ARTICLE

EXAMINING THE EFFECT OF THE DELPHINIUM ON TOXOPLASMA

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ABSTRACT

Introduction: *Nowadays, parasitic diseases because of the similarity of the properties of eukaryotic cells in terms of therapeutic are faced with some problems which in some cases leads to discontinuation of the chemical treatment and since from ancient times in IRAN, herbal treatment was acquainted. The aim of this study is to determine anti-parasitic plant on the extract of Delphinium on the animal models.* ***Methods:*** *In this study a laboratory method and appropriate number of mouse (Mus musculus) in groups with ten members and control groups has been used and sample mice with toxoplasma gondii parasitic RH strain were infected and then treated it with extract of Delphinium which has been obtained as ethanol form and finally, it compared with treated samples with anti-parasitic drug, results with Prism Chart pad software were explained and analyzed.* ***Results:*** *Results showed that a meaningful statistical relationship in tested samples with the extract of Delphinium and pharmaceutical samples will be obtained. In these results, weight changes between control and test group with 95.5% confidence was observed in high dose groups. In hepatomegaly evaluation in control and test groups, wet liver weight evaluation was considered as an index for hepatomegaly and in control and test groups was not observed a meaningful difference and from the other way in splenomegaly evaluation in control and test groups, wet liver weight was measured as an index for splenomegaly that there was not a meaningful difference in this case.* ***Conclusion:*** *It is obvious that results obtained from statistical samples and findings cannot be used of delphinium extract like traditional treatment methods for the other medicinal plants for toxoplasma gondii parasite infection treatment.*

KEY WORDS
Toxoplusma gondii, Delphinium, traditional medicine, Mus musculus, Toxoplasmosis.

Published: 10 October 2016

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INTRODUCTION

Today, along with growth and cultural and social development of communities, increasing many different diseases with different reasons, especially drug-resistant, especially in poor communities is ongoing. In all countries of the world inclusive our country, parasitic diseases and health problems, especially in less developed regions have a wide range. Undoubted, attention to various aspects of human life and attention to the treatment of parasitic diseases in the way that have less harmful effects and be economically more cost-effective are important factor in the development of any human society. On the other hand some parasitic diseases such as Toxoplasma gondii as one of the most common parasitic infection of humans and other homeothermies with a very wide geochartical spread has attracted the attention of many scientific circles.

Since the Toxoplasma gondii, as an mandatory intracellular single-celled parasite can lead to toxoplasmosis by eating raw and half cooked meat, blood transfusions, organ transplants or rarely happens by random insemination in laboratory events or on congenital by the transmission of cause of disease (Tachyzoite) from an infected mother through the placenta to fetus occur.

Given that today, even in progressive societies, maintenance of pets, especially cats can be seen in abundance, as a result, man is exposed to the catching of parasitic infection in the every period of his life from fetus life in the womb to childhood and adult life. In the present era, occurrence of Toxoplasmasis infection, always are considered to be in the two serious and dangerous population groups. First group, patients suffering from immunodeficiency or members use immunosuppressants for some different reasons and second group are fetuses that their mothers are infected to the severe toxoplasmosis during pregnancy which in the 61 percent, there is the probability of infection transition to the fetus through the placenta.

Maternal infection during the first trimester of pregnancy leads to abortion in most cases and mother infection in the second trimester of pregnancy and third trimester of pregnancy cause complications in the fetus and congenital toxoplasmosis lead to clinical protests such as chorioretinitis (usually two-way), mental and motion retardation, anemia, jaundice, splenomegaly, pneumonia, microcephaly, hydrocephalus and intracranial calcification and epilepsy in infants are the most common symptoms and lesions of the central nervous system and is said that Toxoplasma gondii chorioretinitis cause 35 percent of chorioretinitis in infants and adults [6].

