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EFFECT OF ALCOHOL EXTRACT OF GREEN TEA PLANT *CAMELLIA SINENSIS* AS A THERAPEUTIC TREATMENT OF PARASITE *ENTAMOEBA HISTOLYTICA*

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Abstract

This study aimed to investigate the effect of various concentrations of the extract of the green tea plant *Camellia sinensis* on the parasite causing the bloody diarrhea *Entamoeba histolytica* in laboratory mice where the extract of alcohol for green tea concentrations (100,150) mg/kg of the weight of the mouse has been used for 10 consecutive days It was found that the effect of the 100 mg / kg green extract of green tea reduced to decrease the number of cysts in the mice stool with therapeutic efficiency (84%) on the tenth day of treatment, while the effect of 150 mg / kg was the highest therapeutic efficiency on the fifth day of treatment (86.6%) either in the day 10 was equal with 100 mg / kg concentration (84%), indicating that the two concentrations had a reduced susceptibility and could be used as a treatment against amoebic dysentery.

Key words : Amoebic dysentery, alcohol extract, green tea plant, therapeutic treatment.

Introduction

Entomoeba histolytica is an infection of the intestinal tract that causes the human disease known as amoebiasis, whether in the appearance of symptoms or not. The disease of amoebic dysentery is widespread in the tropics and sub-tropics, which suffer from poor health and living conditions (Pham *et al*., 2011). Amoebic dysentery comes third in terms of Mortalites parasitic diseases after Malaria and schistosomiasis (Samie *et al*., 2012).

Amoebic tissue settling in the large bowel cavity of the human because of its organic matter and types of bacteria that provide the environment suitable for feeding and growth and multiply these amoeba. The life cycle of the parasite is direct and the infection is transmitted from water and food contaminated with the mature cysts. Excystation releases the trophozoites phase that invades the mucous layer of the large intestine (Ralston and Petri, 2011). The pathogenic nature of the amoebic tissue causes ulceration in its walls of large intestine and this deep ulcer may be lead to peritonitis, which in turn leads to death (Shannon *et al*., 2013). The parasite may infect other organs of the body such as the lungs, brain and skin. The most common symptoms associated with infection are severe cramping, dysentery, fatigue, ulcer ulcers with abdominal pain in the lower right quadrant of abdomen, tenesmus, and bloody stool with mucus. intestinal amoeba has a startling start, symptoms and various signs, which makes diagnosis difficult, especially when fever and bloody diarrhea are not present in most cases (Ximénez *et al*., 2011).

Amoeba notes in faeces in the following forms: vegetative phase or trophozoite, precyst and cyst, the infectious phases where these cysts reach the human through Water and food contaminated by flies or food brought in contaminated hands (Pham *et al*., 2011).

Amoeba is spread all over the world and depends on the environmental conditions and the extent of care for cleanliness, where there is a lot of slums and places and poor and lack of sanitary and public conditions and lack of drinking water or lack of availability and the washing of fresh vegetables as well as the introduction and use of human waste and water discharges and excuses treated as fertilizer to many of farms (Pham *et al*., 2011).

Due to the use of traditional medicines against this disease, which has a clear side effects, the study aimed to use vegetable extracts, green tea and different concentrations as a therapeutic attempt against the disease of amoebic dysentery. Medical plants are an alternative treatment in acute cases of infectious diseases, and can be counted as a potential source of effective new antibiotics, with no resistance from pathogenic strains. The most prominent of these plants Medicines are those used in the traditional medicine of peoples;So it is necessary to evaluate the possibility of using these plants on the basis of scientific treatment of infectious diseases resulting from common pathogens so researchers sought to extract active substances in natural plants to become an important source for the manufacture of drugs and green tea plant one of the most important medicinal plants used in the treatment of many cases Such as cardiovascular disease and certain types of cancer, promote oral health, reduce high blood pressure, help control body weight, possesses antibacterial properties and viruses, offers protection against ultraviolet radiation, and increase bone mineral density (Cabrera *et al*., 2006), because it contains active substances such as flavonol, which strengthens the immune system and caffeine, which strengthens the heart and strengthens the central nervous system and other essential substances act as antioxidants oxidation such as carotene, magnesium, polyphenols and selium (Kishi and Matsuoka, 2010).

Materials and Methods

Collection of plant specimens

Green tea has been obtained from local markets in Samarra in a powdered form of dried leaves stored in a 5 kg bag. This type of Chinese green tea was filled in Syria - Damascus and proved that *Camellia sinensis* is the current scientific name of green tea , As this plant was classified by a specialist in the science of plant classification.

