

Antigiardial Activity of Garlic (*Allium Sativum*) on White Mice

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Abstract

This study aims to determine the effect of cold water, hot water and alcoholic extracts of *A. sativum* on intestinal flagellate, *G. lamblia*. The oral administration method is used for one week to evaluate the effect of these plant extracts. mice are divided to several groups, each group administrates one type of plant extract. Increasing doses are tested. Another group administrates a vermifuge drug, metronidazole. The control group was administrated an equal amount of normal saline. The oral administrated is achieved twice a day for all mice groups.

The results showed that garlic cold water extract is the best, it exerted significant effects on *Giardia lamblia* without any side effects. Total serum bilirubin, total serum protein and plasma fibrinogen were determined as measurements connected with liver functions to investigate liver change due to treatment with the present plant extracts. Packed cell volume and hemoglobin level also determined. In these aspects, no significant different is noted in the treated mice compared with control group.

Key word : *G.lamblia*, *Giardia lamblia*, *A.sativum*, *Allium sativum*

الخلاصة

تهدف الدراسة الحالية الى تحديد تأثير المستخلص المائي الحار و البارد والكحولي لنبات الثوم في طفيلي الجيارديا لامبليا في الجسم الحي وقد استخدمت طريقه التجريب الفموي للفئران لمدة اسبوع واحد لملاحظة تأثير المستخلص . حيث قسمت الفئران الى مجاميع كل مجموعه جرعت نوع واحد من خلاصة النبات كما جرعت مجموعة من الحيوانات علاج الميترانيدازول وجرعت مجموعة السيطرة محلول الملح الفسلجي علما بانه تم تجريب كل المجاميع مرتين يوميا .واظهرت النتائج ان المستخلص النباتي البارد كان افضل وقد اثر معنويا في طفيلي الجيارديا لامبليا بدون اي اعراض جانبية, وقد حدد كل من البليروبين الكلي في المصل والبروتين والبلازما كمعايير لقياس كفاءة وضايف الكبد وملاحظة تأثير المستخلص خلال فترة المعالجة كما تم تحديد حجم الخلايا المرصوفة ومستوى خضاب الدم وقد لوحظ عدم وجود تغيرات معنوية لمجاميع الفئران المعالجة مقارنة مع مجموعة فئران السيطرة .

الكلمات المفتاحية: الجيارديا لامبليا، نبات الثوم

Introduction

Giardia lamblia, one-celled flagellated protozoan has a simple life cycle consisting of two stages: trophozoite and cyst. It considered one of the most common intestinal parasites in the world, occurring in both industrial-ized and developing countries with an estimated 2.8 million new cases annually. The pathogenesis of *Giardia lamblia* infected mainly responsible for diarrhea and anorexia especially in children rather than adults (Appelbee *et. al* , 2012).

Metronidazole the most widely employed drug for treating Giardiasis, its use can lead to drug resistance and potential risks of mutagenicity and carcinogenicity. In addition, its side effects such as headache, dry mouth, metallic taste, glossitis, and urticaria caused by lengthy treatment or high doses have also been described (Mosby,2011).Therefore, new anti-protozoal drugs with high effectiveness and low toxicity are urgently required for treatment of these parasitic protozoa. Medicinal plants used in the treatment of these diseases can be an alternative resource for novel anti-protozoal drugs. The use of *Allium sativum* plant including shallot, garlic and onion as medicinal plants predates written history (Hann,1996) Evidences of their potency against bacteria, fungus, viruses, protozoa, helminthes, tumors and thrombosis were proven (Milner,1996). The most important chemical compounds of garlic are the organosulphur compound including allicin which was thought to be responsible for these properties.*A. sativum* or garlic has been used as both food and

medicine in many cultures for thousands of years, dating at least as far back as the time that the Giza pyramids were built. It has been recognized not only as a spice but also as a substance which exerts a control on microorganisms (Hariss,2001)

A.sativum is remarkable for a number of potentially active chemical constituents. It contains seventeen amino acids as arginine, at least 33 organosulphate compounds as aliin and allicin, eight minerals (germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc), enzymes as allinase, and the vitamins A, B1 and C. The physiological activity of dietary *A. sativum* is attributed to allicin (diallyl thiosulphinate), which is one of the organosulphate compounds found in the bulb. It is responsible for the anti-microbial properties and the characteristic flavor of fresh garlic(Ayas,2008).

