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Study the protective effect of *Matricaria chamomilla* flower extract against the toxicity of *Entamoeba histolytica* induces liver and renal dysfunctions in adult albino male rats

Abeer A. Ali^{1*}, Shahad Abbas Alattar²

¹Technical College of Kirkuk/North Technical University, Kirkuk, Iraq.

²Veterinary Medicine College/Kirkuk University, Kirkuk, Iraq.

Abstract

The present study was designed to show the potential role of *Matricaria chamomilla* flower extract against toxicity of *Entamoeba histolytica*. The study used 20 adult albino male rats that divide randomly to four groups (each group consist 5 rats); control group received ad libidum, group of rats administrated with 1×10^3 cyct/ml suspension for seven days, group of rats administrated with 1×10^3 cyct/ml suspension and treated with 50mg/kg extract for month, group of rats administrated with 1×10^3 cyct/ml suspension and treated with 100mg/kg extract for month. The results show high significant increased ($P < 0.05$) in levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), urea, creatinine and uric acid in rats administrated with 1×10^3 cyct/ml suspension. Oxidative stress factor in this group show significant increased ($P < 0.05$) in levels of MDA (malonedialdehyied) and significant decreased ($P < 0.05$) in levels of glutathione (GSH) and catalase compared with control group. While, after used *M. chamomilla* flower extract with *Entamoeba histolytica*, the results showed non significant changes ($P < 0.05$) in liver and kidneys function parameters. Also, MDA, GSH and catalase showed non significant changes ($P < 0.05$) compared with control group. It was concluded that flower extract has been potential role against toxicity of *Entamoeba histolytica* in adult male albino rats.

Keywords: *Matricaria chamomilla*, *Entamoeba histolytica*, liver function, kidneys function, MDA (malonedialdehyied), glutathione (GSH), catalase.

دراسة التأثير الوقائي لمستخلص ازهار نبات البابونج ضد اميبا الزحار التي تحت ضعف وظائف الكبد والكلى في ذكور الجرذان البيض البالغة

عبير عباس علي^{1*}، شهد عباس علي²

¹كلية التقنية كركوك، الجامعة التقنية الشمالية، كركوك، العراق.

²كلية الطب البيطري، جامعة كركوك، كركوك، العراق.

الخلاصة

صممت الدراسة الحالية لبيين الدور الفعال لمستخلص ازهار نبات البابونج ضد سمية اميبا الزحار. استخدمت الدراسة 20 جرذ ذكر بالغ وقسمت الى اربع مجاميع (كل مجموعة تضم 5 جرذ): مجموعة السيطرة اعطيت حماية طبيعية، مجموعة الجرذان المجرعة 1×10^3 كيس/ملمتر من العالق لمدة اسبوع، مجموعة الجرذان المجرعة 1×10^3 كيس/ملمتر من العالق ومعالجة 50mg/kg من المستخلص النباتي لمدة شهر،

الجرذان المجرعة $10^3 \times 1$ كيس/مليتر من العالق ومعالجة 100mg/kg من المستخلص النباتي لمدة شهر. أظهرت النتائج زيادة عالية المعنوية ($P < 0.05$) في مستويات ALT، AST، ALP، البيريا، الكرياتينين وحامض اليوريك في الجرذان المجرعة $10^3 \times 1$ كيس/مليتر من العالق. أيضا، عوامل الجهد التأكسدي في هذه المجموعة أظهرت زيادة عالية المعنوية ($P < 0.05$) في مستويات MDA وانخفاض عالي المعنوي ($P < 0.05$) في مستويات الكلوتاثيون والكاتاليز مقارنة مع مجموعة السيطرة. لكن بعد استخدام مستخلص ازهار نبات البابونج *M. chamomilla* مع $10^3 \times 1$ كيس/مليتر من العالق، أظهرت النتائج عدم وجود اي تغيرات لمعنوية ($P < 0.05$) في معايير قياس وظائف الكبد والكلية. أيضا مستويات MDA والكلوتاثيون والكاتاليز أظهرت عدم وجود اي تغيرات لمعنوية ($P < 0.05$) مقارنة مع مجموعة السيطرة. يستنتج بان لمستخلص الازهار فعالية ضد سمية اميبا الزحار في ذكور الجرذان البالغة.

Introduction

Matricaria chamomilla (MC) or German chamomile, also spelled chamomile, is one of the most used in medicine field. The plant has been included in the pharmacopoeia of 26 countries [1]. Its found in different countries such as Asia, Australia, North Africa, North and South America [2]. Two species of *Matricaria* are used in herbalism, *Chamaemelum nobile* and *Matricaria chamomilla* [3]. *Matricaria chamomilla* consist of different types of potential compounds including; terpenoids α -bisabolol, polysaccharides, essential oils, mineral elements, fatty acids, flavonoids, and other phenolic [1, 4]. *Matricaria chamomilla* has been different actions in medical line include antibacterial, anti-inflammatory, antifungal, anti-ulcer, antispasmodic, antiviral, and sedative effects [5]. *Matricaria chamomilla* is used internally mainly as sluggish digestion, for diarrhea and nausea; the urinary tract and for painful menstruation. Externally, may be applied to wounds slow to heal, skin eruptions, infections, such as shingles and boils, throat, hemorrhoids, and eyes [2, 6].

