



INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by Pharmascope Publications

Journal Home Page: www.pharmascope.org/ijrps

Comparison between the effect of metronidazole and some plant extracts on parasite *Cryptosporidium parvum* in vivo

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Article History:	ABSTRACT
Received on: 11.10.2018 Revised on: 23.12.2018 Accepted on: 26.12.2018	The current study included the test of the efficiency of water extract for pomegranate peel and water extract from pumpkin seeds in the treatment of parasite <i>Cryptosporidium parvum</i> within the body of the organism and compared its effectiveness with metronidazole in the treatment to eliminate the same parasite within the body of the organism, was relied on (50) mouse mosquito moss age (8-10 weeks) and weight (25-30 g), the mice were first examined as soon as they were obtained to confirm their safety and the absence of previous intestinal parasites, then they were injected with parasite bags that collapsed to cause the disease, and the mice remained under observation until they were confirmed to obtain the infection, the mice were treated the first group was given the dose 392.0 mg/1.0 ml/mouse of metronidazole, the second group with a dose of 657.14 mg/1.0 ml of water extracts for pomegranate solutions, the third group took 1000 mg/1.0ml /g of pumpkin extract from Pumpkin seeds, plus a positive control group of 2x10 cysts/2.0 ml of bag suspension and 1.0 ml of normal saline solution, the results showed to absence of cysts of parasites completely on the sixth day of the group that took metronidazole and on the seventh and eighth days of the two groups that used water extracts to peel the pomegranate and seeds of the pumpkin respectively for the positive control group, and that both the drug and the plant extracts achieved results of 100% in the treatment of infected mice and the elimination of parasite, and it seems clear that the highest efficiency was the share of the drug metronidazole followed by plant extracts.
Keywords:	
Cryptosporidiosis, Metronidazole, Plant extract	



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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v10i1.1831>

Production and Hosted by

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INTRODUCTION

The Parasites, are one of a biological pathogen that affects many members of the body organism causing various diseases, intestinal parasites, are a type of the parasites endemic to the intestines of the host, which are widely spread all over the world more than 3,000 million people are infected with one or more intestinal parasites during their life

(Al-Abodi *et al.*, 2015; Al-Mosa, 2002). Cryptosporidiosis, a disease caused by intracellular intestinal parasite *Cryptosporidium* sp., is considered to be one of the most common pathogens that cause diarrhea in animals and human, may lead to death especially in young children, the elderly and immunocompromised individual, especially in AIDS patient (Rossle and Latif, 2013), there are two common *Cryptosporidium* species in humans is *Cryptosporidium parvum* and *Cryptosporidium hominids*, are responsible for more than 90% of status cryptosporidiosis in human (Xiao and Ryan, 2004).

Given the importance of the disease and its effects on humans and their environment, it is natural for researchers to find medical drugs to treat this disease such as the phylogenetic drug (Metronidazole): is one of the most commonly used drugs in the case of intestinal parasitic infections, its chemical composition $C_6H_9N_3O_3$ and molecular weight:

15.101, it is pale white powder to yellow crystalline, dissolves with water and alcohol (Evans *et al.*, 2003), but in general due to the side effects caused by chemically manufactured medicines on public health, the researchers have resorted to finding alternatives to treat some of the diseases and parasitic infections and they pointed to the medicinal plants as alternative drug, for example Pomegranate is considered one of the most important medicinal plants and belongs to the Punicaceae family, which contains one species is Punica, the outer shell of the pomegranate contains tannic acid and alkaloids, Pomegranate husks have been widely used to treat diarrhoea because it works to change the nature of the stomach and intestine through the intestinal proteins which reduce the leakage of fluids, as well as its ability to kill germs, and adsorb toxins, Bacterial, acts astringent, antihelminthic, diuretic and coolant for body temperature (Marbun, 2017; Shaker *et al.*, 2018), while pumpkin is also considered from an important medicinal plants that have many benefits, in relation to parasites pumpkin seeds are antidepressants and also reduce heart disease, atherosclerosis, sores and leprosy (Ehlla and Mourad, 2015). Pumpkin is plant cultivated in warmer countries, many experiments, and research demonstrated pumpkins have an important role in some a therapeutics status such as inhibition of prostatic, anti-ulcer inflammation, as well as possibility play a role in injury healing, and in hypoglycemic (control diabetes) (Eneh, 2018; Obada, 2015).

The present study was conducted to compare the effect of Metronidazole, compare with the aqueous extract for pomegranate and Pumpkin as a drug on cryptosporidium sp. parasites inside the living body depending on experimental infection for the laboratory mice.