Alcoholic extraction of green tea *Camellia sinensis*

The green tea leaves were directly transferred to fine powder using an electric grinder and then followed (Alzarvi, 2003) to obtain the extract. 50 g of dry powder was weighed and placed in a glass cylinder with 1000 ml and the distilled water was added and the final volume was completed to 1 liter and left for half an hour. In the Horizontal Shaker and at a medium speed of 35°C. The sample was left to settle for 24 hours, after that which it was filtered with three layers of gauze to separate the large plankton and then using the centrifuge at 3000 rpm for 15 minutes to separate the small plankton, then the starter was filtered using filter paper Whatman N. 0.1 Then the water was evaporated from the extract under low pressure at temperature 40^0C using machine Vacuum Evaporatory, then save the extract after drying in dark glass bottles with covers of the court and in condition without moisture, and so were obtained extract powder the placed in incubater at temperature 37^0C for 48 hours for dry powder extract then placed in sealed tube with aluminium foil and kept in the refrigerator at 4^0C untile use.

Preparation of reagents used

1. Reagents of alkaloid

The Dragendroff Reagent detector was present according to its method (Mahmoud, 2008). The Mayer Reagent and Wagner's Reagent were attended by Salem (2015), Hager Reagent and Tannic acid Reagent (Harborne, 2005).

2. Flavonoids Reagents

Includes several statements Shinoda's Reagent, Ferric Chloride Reagnt and Zinc-Hcl Reduction. Lead Acetate Reagent (Harborne, 2005). Sodium Hydroxide Reagents Alkaline Reagents (Roopashree *et al.*, 2008).

3. Steroid Reagents

Salkowski Reagents (Edeoga, 2005). Bühner Liberman - BurChards Reagents (Majaw and Moirangthem, 2009; Al-Abid, 1985)

4. Carbohydrate reagents carbohydrates reagents

Anthron detection and Molish detection and Benedict detection (Harborne, 2005)

5. Glycosides Regents

Keller-Kiliani's Reagent, Sodium Hydroxide Reagent and Terpenes Reagent (Al Sheikhly *et al*., 1993).

6. Phenolic compounds and Tannins Reagents

Ferric Chloride Reagent ,Gelatin Reagent and Lead Acetate Reagent Detection (Harborne, 2005; Al-Janabi, 2014).

7. Saponins Reagnt

Mercury Detector Mercuric Chloride Reagents, Quinones Reagent (Salem, 2015).

8. Detection of Resins

The method followed in Al-Janabi (2014)

9. Detection of Coumarines

Detect coumarin as stated in Al-Janabi (2014)

10. Detection of volatile oils :

The method of detection of essential oils was adopted as reported in Indian Herbal Pharmacopoeia (Indian,

1998).

Laboratory animals

The mouse was used by the Mus Musculus Balb laboratory, which was obtained from the pharmacology lab in the General Company for the manufacture of pharmaceuticals and medical supplies in Samarra. The mice were examined to ensure that they were free of parasite cysts. The number of 20 mice was divided into 4 groups and each group consisted of 5 mice. They weighed between 20-25 g and were 4-5 weeks old. Cages were placed in the laboratory animal room at a temperature of 25^0C. The cages were cleaned and the brush changed daily.

Samples of parasite

The *E. histolytica* parasite samples were obtained from the reviewers and residents of Samarra General Hospital, who suffer from severe to moderate diarrhea and in most cases have hemorrhagic diarrhea. The samples were diagnosed in the manner used by (Singh *et al*., 2009) and the parasite was isolated by (Clark and Diamond , 2002) The dose was determined by calculating the number of cysts in the size of (0.1) ml and the dose was determined by 1 × 10 sachets infection dose for each mouse taken orally. After the injection dose was determined, the mice were vaccinated with parasite cysts Oral examination of these bags in the infected mices stools daily and for three days after, the infection was confirmed by the infection by preparing several swabs from infected faeces on a glass slide and examined under a microscope and watching the parasite and its various stages by using Various dyes such as local iodine dye and its diuretic solution.

Design of experience:

20 mouse were used and divided into four groups:

Group 1 : positive control group was injected with parasite

Group 2 : negative control group left untreated

Group 3 : Treatment group with alcohol extract of green tea at a concentration of 100 mg / kg

Group 4 : Treatment group with alcohol extract of green tea with a concentration of 150 mg / kg.