Infections by *Entamoeba histolytica* lead to different clinical manifestations and disease, including dysentery, diarrhea, and hepatic liver abscess. Approximately 50 million person in world are affected by *E. histolytica* and approximately 100,000 person die every year in world [7]. Infection by this parasite is occurs when the host ingests cyst phases in contaminated food or water. After that, trophozoites are released in the small intestine, may colonize the large intestine and then may invade through the intestinal epithelium to cause colitis or liver abscesses [8]. trophozoite may be transported and invade by the blood to extra-intestinal locations such as liver, lungs kidneys and brain [9-10].

Materials & methods

Animal model

In this study twenty adult male albino rats, (wt 225-275 gm with age 5-8 month) obtained from Veterinary college/ Kirkuk University, and kept on standard pellet diet and water for two weeks to insure its normal and there aren't any infection.

Plant Extraction

Flowers of *M. chamomilla* were collected locally from different places in Kirkuk city. Dried flowers reduced to a fine powder with grinder. The powder plant (50 g) was put in 500 ml of distilled water, the mixture was concentrated to dryness using a rotary evaporator then it was stored at a temperature of 4°C until use [11].

Entamoeba histolytica

Trophozoite and cyst phases of *Entamoeba histolytica* were collected from feces of patients whose infected with parasite in private laboratory in Kirkuk city. Diagnosis way was done by using microscope according to the way of [12]. The purification of trophozoite and cyst was done according to the way of [13]. The estimated of cyst phases was done by using Hemocytometer. The suspension was prepared for cyst phase and administrated to rats with dose 1×10^3 cyct/ml, the infection of rats was insured by testing the feces of rats to diagnosis trophozoite and cyst by using smear way [14].

Experimental design

Twenty adult male albino rats were used and divided as follow (each group consist five rats):

- I. Rats were received standard pellet diet only for seven days and then killed.
- II. Rats were received 1×10^3 cyct/ml suspension of cyst phase for seven, and then killed.
- III. Rats were received 1×10^3 cyct/ml suspension of cyst phase and then treated by 50mg extract in for month, and then killed.

IV. Rats were received 1×10^3 cyct/ml suspension of cyst phase and then treated by 100mg extract in for month, and then killed.

Prepare of blood solution

The blood collecting from rats by cardiac puncher, under anesthesia, and put in test tubs. After clotting, the tubes were centrifugation for 10 min to obtain sera. The serum was taken and stored by deep freezing until used.

Homogenization

Organs (liver & kidneys) were removed immediately and the put in glass dish contents 0.9% NaCl buffer for washing and removed the blood. To oxidative stress factors determination, 10% from organ weight was dissolved with buffer (PH 7.4) and the organ tissue was crashed by use ceramic mortar. Then mixture was centerfigation for 10 min. Supernated was taken and stored by deep freezing until used [15].

Measurements

ALT, AST & ALP

Serum ALT, AST & ALP were measured by technique according to the instructions of manufacturer company kit (Randox).

Urea, creatinine & uric

Serum urea, creatinine & uric acid were measured according to the instructions of manufacturer company kit (Randox).

Plasma Peroxidation levels (MDA)

MDA (malonedialdehyied), was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer [16].

Glutathione (GSH) and Catalase

GSH level estimated bymixed 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer [17]. Catalase was measured by using the procedure of Biovision-USA kits.

Statistical analysis

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one way analysis of variance (ANOVA).

Results

Liver function tests

The levels of ALT (98.15 ± 8.27 IU/L), AST (104.55 ± 4.88 IU/L) and ALP (119.1 ± 7.92 IU/L) in rats administrated with 1×10^3 cyct/ml suspension show high significant increased ($P < 0.05$) compared with control rats (32.2 ± 3.96 , 46.15 ± 4.45 and 64.7 ± 4.38 respectively). The levels of ALT (56.6 ± 4.81), AST (76.25 ± 10.25) and ALP (89 ± 10.32) in rats received with 1×10^3 cyct/ml suspension and received 50mg/kg extract show high significant increased ($P < 0.05$) compared with control rats. While, the levels of ALT (33.45 ± 7.85), AST (42.1 ± 2.97) and ALP (68.75 ± 5.3) in rats administrated 1×10^3 cyct/ml suspension and treated with 100mg/kg extract show no significant decreased ($P < 0.05$) compared with control rats as shown in Table-1.