MATERIAL AND METHODS

Parasite collection: The parasite was obtained in its trophozoites and cyst phases of infected people suffering from acute diarrhoea references to the general hospital in Al-Qadisiyah Governorate, the samples were collected in Sterilized plastic bottles, the parasites were used in events Infection is experimental in laboratory mice, fecal samples taken from people with parasite were examined by examining each sample with Modified Ziehl Neelsen stain and flotation method by using Sheather's Solution and detected of parasites cysts, the cyst phase has been purified and calculated according to (Malgwi *et al.*, 2014) for obtaining a dose of infection, where the mice were infused with parasites with concentration 2×10^3 cyst (2.0ml).

Plant extract: The water extract was prepared for the pomegranate peel according to what was men-

tioned (Malgwi *et al.*, 2014), while the water extract of the pumpkin seeds was prepared according to the method (Al-Ammash *et al.*, 2012).

Experimental animals: The current study was conducted in the laboratory of the department of life Sciences, faculty of Science, university of Al-Qadisiyah, in the current study (50) mice *musculus muses* at age (8-10 Week) and weight (25-30 g), the mice's faeces were first examined as soon as they were obtained to confirm their safety and not to be infected with an earlier intestinal parasite after that they were placed in plastic cages for the breeding of these experimental animals and at the rate of seven mice in each cage, the appropriate environmental conditions (heat, ventilation and lighting, specialized care, and sterile drinking water were provided by private bottles), as well as the purification cysts of the parasite and its injection using the special stomach tube, after that the mice were placed in cages Clean free of sawdust and their faeces were examined in a direct smear method in the third day of the investigation incidence of infection as in study of (Vazini *et al.*, 2017) , the infected mice were then divided into clusters the basis of similarity in the incubation period, each group contains 10 mice, as follows:

1. Negative control group: take dose 1.0 ml of normal saline solution.
2. Positive control group: this group is taking dose 2×10^3 cyst / 2.0 ml cysts suspended, the rats' faeces were examined and after the infection was detected directly, inject 1.0 ml of the normal saline solution.
3. Infection mice group take dose 392.0 mg / 1.0 ml/rat of solution metronidazole, this drug is used to treat people Adults with a dose of 1000 mg /day/person, and on the basis of that determined therapeutic dose of mice.
4. Infection mice group take dose 657.14 mg / 1.0 ml / rat of solution water extract for pomegranate crops.
5. Infection mice group take dose 1000 mg / 1.0 ml/rat of solution water extract of pumpkin seeds.

The therapeutic dose was selected from the plant extracts studied depending on the LD (LD50) specified for each extracted, according to the (Malgwi *et al.*, 2014), the process of cyst counting continued during treatment, and until the end of infection, the following equation was applied for measuring the therapeutic efficacy of both drug and plant extracts (Vazini *et al.*, 2017).

Table 1: Average number of cysts of treated mice during the treatment period compared with the control group

Groups	Average cysts preparation rate ± standard error (cysts/ gm of stool) Day after therapeutic					
	Immediately after the infection	1	2	3	4	
Group of treated mice	Metronidazole	881.02 ±44.12 D	472.00 ±18.33 D	244.00 ±16.99 F	121.09 ±9.00 F	661.00 ±5.01 F
	Aqueous extract of pomegranate	911.00 ±33.55 CD	488.00 ±18.33 D	299.00 ±9.09 E	219.00 ±12.99 E	121.00 ±7.00 E
	Aqueous extract of pumpkin seeds	899.00 ±40.00 A	799.00 ±44.22 C	599.99 ±35.44 D	422.08 ±20.00 D	211.00 ±16.31 D
	Control group	933.00 ±12.33 AB	1101.0 ±12.33 A	1199.00 ±11.11 A	1210.33 ±10.99 A	1219.33 ±10.59 A

The vertically similar characters indicate no significant differences below the probabilistic level p<0.05

Table 1: Average number of cysts of treated mice during the treatment period compared with the control group (Contd....)

Groups	Average cysts preparation rate ± standard error (cysts/ gm of stool) Day after therapeutic					
	Immediately after the infection	5	6	7	8	
Group of treated mice	Metronidazole	881.02 ±44.12 D	28.00 ±2.22 F	0.00 F	0.00 F	0.00 D
	Aqueous extract of pomegranate	911.00 ±33.55 CD	58.99 ±4.99 E	29.00 ±3.91 E	0.00 E	0.00 D
	Aqueous extract of pumpkin seeds	899.00 ±40.00 A	129.00 ±5.88 D	56.09 ±5.09 D	30.00 ±5.00 D	0.00 D
	Control group	933.00 ±12.33 AB	1277.90 ±10.99 A	1171.20 ±10.88 A	999.40 ±10.51 A	701.0 ±10.22 A

* Similar figures indicate that there are significant differences below the probability level p<0.05

Table 2: Table 2: Effectiveness of metronidazole and plant extracts under study in the experimental treatment of *C. parvum* in white mice

Groups	Therapeutic efficiency%			
	Days after therapeutic	Metronidazole	Aqueous extract of pomegranate	Aqueous extract of pumpkin seeds
1		58	52	29
2		82	73	41
3		89	84	59
4		94	90	70
5		98	92	82
6		100	97	90
7			100	96
8				100

Efficacy of treatment = Average number of parasite cysts in positive control group - average number of parasite cysts in therapeutic group / Average number of parasite cysts in positive control group * 100, the results of the calculation of the

preparation of parasite cysts were analyzed using the (ANOVA) analysis of variance test as well as the use of the Chi-square test to analyze the results of the therapeutic efficacy of the drug and the plant

extracts under study, the differences were determined at a potential level ($p < 0.05$).