Method of calculating the number of cysts in the faeces

The faeces are collected every 24 consecutive hours according to method (Ghazal, 1974) by placing the mice used for this purpose in a clean cage containing a small amount of sawdust. The faeces is weighed by a sensitive electrical balance and It is taken from 0.2 g and placed in a test tube containing 5 cm 3 of normal water and left in Moisturize for half an hour and then mix with a stick of wood,the solution is filtered by 6 layers of gauze and then completed to 10 cm 3 by distilled water. The leachate is placed in the centrifuge for 1000 rpm for 10 minutes, then a drop is taken from the surface of the solution and placed on a thick glass counting chamber. The number of cysts is calculated in two squares, And we get the number of cysts in grams stain.

$$\text{No. of cysts in 1 gram} = \frac{\text{No. of cysts into two chamber}}{\text{faece weight sample}}$$

Evaluation of therapeutic efficacy

The therapeutic efficiency of the alcoholic extract of green tea was calculated according to the following equation according to researcher (Xiao and Ring, 1996).

$$\text{Therapeutic efficiency} = \frac{\text{Average no. of eggs cysts in control group} - \text{Average no. of eggs cysts in infected group}}{\text{Average no. of eggs cysts in control group}}$$

Results and Disscution

Experimental infection results

The results of the laboratory infection of the mice in the parasite cysts with a dose of 1×10^3 cysts that these animals are very sensitive to infection, where it was 100% and was observed through a microscopic examination of faeces in a direct smear and concentrated methods of infection and the cysts appeared after 3 days of injury where the signs appeared Clinical trials on mice were experimentally programmed as of the second day after infection, included inactivity, And increased symptoms on the third day, represented by anorexia, increased drinking water, and increased defecation, and increased the number of cysts of eggs from the third day these results correspond to those reached with Al-Janabi (2014) and disagreed with what (Stanley, 2004) who observed the don't occurs by injecting them into the parasitic cysts of the parasite that natural infection occurs only in humans.

Chemical qualitative detection of active compounds in green tea extract

The results of the chemical extracts of the green tea extract on the following compounds showed in table 1 alkaloids, saponins, flavonoids, phenols, glycosides, tannins and terpenes. These compounds have anti-bacterial activity. This is in line with what was stated (AlSaddh, 2003; Salem, 2015; Singh and William, 2009).

Table 1 : The active compounds in the alcoholic extract of green tea.

The alcoholic extract	The active compounds
+	alkaloids
+	flavonoids
+	carbohydrate
+	glycosides,
+	terpenes.
+	tannins
+	phenols
+	quinones
+	coumarines
+	resins
+	saponins
-	steroids

Green tea extract result

Table 2 showed a difference between the two concentration used in the treatment of mice programmed with amoeba parasites. The number of parasite cysts decrease after the first treatment day in concetration100 mg / kg with therapeutic efficacy (61.1%). The concentration of 150 mg / kg showed a higher therapeutic efficiency (64.4%).

Table 2 : Therapeutic efficiency of the green extract of green tea at a concentration of 100,150 mg / kg on the first day after treatment.

Concentration of the extract	No. of cyst after 1st day	Therapeutic efficiency %
100	3500	61.1
150	3200	64.4
Negative control group	9000	

Table 3 show decreased the number of parasite cysts after the second treatment day with a concentration of 100 mg / kg with therapeutic efficacy (68.7%), while the concentration of 150 mg / kg showed a higher therapeutic efficiency of 75.8% .

After the third day of treatment, parasite cysts continued to decrease and continued to increase the therapeutic efficiency as shown in table 4. The number of parasite cysts decreased after 3 days of treatment at 100 mg / kg with therapeutic efficacy (73.9%). The concentration of 150 mg / kg showed Highest therapeutic efficiency (83.4%).

After the fourth day of treatment, parasite cysts continued to decrease and continued to increase the therapeutic efficiency as shown in table 5. The number of parasite cysts decreased after 4 days of treatment at

Table 3 : Therapeutic efficiency of the green extract of green tea at a concentration of 100,150 mg / kg on the 2nd day after treatment.

Concentration of the extract	No. of cyst after 2nd day	Therapeutic efficiency %
100	3500	7.16
150	2700	75.8
Control group	11200	

Table 4 : Therapeutic efficiency of the green extract of green tea at a concentration of 100,150 mg / kg on the 3rd day after treatment.

Concentration of the extract	No. of cyst after 2nd day	Therapeutic efficiency %
100	3500	7.16
150	2700	75.8
Control group	11200	

Table 5 : Therapeutic efficiency of the green extract of green tea at a concentration of 100,150 mg / kg on the 4rd day after treatment.

Concentration of the extract	No. of cyst after 4th day	Therapeutic efficiency %
100	2700	74.2
150	1600	84.7
Control group	10500	

100 mg / kg with therapeutic efficacy (74.2%). The concentration of 150 mg/kg showed Therapeutic efficiency reached (84.7%).

The number of parasite cysts decreased after 5 days of treatment with a concentration of 100 mg / kg with therapeutic efficacy (73.3%). The concentration of 150 mg/Kg showed a therapeutic efficiency reached (86.6%).

Table 7 shows a decrease in the number of eggs cysts presented after the sixth day of treatment with therapeutic efficacy (73.3%) for 100 mg/kg concentration and efficacy (81.6%) at 150 mg/kg.

Table 8 shows the continued decrease in the number of egg cysts presented after 7 days of treatment with therapeutic efficacy (76.3%) for 100 mg/kg concentration and efficacy (80%) at 150 mg/kg.

