeba histolytica; Thymus serpyllum; Cyperus rotundus; Dysentery

INTRODUCTION

Entamoeba histolytica is an enteric protozoan parasite that causes amebiasis (Ali, 2015). It is the third leading reason for death from parasitic diseases worldwide after malaria and schistosomiasis (Mortimer and Chadee, 2010; Ghasemi et al., 2015). It is estimated that it affects nearly 50 million people worldwide, causing deaths of nearly 40,000–100,000 people annually (Inam et al., 2016; Pineda and Perdomo, 2017). *E. histolytica* is prevalent worldwide, being most spread in tropical and subtropical regions (Al-Areeqi et al., 2017; Yimer et al., 2017; Sastry and Bhat, 2018), especially in developing countries (Peterson et al., 2011; Gwairgi and Ghildyal, 2018). There are more than 20 species of *Entamoeba*, with varying disease-causing potential and host specificity (Nozaki and Bhattacharya, 2015). Unfortunately, many species of *Entamoeba* are morphologically indistinguishable from *E. histolytica*, including the potentially pathogenic but rare *E. nuttalli* (Levecke et al., 2015), as well as many non-pathogenic species of *Entamoeba*, including *Entamoeba dispar*, *Entamoeba moshkovskii,* and *Entamoeba bangladeshi* (Nozaki and Bhattacharya, 2015; Jirků-pomajbÍková et al., 2016).

Thymus serpyllum is an important medicinal herb because it contains antimicrobial agents and is rich in various active substances such as thymol, carvacrol, paracymene, and terpinene (Nabavi et al., 2015). *Cyperus rotundus* is a perennial medicinal plant and is used globally in traditional medicine to treat various diseases (Singh et al., 2012; Lydia and Sudarsanam, 2014; Peerzada et al., 2015). The plant contains many active compounds such as flavonoid, saponin, glycosides, alkaloid, starch, monoterpene, and many other secondary metabolites (Sivapalan and Jeyadevan, 2012; Singh et al., 2012; Al-Snafi, 2016), as many studies have proven it to be analgesic, anti-allergic, anti-inflammatory, anti-diarrheal, anti-reactive, and anti-worm (Kamala et al., 2018). There are five types of immunoglobulins, A, D, E, G, and M and the first four species are found in the immune response to parasites (Abd-Alla et al., 2006; Macpherson et al., 2008).

MATERIALS AND METHODS

Isolates of *E. histolytica*

E. histolytica was isolated from positive stool samples obtained from children in pediatric hospital in Kirkuk city

governorate from a period between November 2018 and April 2019. Children selected for this study were usually suffering from diarrhea, dysentery, and vomiting. The sterile plastic container was used during the transport of stool from the laboratory of parasitology in the pediatric hospital to the advanced laboratory of parasitology in the Department of Biology/College of Education for Women. The stool samples were examined using two direct smears method first in normal saline 0.9% and the second one in Lugol's iodine 1% to the screening on *E. histolytica* trophozoites and cysts stage, respectively (Cheesbrough, 2005).

Experimental Design

Sixty mice were used to conduct the experiment. These mice were divided into 10 groups, each with six mice. The mean of the number of cysts or trophozoite stages of the parasite was then calculated for each group of infected mice before treatment. Treatment with aqueous extracts was given orally and with drinking water at specific doses. The percentage of parasite efficacy was calculated for each of the infected mice (positive control) and the groups of mice 25 days after giving them the treatment, according to the following equation:

Parasite efficacy = (number of cysts or trophozoite stages post-treatment/number of cysts or trophozoite stages pre-treatment) × 100 (AL-Mukhtar and Barwari, 2008).

Group 1: Six mice each was orally administered normal saline for 25 days.

Group 2: Positive control, mice were infected with *E. histolytica* according to Lemee et al. (2000).

Group 3: Six mice each were administered 2mg/ml b. wt. of the fresh aqueous extracts of *C. rotundus* for 25 consecutive days

Group 4: Six mice each was administered 3 mg/ml b. wt. of the fresh aqueous extracts of *C. rotundus* for 25 consecutive days

Group 5: Six mice each was administered 4 mg/ml b. wt. of the fresh aqueous extracts of *C. rotundus* for 25 consecutive days

Group 6: Six mice each was administered 5 mg/ml b. wt. of the fresh aqueous extracts of *C. rotundus* for 25 consecutive days.

Group 7: Six mice each was administered 2 mg/ml b. wt. of the fresh aqueous extracts of *T. serpyllum* for 25 consecutive days.

Group 8: Six mice each was administered 3 mg/ml b. wt. of the fresh aqueous extracts of *T. serpyllum* for 25 consecutive days.

Group 9: Six mice each was administered 4 mg/ml b. wt. of the fresh aqueous extracts of *T. serpyllum* for 25 consecutive days.

Group 10: Six mice each was administered 5 mg/ml b. wt. of the fresh aqueous extracts of *T. serpyllum* for 25 consecutive days.