This parasite in patients with immune deficiency also causes encephalitis, meningoencephalitis, hemolytic anemia, pneumonia, myocarditis, pericarditis, hepatitis and polymyositis which are the most common manifestation of the people involved in the central nervous system (encephalitis Toxoplasma)[7] .
Currently, the preferred treatment of toxoplasmosis is combination of synergistic, pyrimethamine and a sulfonamide such as sulphadiazine. This treatment causes problems for clinicians, especially for people with severe disorders of the immune system, such as patients with HIV. Almost 50 percent of patients with HIV treated with the combination of pyrimethamine – sulphadiazine show symptoms of severe poisoning that led to discontinuation of treatment. Pyrimethamine is causing osteoporosis and blood poisoning, and also due to the creation of teratogenic effects should not be taken in the first trimester. Sulfadiazine in addition to crystalluria and hematuria cause hypersensitivity reaction in people.

This combination is not able to completely eradicate the parasite in the host and when the parasites as cysts in the brain are deployed will not be affected by this combination. In patients with immunosuppressants suffering from toxoplasmosis, treatment should be continued during the life and in most cases, as soon as not to use the drug, severity of illness can be seen, on the other hand, drugs are potentially toxic and due to this, sometimes, usage of them in long time in patients with HIV, make some unwanted severe effects that cause to giving up the drug and furthermore because pyremithamine is teratogenic and its usage dose not advice in pregnancy periods[9]. Human at the first according to experience for treatment of their illness used plants which are close their environment and now according to world health organization's statistics, near 80 percent of world population use the herbal drugs that this statistic s is not developed countries is higher and in developed countries are lower. Because of some problems caused by chemical drugs and also drug resistance, thinking of building and using the herbal plant in some parasitic disease in cow and sheep in Iran and world is increasing as a need [10].

MATERIALS AND METHODS

In this study which is based on the use of pharmaceutical laboratory studies, at first we collect delphinium plant from Tehran and Lorestan (near Borujerd), in the spring, its growing season and due to herbal extract which is existing in the delphinium, first, dry the plant organs in the environment and then divide it to some small pieces then to check all the existing compounds in plant extract, drench the chopped plant with methanol in the 1000 milliliter, then after 48 to 72 hours, separate aboveground organs of plant from the solution and then to more purification in case of additional materials, the solution with green color with the use of What man Filter Paper filtered. Finally, under vacuum, solvent extracted from solution and the cured extract obtained.

In this study, toxoplasma gondii parasite RH strain used which was always passaged in the parasitology field of Pasteur institute of Iran. Due to proliferation of parasites, first 3 mouse heads infected to parasite RH strain which were infected to this strain from intra peritoneal and disease symptoms showed clearly, killed by ether then to reach to peritoneal fluid which contain a lot of tachyzoite inner and out of cell, sterilized physiological saline entered to them and regain. Result suspension under microscope in the black field was studied and after dilution, if there is 20 to 25 tachyzoite in each field of view of lens 40 and absent of microbial contamination and after counting neubauer slide, from tachyzoite to peritoneal of mouse, near 10000 tachyzouites was injected to the peritoneal of tested mouse to infect to severe toxoplasmosis disease and it can be said that this amount of parasite RH strain is deadly for mouse and during 5 to 6 days lead to animal's death.

For grouping to treatment , 40 healthy male mice with weight near 30g from pedigree animal field of Pasteur Institute of Iran was provided and in 4 different groups was grouped and were studied during 8 days. Herbal extract solution, v / v 10% ethanol 96 degrees and 90% physiological saline, three concentrations (1,10,100 milligrams per ml) provided. mice classified in groups of 10 and each group for 7 days one of the tested concentrations of plant extracts made extraversion to the possible effects of solvents (toxicity). Also, 40 mice weighing approximately 30 grams of the Pasteur Institute of Iran was prepared from animal pedigree and suspensions containing 10,000 Tachyzoites of parasites and was injected to the intra peritoneal to 30 mice of these mice and then mice were classified to 4 groups with 10 members.

The first group of patient mice treated with 200 ml of extract solvent in the survival time every day and the second group each day with 200 ml of the drug extract at a concentration of 100 mg per ml were treated during survival. The third group as control group served to compare the survival of patients treated with studied mice without treatment which were used and fourth group were placed as environmental control in the environment.