At the eighth day of the treatment, the number of cysts bags shown in table 9 with therapeutic efficiency (78%) was reduced to 100 mg/kg concentration and efficacy (80%) at 150 mg/kg.

When treatment continued on the ninth day, the number of egg cysts shown in table 10 was shown to be efficiently (80%) for both concentrations.

Table 6 : Therapeutic efficiency of the green extract of green tea at a concentration of 100,150 mg / kg on the 5th day after treatment.

Concentration of the extract	No. of cyst after 5th day	Therapeutic efficiency %
100	2400	73.3
150	1200	86.6
Control group	9000	

Table 7 : Therapeutic efficiency of the green extract of green tea at a concentration of 100, 150 mg / kg on the 6th day after treatment.

Concentration of the extract	No. of cyst after 6th day	Therapeutic efficiency %
100	1600	73.3
150	1100	81.6
Control group	6000	

Table 8 : Therapeutic efficiency of the green extract of green tea at a concentration of 100,150 mg / kg on the 7th day after treatment.

Concentration of the extract	No. of cyst after 7th day	Therapeutic efficiency %
100	1300	76.3
150	1100	80
Control group	5500	

Table 9 : Therapeutic efficiency of the green extract of green tea at a concentration of 100, 150 mg / kg on the 8th day after treatment

Concentration of the extract	No. of cyst after 8th day	Therapeutic efficiency %
100	1100	78
150	1000	80
Control group	5000	

Table 10 : Therapeutic efficiency of the green extract of green tea at a concentration of 100, 150 mg / kg on the 9th day after treatment

Concentration of the extract	No. of cyst after 9th day	Therapeutic efficiency %
100	900	80
150	900	80
Control group	4500	

When treatment continued on day 10, the number of egg cysts shown in table 11 was consistently reduced with therapeutic efficiency (84%) for both concentrations.

The previous results showed that both high concentrations of extracts had a significant effect on

Table 11 : Therapeutic efficiency of the green extract of green tea at a concentration of 100, 150 mg / kg on the 10th day after treatment

Concentration of the extract	No. of cyst after 10th day	Therapeutic efficiency %
100	800	84
150	800	84
Control group	5000	

parasite elimination and treatment of the pathogenic effects of the study compared to the medium and low concentrations. The number of outpatient cysts with the faeces decreased. These results were consistent with (Singh and William, 2009) on the effect of green tea extract on Amoeba parasite condition for the tissue, this can be attributed to the effective therapeutic effect of plant extracts and the drug because they contain effective substances such as alkaloids, phenols, tannins, flavonids, glycoasides, saponins, and resins that may eliminate the parasite inside the colon, thus inhibiting the parasite and preventing it from dividing and multiplying within the colon cavity. Thus, the parasite does not continue to cause pathogenicity and severe tissue changes in the colon tissue. Alkaloids act as catalysts for cellular immunity. Caffeine is composed of alkaloids (Richard , 2007), which have the ability to break down the cellular wall (parasitophorous vacuole) and the containment of proteins and fats, and thus the destruction of the parasite, as well as the advantage of alkaloids ability to interfere with DNA, while Tannins can inhibit the enzymes and proteins transport and located in the cell membrane. The mechanism of action of phenolic compounds and tanins is due to their ability to precipitate proteins because of the formation of hydrogen bonds between the groups of hydroxyl phenols, nitrogen compounds and proteins, due to inhibiting necessary enzymes in living organisms.

That green tea contains gallic acid, which creates an environment unsuitable for parasite growth and vitamin A works to strengthen the mucous membranes that help prevent parasitic adhesion to the wall of the colon, while phosphorus builds and supplies energy to the body's cells (USDA, 2003), other reasons that have a great effect on the treatment of amoeba parasites are the presence of zinc, which prevents severe indigestion, the effect of vitamin K, which helps in the healing of incurable wounds and facilitates intestinal passage, preventing severe constipation (Rizk, 2008) thus narrowing the chance adhesion parasite in epithelium cells in intestine opportunity and some studies have confirmed that the use of high concentrations of plant extracts is more efficient than the low concentrations of intracellular

parasites (Al-Masoudi, 2001).

Green tea had clear effects on other parasites and bacteria. The results were consistent with the study of (Salem, 2015) on effect the extracted on *Cryptosporidium paravium* parasites. The study of (Akroum and Lalaoui, 2009; Chakaraborty and Chakraborti, 2010) showed an inhibitory effect for the growth of negative and positive bacteria of gram stain, as the alcohol extract inhibited the growth of bacteria (Aldakele, 2006) that the green tea extract is effective against *Bacillus fastidosus, Staphylococcus aureus*, and this is the case for the treatment of Bacteria negative gram stain such us *Salmonella cholerasuis*.

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