Statistical Analysis

The statistical analysis system (SAS 2010) was used in this study the effect of the different factors in the studied traits. The differences between the means were compared with the least significant difference of LSD, and the percentages were compared with the Chi-square test.

RESULTS AND DISCUSSION

Estimation of Immunoglobulin's Level

The study showed differences in the level of IgG, IgA, and IgM in mice infected with *E. histolytica* compared to the control group [Table 1] and treatment with the aqueous extracts of *C. rotundus* in different concentrations [Table 2] when compared with the control group and the concentration of mg/dl was studied. Statistical analysis showed significant differences between the different groups at $P = 0.05$.

Several studies have indicated that IgM, IgG, and IgA antibodies have increased in acute and chronic cases of *E. histolytica* infection (Al Quraishi and Al-Sultany, 2017).

Table 1: Levels of immunoglobulin IgM, IgG, and IgA in mice serum infected with *Entamoeba histolytica* and control group

Types of immunoglobulin	Infected mice	Control
IgA	544.30±55.1*	104.65±8.20
IgG	1610.5±85.0	530.6±19.11
IgM	366.00±61.1	211.88±10.88

(*Mean±SD). IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M

Table 2: Fluctuation of different immunoglobulin levels after using of aqueous extract of *Cyperus rotundus* in different concentrations in mice infected with *Entamoeba histolytica*

Concentrations of aqueous extract of *Cyperus rotundus* (mg/ml)	Immunoglobulin		
	IgA	IgG	IgM
2	112.80±11.26*	537.3±11.91	255.10±24.1
3	124.23±10.80	539.2±31.9	264.06±19.12
4	127.70±25.7	616.8±18.91	273.55±12.89
5	128.10±32.5	698.8±27.8	305.22±16.69

(*Mean±SD). IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M

Table 3: Fluctuation of different immunoglobulins after using of aqueous extract of *Thymus serpyllum* in different concentrations in mice infected with *Entamoeba histolytica*

Concentrations of aqueous extract of *Thymus serpyllum* (mg/ml)	Immunoglobulin		
	IgA	IgG	IgM
2	124.52±8.48*	545.9±85.5	159.78±12.02
3	127.36±6.99	642.5±60.8	171.30±10.07
4	143.88±18.32	713.1±14.74	188.16±18.35
5	239.60±24.0	806.4±16.31	315.30±27.3

(*Mean±SD). IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M

The current study the an increase in the IgM, IgG, IgA, of infection with E. histolytica,this result is close to AL-Kahfaji (2010) who recorded an increase in the IgM, IgG, IgA, and IgE antibodies in the infected patients E. histolytica compared with the control group. An increase in the IgM, IgG, IgA, and IgE antibodies in the infected patients *E. histolytica* compared with the control group.

In Kirkuk, Anwar (2014) observed an increase in IgM, IgG, and IgA levels in people who *E. histolytica* parasites. Another study indicated an increase in the level of IgA and IgG antibodies in animals infected with parasite while no significant differences were observed in IgM (Houpt et al., 2002; Jarillo-Luna et al., 2002). In the present study, the results showed a significant increase of $P < 0.05$ in IgA, IgG, and IgM antibody levels in mice infected with *E. histolytica* compared to levels in control groups.

Animals treated with different concentrations of 2, 3, and 4 mg/ml of aqueous extract of *C. rotundus* plant showed a decrease in the level of antibodies and increased at a concentration of 5 mg/ml. This may be due to the important therapeutic role of this plant extract through its active substances such as glycosides, resins, and alkaloids, in addition to the acidic environment, these factors led to the reduction of the parasite numbers in the lumen of the large intestine and thus the damage caused by the parasite and thus the reduction of the acute inflammatory state due to the low level of antibodies to the serum of animals (AL-Kahfaji, 2010). Concentrations 2 and 3 were to be better compared to control and treated groups.

As for the groups treated with aqueous extract of *T. serpyllum* plant in different concentrations 2, 3, and 4 mg/ml, it was observed that the level of antibodies decreased and increased at a concentration of 5 mg/ml [Table 3]. The decrease in the level of antibodies may be due to the fact that its essential oil contains active compounds such as thymol and carvacrol which have an effect on the disappearance of the parasite within the large intestine and therefore the decrease in the level of antibodies treated with aqueous extract of *T. serpyllum* and the return of the immune status to the normal level and the level of serum proteins gradually decreased, and the concentrations of 2, 3, and 4 mg/ml gave comparable results compared with groups while increased at a concentration of 5 mg/ml.

CONCLUSION

The results of the present study showed a significant increase IgG, IgM, and IgA levels in mice infected with *E. histolytica* in general, compared to the control and treated groups of aqueous extracts of *C. rotundus* and *T. serpyllum*. This indicates the efficacy of these extracts as treatment and after further studies on all active ingredients in both plants.

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ETHICAL APPROVAL

Ethical approval has been collected and preserved by the authors concerning standards or university standards.

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