In each group, 24 hours after gavaging, animal were studied to study the disease amount or animal survival and cosmetic changes. In patient groups, time period between infection and animal death in each group was recorded and groups were compared in average time of survival with each other.

RESULTS

As can be seen in [Table 1], in the survey of solution's effect on healthy mice, weight of liver and spleen in each test group was examined and in the first and third group, 9 mice and in the second group, 8 mice were survived at the end of test time.

Table1: survey of liver and spleen weight in tested samples

Spleen's weight	Liver's weight	number	Spleen's weight	Liver's weight	number
0/31	1/58	1	0/23	1/51	1

0/20	1/53	2
0/18	1/66	3
0/29	1/71	4
0/17	1/75	5
0/27	1/67	6
0/24	1/64	7
0/34	1/77	8
0/51	1/60	9
0/27	1/65	MEAN
0/03	0/02	SEM

Weight of liver and spleen in gavaged mice with 100 mg/ml

0/25	1/59	2
0/33	1/81	3
0/17	1/44	4
0/29	1/79	5
0/26	1/68	6
0/18	1/46	7
0/36	1/82	8
0/24	1/55	9
0/25	1/62	MEAN
0/02	0/05	SEM

Weight of liver and spleen in gavaged mice with 1 mg/ml

Spleen's weight	Liver's weight	Number
0/19	1/5	1
0/33	1/68	2
0/21	1/37	3
0/25	1/53	4
0/38	1/87	5
0/40	1/98	6
0/45	2/3	7
0/51	2/44	8
0/34	1/8	MEAN
0/04	0/13	SEM

Weight of liver and spleen in gavaged mice with 10 mg/ml

In survey of effects on healthy mice group with plant extract solvent gavage, results of [Table 2] was seen as below and seen that these mice group, were gavaged for only seven days with plant extract solvent to survey, effect of toxicity of solvent. This group has 27.7 average weight which at the end of seventh day reduce to 25.9.This group was with 9 alive mice at the end of seventh day and the below table conclude weight of spleen and liver of this group at the end of this test. As you can see at chart 1, with respect to no special toxicity was observed at examination test of toxicity in different groups, high dose with 100 mg/ml was selected for patient treatment.

Table 2: weight of liver and spleen in healthy mice group with plant extract solvent gavage

Spleen's weight	Liver's weight	number
0/34	1/64	1
0/42	1/76	2
0/21	1/44	3
0/36	1/64	4
0/12	1/12	5

0/20	1/43	6
0/47	1/77	7
0/43	1/76	8
0/20	1/42	9
0/30	1/55	MEAN
0/04	0/07	SEM

Due to weight changes in control and test groups, chart was drawn with Prism Chart pad software and control and test group was examined from statistical which was accorded to chart number 2 with 95.5 % significant differences in High Dose group.

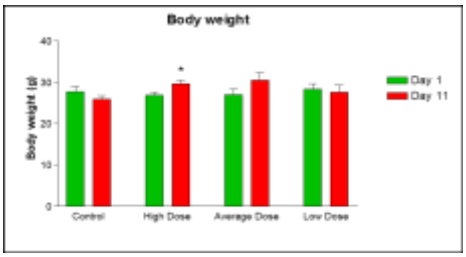

Fig. 1: Chart of toxicity amount in delphinium's extract.

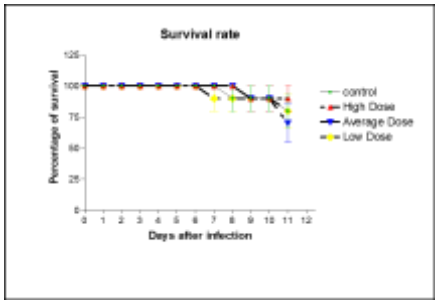

Fig. 2: Chart review of weight change in control and test groups.

Also, comparison of survival percent in control and test group according to chart number 3 which was drawn by Prism Graph pad software, control and test group was statistically analyzed which there was not any significant difference. (n=10mice/group, ANOVA).