RESULTS AND DISCUSSION

Several studies have examined the effect of plant extracts on pathogens, intestinal parasites have a large share of these studies, such as (Fallahi *et al.*, 2016) on comparing the effect of metronidazole and Hydroalcoholic extract of Rosemary in the treatment of *G. lamblia* infection, and showed that rosemary has high effects and can an alternative to metronidazole in the treatment of giardiasis infection, as well as the study of (Rossignol, 2010) the effect of olive leaves, *Satureja khuzestanica*, and *Allium sativum* extracts on *G. lamblia* cysts compared with metronidazole. The best treatment is the extraction of olive leaves and *Satureja khuzestanica* respectively afterwards metronidazole and finally *Allium sativum*, and study of about effect of alcohol extract of green tea as a therapeutic of *Entamoeba histolytica*, the present study is a complementary part of the previous studies in the same field, a comparison was made between the effect of metronidazole and two different plant extracts on cryptosporidium parasite in vivo the organism, the results in table [1] show that *Cryptosporidium parvum* parasites were approximately equal in the treated rat groups and the positive control group that will use the saline solution at the beginning of the infection, ranging from 881 to 993 cysts / g of stool, the rate of dropping cysts gradually began in the first group starting from the first day of treatment with drugs and extracts to the absence of cysts of parasites completely on the sixth day of the group that took metronidazole and on the seventh and eighth days of the two groups that used water extracts to peel the pomegranate and water extract from the seeds of the pumpkin respectively for the positive control group, which continued to be phased out by physiological solution after infection, it was observed that the number of parasitic cysts increased during the first five days after the infection and then gradually decreased until it reached 701 cysts/gram of stool.

The results indicated in table (2) that both the drug and the plant extracts achieved results of 100% in the treatment of infected mice and the elimination of parasite, and it seems clear that the highest efficiency was the share of the drug metronidazole followed by the water extract of pomegranate peel and finally the water extract of pumpkin seeds.

The results of the present study are consistent with what is stated in (Sanad and Al-Ghabban, 2011). When noted that the drug metronidazole took longer than it was *Thymbra spicata* in the case of the water extract of the thyme For treating white mice it but the results of the study did not match (Al-Alousi, 2004) In the case *G. lamblia* required for

the treatment of the experimental infection of the parasite mice with metronidazole were 10 days, with therapeutic efficacy [93.33%], while coincided with in the fact that the drug is more efficient than water extract For garlic, which gave 70% efficiency after 10 days of treatment, (Fahmy *et al.*, 2009) indicated the efficiency of the alcoholic extract of pomegranate (71%) treatment of Cryptosporidiosis in the white mice at a concentration of 500 mg/kg., in study of (Al- Rifai, 2006) The methanolic extract was used to peel pomegranate fruits for treatment mice infected with *Schistosoma mansoni* parasites with continued stimulation of mice infected with the parasite for only three days and found that the extract has given a therapeutic efficiency of 72%, as well as (Kubaisi *et al.*, 2007) indicated that the extract of pomegranate gave a therapeutic efficacy of 53% in the treatment of Cryptosporidiosis when concentrating 1000 mg/kg.

The results of the present study show that the infected mice treated with plant extracts took longer than the treated mice with a drug to reach the healing stage. This may be due to the lack of sufficient plant extracts to affect the parasite as a result of being absorbed from the walls of the intestines due to its easy absorption compounds, but over time the concentration of the extract became more efficient because reduced intestinal absorption, and therefore appear the direct effect of plant extracts on the parasite (Gardner and Hill, 2001).

The difference in duration may be due to treatment with the metronidazole drug to the presence of the parasites and hence the different resistance of these strains to the drug, the evolution of resistance of certain strains of the parasite, study of (Sanad and Al-Ghabban, 2011) suggests to that the genetic variation between the parasites it may be reflected in parasitic resistance and aggression, Also may produce resistance of the parasitic ability to reduce the access of the drug through (Buret *et al.*, 2005), or it results from its ability to make complex changes to genes responsible for the encoding of parasite membrane proteins (Bradley *et al.*, 2015), but in a manner general the mechanical resistance of the neidazole is still unclear, It is not understood keep nitroimidazoles and other drugs dependent on compounds the most effective drugs and the availability of treatment for the disease of tourism the drugs (benzazole, mependazole, and metronidazole) are present in the list (WHO) of the World Health Organization (WHO) in preparation for the treatment of diseases (Vazini *et al.*, 2017).

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