Table 1-The levels of ALT, AST and ALP in serum

Parameters Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
I	32.2 ± 3.96 c	46.15 ± 4.45 c	64.7 ± 4.38 c
II	98.15 ± 8.27 a	104.55 ± 4.88 a	119.1 ± 7.92 a
III	56.6 ± 4.81 b	76.25 ± 10.25 b	89 ± 10.32 b
IV	33.45 ± 7.85 c	42.1 ± 2.97 c	68.75 ± 5.3 c

Note: same letters mean non-significant changes and different letters mean significant changes.

Kidney function tests

The levels of urea (82.1 ± 5.37 mg/dl), creatinine (2.66 ± 0.32 mg/dl) and uric acid (5.6 ± 0.3 mg/dl) in rats received with 1×10^3 cyct/ml suspension show high significant increased ($P < 0.05$) compared with control rats (35.55 ± 4.88 , 0.93 ± 0.25 and 2.37 ± 0.31 respectively). The levels of

urea (54.6 ± 6.22), creatinine (1.9 ± 0.57) and uric acid (4.3 ± 0.46) in rats received with 1×10^3 cyct/ml suspension and received 50mg/kg extract show high significant increased ($P < 0.05$) compared with control rats. While, the levels of urea (39.6 ± 5.1), creatinine (1.03 ± 0.15) and uric acid (2.47 ± 0.31) in rats received with 1×10^3 cyct/ml suspension and treated with 100mg/kg extract show no significant changes ($P < 0.05$) compared with control rats as shown in Table-2.

Table 2-The levels of urea, creatinine and uric acid in serum

Parameters Groups	urea (mg/dl)	creatinine (mg/dl)	Uric acid (mg/dl)
I	35.55 ± 4.88 c	0.93 ± 0.25 c	2.37 ± 0.31 c
II	82.1 ± 5.37 a	2.66 ± 0.32 a	5.6 ± 0.3 a
III	54.6 ± 6.22 b	1.9 ± 0.57 b	4.3 ± 0.46 b
IV	39.6 ± 5.1 c	1.03 ± 0.15 c	2.47 ± 0.31 c

Oxidative stress parameters (MDA, GSH and catalase)

The levels of MDA (2.02 ± 0.12 mmol/l), GSH (0.337 ± 0.047 mol/l) and catalase (0.88 ± 0.03 mmol/l) in rats received with 1×10^3 cyct/ml suspension show high significant changes ($P < 0.05$) compared with control rats (1.62 ± 0.08 , 0.593 ± 0.031 and 1.19 ± 0.08 respectively). The levels of MDA (1.85 ± 0.06), GSH (0.407 ± 0.021) and catalase (0.98 ± 0.03) in rats received with 1×10^3 cyct/ml suspension and received 50mg extract show high significant changes ($P < 0.05$) compared with control rats. While, the levels of MDA (1.64 ± 0.05), GSH (0.597 ± 0.038) and catalase (1.18 ± 0.02) in rats received with 1×10^3 cyct/ml suspension and treated with 100mg extract show no significant changes ($P < 0.05$) compared with control rats as shown in Table-3.

Table 3-The levels of MDA, GSH and catalase in serum

Parameters Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
I	1.62 ± 0.08 c	0.593 ± 0.031 a	1.19 ± 0.08 a
II	2.02 ± 0.12 a	0.337 ± 0.047 c	0.88 ± 0.03 c
III	1.85 ± 0.06 b	0.407 ± 0.021 b	0.98 ± 0.03 b
IV	1.64 ± 0.05 c	0.597 ± 0.038 a	1.18 ± 0.02 a

In liver

The levels of MDA (1.29 ± 0.03 mmol/l), GSH (0.163 ± 0.015 mol/l) and catalase (0.457 ± 0.049 mmol/l) in rats received with 1×10^3 cyct/ml suspension show high significant changes ($P < 0.05$) compared with control rats (1.05 ± 0.04 , 0.32 ± 0.02 and 0.793 ± 0.055 respectively). The levels of MDA (1.18 ± 0.031), GSH (0.25 ± 0.026) and catalase (0.663 ± 0.038) in rats received with 1×10^3 cyct/ml suspension and received 50mg extract show high significant changes ($P < 0.05$) compared with control rats. While, the levels of MDA (1.013 ± 0.05), GSH (0.34 ± 0.017) and catalase (0.847 ± 0.042) in rats received with 1×10^3 cyct/ml suspension treated with 100mg/kg extract show no significant changes ($P < 0.05$) compared with control rats as shown in Table-4.