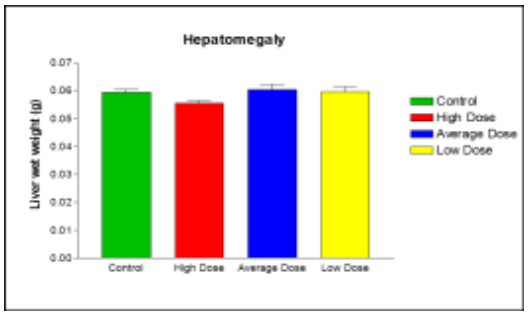

Fig. 3: comparison of survival percent in control and test group.

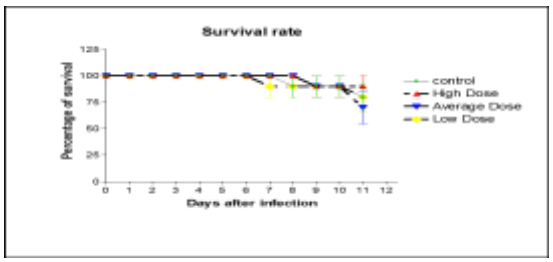

Fig. 4: Chart review of weight change in control and test groups.

Also, in comparison with survival percent in control and test group according to chart number 3 which were drawn by Prism Graph pad, control and test group were analyzed which there was not any significant difference(n=10mice/group, ANOVA).

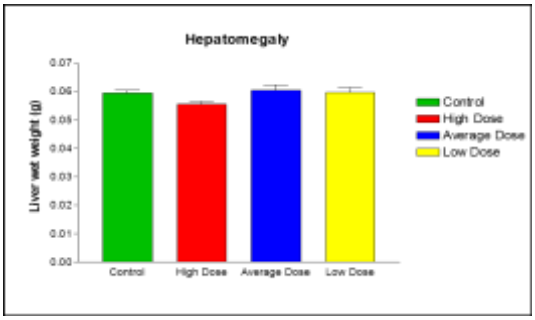

Fig. 5: Comparison of survival percent in the test and control groups.

In hepatomegaly assessment, in control and test groups according to chart number 4, wet weight of liver was considered as an index for hepatomegaly and under Prism Graph pad, control and test group was analyzed statistically which there was no significant difference(n=10mice/group , ANOVA).

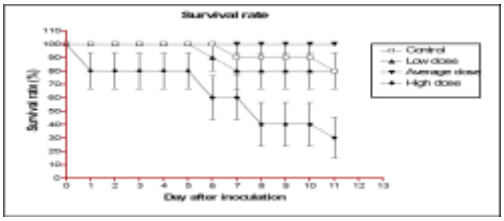

Fig. 6: Hepatomegaly evaluation in experimental and control groups

In splenomegaly evaluation in control and test groups, spleen's weight was examined as an index for splenomegaly and according to chart number 5 which was drawn by Prism Graph pad and control and test group was analyzed statistically and there was not any significant difference. (n=10mice/group, ANOVA).

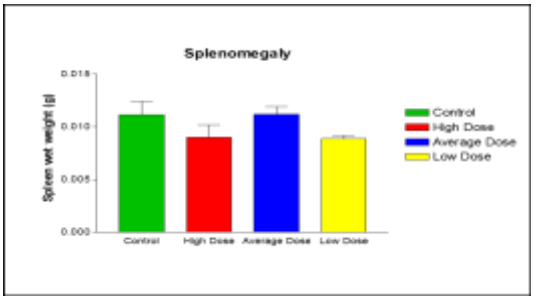

Fig. 7: splenomegaly evaluation in control and test group

In the study, patient mice had pharmaceutical solvent gavge that for 6 days, volume of 10% of ethanol 96 degrees and 90% of physiological saline was feed. In the first and second day of sickness, no difference between these groups of mice and healthy mice were observed. But on the third day of sickness, slight symptoms that include decreased mobility, secluding and lethargy was clear.