Materials and methods

Plant extract :

-Water extract preparation:

Garlic cloves were blended with water in a ratio of 1:2 (garlic: distilled water (Chowdhury,2008). The mixture was first filtered with filter paper through a Buckner funnel, then centrifuged at 3,500 rpm for 30 seconds, then the supernatant were put in oven at 60C° for dried and then weighted extract which used to preparation and obtaining the suitable concentrations. Hot extract was prepared by using boiled distilled water.

-Alcoholic extract preparation:

Method of (Harborn,1985) was used to prepared alchohlic extract of garlic by putting garlic cloves in thimbles of soxhlet extractor with ethylic alcohol in a ratio of 1:5 (garlic: ethylic alcohol) for 24 hours then the extract was transformed to oven with 60C° in order to evaporate alcohol. Finally the extract was kept in refrigerator until used.

Laboratory animals:

In this study used Mice Balb\c (6-8 weeks and weight 20-25 gm), mice were infected with *Giardia lamblia* through intraesophageal . After that, direct smear technique was used to test the infection of mice with *Giardia lamblia*.

-Administration of laboratory animals :

In this experiment we used 50 mice and grouped into two groups, first group with 25 mice administrated *A. sativum* watery extract with doses (600,1200,1800 and 2400) mg\kg and normal saline (0.85%)of mice weight for each five of them. The second group of mice (25) was also administrated with 300, 600, 900 and 1200 mg\kg of *A. sativum* alcoholic extract and metronidazole(20mg\kg). Oral administration was achieved twice a day for all mice groups.

5-Statistical analysis:

Analysis of variance (F) and Chi-square was used to analyze data, Probability values less than 0.05 and 0.01 were considered statistically significant (Niazi,204).

Results and discussion:

-Mice tolerance to water extract of *A.sativum*:

As shown in table 1 and 2 the mice tolerate to the cold and hot watery extracts of *A. sativum* until dose 2400mg\kg, this may be due to low toxicity of cold and hot extracts. (Al-Mosawy,2000) confirms that mice tolerate water extract of *A. herba-herba* in 2000 and 4000 mg\kg for ninth day of administration.

Table (1): Mice tolerance to cold water extract of *A.sativum*.

Dose(mg/kg)	Number of a live mice after:			
	One day	Three days	Five days	Seven days
600	5	5	5	5
1200	5	5	5	5
1800	5	5	5	5
2400	5	5	5	5
Normal saline (0.85%)	5	5	5	5

Table (2): Mice tolerance to hot water extract of *A.sativum*.

Dose(mg/kg)	Number of a live mice after:			
	One day	Three days	Five days	Seven days
600	5	5	5	5
1200	5	5	5	5
1800	5	5	5	5
2400	5	5	5	5
Normal saline (0.85%)	5	5	5	5

-Mice tolerance to alcoholic extract of *A.sativum*:

table (3) showed results of administration of alcoholic extract of *A. sativum* , where mice tolerated the doses 300, 600 and 900 mg/kg of body weight don't caused death for mice while administration of dose 1200mg/kg caused the death of one mouse in 5th day, also another mouse died in 7th day of administration. That was due to effect of component of *A. sativum* extract. Garlic contains a higher concentration of sulfur compounds such as alkaloids and phenols which are responsible for its effects (Al-Rawi,1988) .

Table (3): Mice tolerance to alcoholic extract of *A.sativum*.

Dose(mg/kg)	Number of a live mice after:			
	One day	Three days	Five days	Seven days
300	5	5	5	5
600	5	5	5	5
900	5	5	5	5
1200	5	5	4	3
Normal saline (0.85%)	5	5	5	5

F calculate for doses: 0.98

F calculate for days: 2.45

F tabled at 0.01= 5.95

F tabled at 0.05= 3.49.

-Effect of *A. sativum* water extract on *G.lambli*a in mice:

The results in table (4) showed that cold extract was active in killing *G.lambli*a at 2400mg/kg of mice body weight, this attributed to Garlic contains at least 33 sulfur compounds, several enzymes,17 amino acids, and minerals such as selenium. It contains a higher concentration of sulfur compounds than any other *Allium* species. The sulfur compounds are responsible for garlic's pungent odor and many of its medicinal effects. It also contains approximately 1% alliin (S-allyl cysteine sulfoxide).One of the most biologically active compounds, allicin(diallyl thiosulfinate

or diallyl disulfide) does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes alliin to allicin. Allicin is further metabolized to vinyldithiines. This breakdown occurs within hours at room temperature and within minutes during cooking (Kemper,2011)

Table (5) showed results of hot extract of *A. sativum* which also effective against *G.lamblia* especially when used in dose (2400 mg/kg) but its less effectiveness than cold extract, this may be due to destruction of some active compounds in heating. (Musleh,1995) Confirms that heating had an effect on activity of *A. sativum* against *Pseudomonas aeruginosa*. (Kiesewetter,2013) show that hot extract of *A. sativum* was less effectiveness in inhibition of cyclooxygenase which cause thrombosis compare to the cold extract.