On the fourth day, symptoms such as lethargy, secluding and animal concentrating in the corner, not wanting to eat food, flanks going deep, hair bristling, closing the eyelids and sticky stools, more or less in these groups was observed and in the fifth day of sickness, symptoms of sickness was much higher and all mice were secluding in a corner of the cage and in this day, 5 mice were destroyed. On the sixth day, the mice immobilized and on this day,as well as four other mice in this group were destroyed and on the seventh day of study, the last mice in this group were wasted that by using the peritoneal fluid of mice and examining it under a microscope, severe toxoplasmosis cause of death was announced. But in patient mice with high doses of the drug solution 100mg / ml for 6 days with 100 mg ml equivalent to 200 micro liter were gavaged. Average weight of the first day of testing was equal to 34/5 grams and on the first and second day of sickness, there was no difference between the groups of these mice with normal mice but on the third day of sickness, slight symptoms include reduced mobility and secluding and lethargy was

apparent. On the fourth day, symptoms such as animal's lethargy, secluding and concentrating in the corner, not wanting to eat food, flanks going deep, hair bristling, eyelids closing and sticky stools, more or less seen in this group and in the fifth day, sickness symptoms were get more and all mice in this group were secluding on the corner of the cage and one mouse were died. On the sixth day, mice were motionless and six other mice from this group were destroyed. On the seventh day, study of last three mice which were destroyed in this group, with respect to peritoneal fluid providing from these mice and analyzing it under microscope, sever toxoplasmosis cause of death was announced. On the patient mice group lacking treatment which kept in similar conditions like the others groups and was as control patient and no drug neither gavaged nor injected. At the first of our test, average weight was 30.75 gram and at the first and second day of sickness, no difference was between these groups with healthy mice but at the third day of sickness, slight symptoms including mobility reduction, secluding and lethargy was clear. At the fourth day, symptoms including lethargy, secluding, concentrated animal in the corner, not wanting to eat food, flanks going deep, hair bristling, closing the eyelids and sticky stools, more or less seen in this group. In addition, one mouse in this group had vanished. On the fifth day, symptoms was much higher, mice secluded in one corner of the cage and three mice in this group were destroyed. On the sixth day, mice were quite funky and fetide and mice were completely immobile and three others from this group were destroyed.

On the seventh day, the last three mice in this group wasted and due to the peritoneal fluid provided from these mice and examine it under a microscope, acute toxoplasmosis cause of death was announced.
In the study of healthy mice groups and non-gavaged that were kept in similar conditions and other groups were fed the same food and water but were not infected with toxoplasma. No mouse waste and an average weight of the mice on the first day of testing were 15/33 and 25/35 gram at seventh day.

With these findings to examine the effects of delphinium on sick mice for 6 days, 200 ml of the pharmaceutical solution was injected intraperitoneally. On the first day of test, average weight of this group was equivalent to 34 g. In the first and second day of the disease, there was no difference between the groups of mice with normal mice but in the third day, mild symptoms include secluding, lethargy was visible, the fourth day, symptoms such as lethargy and secluding, center concentrating animal, not wanting to eat food, flanks going deep, hair bristling, closing the eyelids was apparent symptoms but on the fifth day, symptoms was much greater and all mice were secluded in a corner of the cage. On this day, 8 mice were wasted. On the sixth day, mice were immobilized and 2 last mice were wasted and by providing the peritoneal fluid of mice and examining it under a microscope, sever toxoplasmosis, cause of death was announced. But in the patient group with intra peritoneal injection of high dose of delphinium for amount of 100 mg / ml to 6 days Just intra peritoneal injection to an amount equivalent to 200 ml with an average weight of 34/3 gram on the first day of testing.