Table (4): Effect of cold watery extract of *A. sativum* on *G.lamblia* in mice.

Dose (mg/kg)	number of <i>G.lamblia</i> in mice after:			
	One day	Three days	Five days	Seven days
600	103	60	29	15
1200	129	65	30	11
1800	114	31	16	3
2400	120	25	12	2
Normal saline (0.85%)	105	100	95	100

F calculate for doses: 47.32

F calculate for days: 21.61

F tabled at 0.01= 5.95

F tabled at 0.05= 3.49.

Table (5): Effect of hot watery extract of *A. sativum* on *G.lamblia* in mice.

Dose (mg/kg)	number of <i>G.lamblia</i> in mice after:			
	One day	Three days	Five days	Seven days
600	95	80	56	20
1200	107	72	47	15
1800	100	54	22	8
2400	112	39	19	4
Normal saline (0.85%)	105	100	95	100

F calculate for doses: 14.32

F calculate for days: 22.6

F tabled at 0.01= 5.95

F tabled at 0.05= 3.49.

-Effect of alcoholic extract of *A. sativum* on *G.lamblia* in mice:

As shown in table (6), the increasing doses of *A. sativum* alcoholic extract was very active in killing *G.lamblia* in vivo, this belongs to concentration of active compound in alcoholic extract compared to watery extract. the antiparasitic activity of *A. sativum* is mainly due to the presence of allicin produced by the enzymatic activity of allinase on alliin. Allicin is considered to be the most potent antibacterial agent in crushed garlic extracts. the mechanisms by which garlic inhibit *G.lamblia* is attributed to the sulphur-containing compounds especially allicin, that shows variety of anti-microbial activities (Ankri,2009) . The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase. (Soffar,2009) used *A. sativum* extract in treat children who infected with *Hymenoleps nana* and so the extract shows strong effect in treatment.

Table (6): Effect of alcoholic extract of *A. sativum* on *G.lambli* i in mice.

Dose (mg\kg)	number of <i>G. lambli</i> i in mice after:			
	One day	Three days	Five days	Seven days
600	90	80	65	16
1200	85	50	25	16
1800	100	70	20	6
2400	110	40	15	2
Normal saline (0.85%)	105	100	95	100

F calculate for doses: 31.9

F calculate for days: 22.8

F tabled at 0.01= 5.95

F tabled at 0.05= 3.49.

-Effect of *A. sativum* water and alcoholic extracts on haematocrit, haemoglobin and some biochemical blood parameters connected with liver function in mice:

As shown in table(7) there is no change in level of haematocrit and haemoglobin in mice that are treated with water and alcoholic extract of *A. sativum* and metronidazole (2400,1200and 20) mg\kg respectively compared to the control group which is treated with normal saline. These may be retain to plant extracts do not effect on red blood cells production,This results agree with (AL-Mosawy,2000) who confirm that the level of hematocrit and Hb remains constant in mice given water and alcoholic extract of *A. herba-herba*. There are also no significant difference in serum total bilirubin and serum total protein in mice when treated with plant extract and metronidazole. Whereas level of plasma fibrinogen slightly increases in mice treated with waterand alcoholic extract of *A. sativum* and metronidazole(2400, 1200 and 20)mg\kg respectively. This is duo to aggregation of chemical compound found in extracts. This results agree with (Ankri,2009) who found increasing in plasma fibrinogen in mice treated with lipopolysaccharides.

Table(7): Effect of *A. sativum* water and alcoholic extracts on haematocrit, haemoglobin and some biochemical blood parameters connected with liver function in mice:

Dosage of treatment	Haematocrit (%)	Hb (g\100ml)	serum total bilirubin (mg\100ml)	Serum total protein(mg\ml)	Plasma fibrinogen (mg\100ml)
Cold water extract (2400mg\kg)	43.1	14.2	1.0	5.9	6.8
Hot water extract (2400mg\kg)	40.9	14.0	0.9	5.1	5.9
Alcoholic extract (1200mg\kg)	42.5	14.5	0.8	6.3	7.9
Metronidazole (20mg\kg)	40.0	14.1	1.1	5.2	8.2
Normal saline (0.85%)	39.4	13.2	0.7	5.0	6.2
Calculated X ²	0.066	0.009	0.022	0.043	0.221

X² tabled at 0.01= 16.59X² tabled at 0.05= 12.59

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