In first and second day of sickness, no difference was between these mice group with healthy mice. But at third day of sickness, slight symptoms of sickness include, reducing mobility, secluding and lethargy was visible and on the fourth day, symptoms such as lethargy, secluding, not wanting to eat food, flanks going deep, hair bristling, closing the eyelids was seen and 2 mice were destroyed. On the fifth day, sickness symptoms were much higher and all mice were secluding on the corner. In this day, 4 mice wasted. At the sixth day, mice were immobilized and the 4 last mice wasted too that according to providing the peritoneal fluid from these mice and analyzing them under microscope, severe toxoplasmosis was announced the cause of death. In patient mice lacked treatment which kept in similar conditions and were considered as control for patients and for this group, no drug was injected. Average weight at the first of test was equivalent to 33/9 gram and at the first and second day of sickness, there were not any difference between these mice with healthy mice. But at the third day of sickness, slight symptoms of sickness include reducing mobility, secluding and lethargy was visible and at the fourth day, symptoms such as lethargy, secluding, center concentrating, not wanting to eat food, flanks going deep, hair bristling, closing the eyelids was seen.

At the fifth day, sickness symptoms were much higher and all mice were secluding at the corner and 6 mice were wasted.

At the sixth day, mice were immobilized and 4 last mice wasted and according to peritoneal fluid providing of these mice and analyzing it under microscope, cause of death was announced toxoplasmosis.

In healthy and patient group which lacked of intra peritoneal injection that kept at similar condition such as other groups and feed similar water and food but they were not infected to toxoplasma parasite, no mice were wasted because of animal house's condition and work condition and average weight of these mice at the first and sixth day were equivalent to 33/8.

DISCUSSION

In a study, the amount of activity and impact of Consolida oliveriana plant flavonoids in the trypanosome cruzi intracellular and extracellular forms was studied by Samira Boutaleb-Charki and colleagues that showed acetylated flavonoid compounds are potent inhibitors of epimastigote epidermal growth with similar activity of benzidazol [13]. In another study, anti plasmoid activity of Atisinium chloride from Aconitum orochyseum plant was studied by Phurpa Wangchuk and his colleagues that this study provide first evidence in support of one of the treatments shown in Greece traditional medicine that these

alkaloids also shows a new potential anti-malarial structure [14]. Doroudgar and colleagues (2007) at a study surveyed concentration of 1, 3 and 5 percent of ethanol plant Artemisia plant hydroalcholic emulsion (Artemisia sieberi) on the wounds that result from leishmaniasis major in BALB/c mice and concluded that the extract of Artemisia in used concentration is not effective on leishmaniasis major and microscopic examination of samples taken from mice under studied were positive.

In another study, flavonoid of Delphinium staphisagria plant on leishmania by Inmaculada Ramirez-Macias and colleagues were studied that studies showed that flavonoid derivatives are active on leishmania infantom and leishmania braziliensis [16]. In a study that was reviewed by Diaz and colleagues, cytotoxic activity of flavonoid derivatives of Consolida oliveriana plant that kaempferol tetra-acetate , quercetin penta acetate, trifolin hepta acetate, considerable toxicity showed[17]. In a study that was for determining for 3 species D. D. glaucum, D. barbeyi and. occidentale from Delphinium family by James A. Pfister and colleagues was done, they resulted that D. glaucum have more toxicity in 3 species. In a study that was done by Grandez and colleagues, four alkaloids olivimine, olividin, 8-O-methylcolumbianine, 7alpha-hydroxycossonidine were separated from atmospheric organ [18].

In another study, alkaloids antioxidant activity of Delphinium linearilobum plant was done by Kolak Ufuk, in this study that spectroscopy technique was used for determination of adversity and structure. In study that was done by Zhou and colleagues, they could to separate three new alkaloids trifoliolasine A (1), trifoliolasine B (3), trifoliolasine C (5)for the first time from the Rannunculaceae family and they grow in china province(10). Khosh zaban and colleagues (2008) in a research reviewed the effect of peganum aqueous extract on severe toxoplasmosis on BALB/c mice and they treat mice with toxoplasma gondii parasite RH strain which were infected as subcutaneously after 24 hours for 7 days. Result of this study showed that peganum extract lead to more survive on animal infected to toxoplasma. In this study, best dosage of increase from the survive time was 300 micrograms/kilograms of mice weight from peganum aqueous extract [19].

In another study, aqueous and alcoholic extract of Piper nigerum ، fratescens
Capsicum ، Curcuma longa , Cinnamomum cassia plants in 100 milligram/kilogram and 200 milligram/kilogram on day was reviewed on the swiss albino mice which was infected from intraperitoneal with 2× ⟦10⟧^2 of tachyzoite for 7 days and it was observed that all treated mice were wasted until seventh day. On the eighth day, existence tachyzoites in peritoneal was counted and compared to patient control group that based this, the Curcuma longa had best effect of alcoholic extract that 100 milligram/kilogram dosage and 200 milligram/kilogram dosage on a day, respectively, 98/6% and 99/2% they had parasites prevention [20]. In a study that was done by Roostaeean and colleagues, anti-malaria activity of Artemisia diffusa plant extract on Plasmodium berghei on malaria mouse model, in vivo ,was surveyed which showed that Artemisia diffusa plant extract can be for growth inhibitor of Plasmodium berghei in mouse body. In a study, activity amount and flavonoid effect of Consolida oliveriana plant on leishmania was studied by C.Marin, S. Boutaleb-Charki and colleagues that showed flavonoid compounds can be growth inhibitor promastigote and leishmania amastigote which flavonoid acetyl compounds can be more active.

In a study which was by Clotilde Marin and his colleagues on laboratory condition on trypanocidal activity from nine separated flavonoid oboveground organs of 'Delphinium staphisagria plant in both acute and chronic phase of chagas disease was studied. Anti-proliferation activity of this material against trypanosome cruzi on the epi mastigote, amastigote and tripomastigote forms was in some cases had stronger activity of anti trypanosomatid and lower toxicity of reference drug, benznidazol.[25]. Khoshzaban and colleagues(2007) in a watery extract effect and garlic pill reviewed on sever toxoplasmosis on BALB/c mouse and in this study, to severe infection in mice intraperitoneal 10000 tachyzoites of toxoplasma gondii of RH strain was injected to mice and 24 hours after infection, treatment started and continued for 7 days and observed that control mouse group died after injection of toxic dosage of parasite along 4 to 5 days while 100 percent of treated mice was alive until fifth day and the result of this study showed that garlic extract lead to survive increase in infected animals with dosage that made it to toxoplasma and the best effective dosage in the case of survive time of 200 milligram/kilogram of mice weights from garlic extract.

CONCLUSION

Generally, based on this study result, with considering results of the other studies in the case of medicinal plants on parasites cases, can concluded that in this study, to study the effect of delphinium plant on severe toxoplasmosios, at first, 40 healthy mice was classified into 4 groups. To one of groups, only drug solvent was gavaged. Three remained group respectively, with solution of plant with 1,10,100 milligram/liter were gavaged. Result of drug toxicity in one week was not considerable. Therefore, according to statistical results we reach to this resultant that plant extract was lack of toxicity and did not effect on liver and spleen of healthy mice.

Then, in the next study, 40 mice were classified on four groups and one group was considered as healthy control group. 30 other mice were infected to toxoplasma gondii RH strain. One control patient group (lack of treatment), one patient group with plant high dosage solution of treatment and the last patient group had pharmatical solvent gavage. After 7 days, only healthy group was remained that showed delphinium does not have considerable effect on toxoplasma parasite as the survive of infected mice in group under

treatment with plant was not considerable in compare to patient mice. It means that plant extract was not able to dextroy the parasite tachyzite or inhibit from their proliferation and at last in the last study, 40 mice was classified in 4 groups. One group was as healthy as a control. 30 other mice were infected to toxoplasma gondii RH strain. One patient control group lack the treatment, one group of patient had treatment with high dosage of plant solution was as intra protenial injection and the last patient group had drug solution as injection in intra pretonial. After 7 days, only healthy group was survived that shows delphinium lack of considerable effect on toxoplasma parasite. As the survive of infected mice in group which was treated with drug was not considerable in comparison with patient mice. It means that delphinium was not able to destroy the tachyzoits of parasites or inhibit their proliferation.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

These researchers appreciate the Islamic Azad University, Pharmaceutical Sciences branch

FINANCIAL DISCLOSURE

None

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