Table 4- The levels of MDA, GSH and catalase in liver

Parameters Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
I	1.05 ± 0.04 c	0.32 ± 0.02 a	0.793 ± 0.055 a
II	1.29 ± 0.03 a	0.163 ± 0.015 c	0.457 ± 0.049 c
III	1.18 ± 0.031 b	0.25 ± 0.026 b	0.663 ± 0.038 b
IV	1.013 ± 0.05 c	0.34 ± 0.017 a	0.847 ± 0.042 a

In kidneys

The levels of MDA (0.4 ± 0.05 mmol/l), GSH (0.117 ± 0.021 mol/l) and catalase (0.32 ± 0.032 mmol/l) in rats received with 1×10^3 cyct/ml suspension show high significant changes ($P < 0.05$) compared with control rats (0.94 ± 0.02 , 0.28 ± 0.026 and 0.53 ± 0.026 respectively). The levels of MDA (0.67 ± 0.04), GSH (0.2 ± 0.01) and catalase (0.44 ± 0.047) in rats received with 1×10^3 cyct/ml suspension and received 100mg extract show high significant changes ($P < 0.05$) compared with control rats. While, the levels of MDA (0.94 ± 0.05), GSH (0.297 ± 0.057) and catalase (0.53 ± 0.032) in rats received with 1×10^3 cyct/ml suspension treated with 100mg/kg extract show no significant changes ($P < 0.05$) compared with control rats as shown in Table-5.

Table 5-The levels of MDA, GSH and catalase in kidneys

Parameters Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
I	0.94 ± 0.02 c	0.28 ± 0.026 a	0.53 ± 0.026 a
II	0.4 ± 0.05 a	0.117 ± 0.021 c	0.32 ± 0.032 c
III	0.67 ± 0.04 b	0.2 ± 0.01 b	0.44 ± 0.047 b
IV	0.94 ± 0.05 c	0.297 ± 0.057 a	0.53 ± 0.032 a

Discussion

Liver function tests (ALT, AST and ALP), kidneys function tests (urea, creatinine and uric acid) and oxidative stress factors (MDA, GSH and catalase) show high significant changes ($P < 0.05$) administrated with 1×10^3 cyct/ml suspension compared with control rats, this results are in agreement with study designed by Chabuket al. (2014) to show the effect of parasite on liver enzymes. The results of study show AST, ALT and ALP enzyme tests increased in all infected animals (*E. histolytica*) when comparison with control group, they suggest *E. histolytica* lead to histological lesions in t liver as apoptosis death of cells as well as changes in biochemical parameters (ALP , AST,ALT) [18]. Also, Al-Kubaissi (2002) to note a high in the level of concentration of the enzyme ALP reached 90 % of the cases with a high level of enzyme AST, ALT in the serum of patients infected with dysentery [19]. Mahmood and Ban (2012) show different lesions in liver of mice that administrated with *E. histolytica* including necrosis and degeneration of hepatocytes with infiltration of lymphocytes [20]. Also, Husein et al. (2013) referred that *E. histolytica* lead to different lesions in liver including liver abscess, necrosis of hepatocytes, hypertrophy of hepatocytes and iltration of lymphocytes [21]. The different lesions of liver may back to the ability of parasite to invasion through the intestine to the other organs such as liver and kidney and lead to degenerative changes [22]. The results also show increased in levels of creatinine, urea and uric acid, The histological and physiological changes may back to ability of parasite to stimulating the factor NF-kB and stimulating the epithelium cells to produce IL-IB that lead to increase the number of inflammatory cells to the site of infection [23]. The oxidative stress and antioxidant levels show significant changes compared with control group the results agreement with Al-Kaky (2006) who referred that the patients with *E. histolytica* show increased in levels of MDA and decreased in GSH compared to control group. Suggest that the increased of MDA levels and decreased in GSH back to the ability of parasite to increase the free radical which induce cytological changes [24].

M. chamomilla is one of the most widely used and well documented medicinal plants in the world. The use of *M. chamomilla* as a medicinal plant dates back to ancient Greece and Rome [25]. *M. chamomilla* contain phenolic compounds as flavonoides such as flavon glycoside , a glycoside apigenin and luteolin. Phenols has important role against Entamoeba species, where the phenols lead to inhibition enzymes of parasite by oxidizing compounds [26]. Al – Maliki (2012) referred that the phenols and alkaloidic compound which were isolated from Matricaria chamomilla flowers , have characteristic inhibition activity against pathogenic bacteria (Staphylococcus aureus and Escherichia coli) [27]. Salama et al. (2011) state that the *M. chamomilla* has been protective affects against cisplatin Induced Renal Injury. They found that Cisplatin caused elevation in all kidney function parameters (urea, creatinine) and significant decrease in GSH (measured in renal tissue). After that, they used *M. chamomilla* extract in the treatment. *M. chamomilla* extract with cisplatin provided the best protection to the kidney by reducing the levels of urea, creatinine and increased GSH levels closer to the normal ranges [28]. About the protective effect of in the liver functions parameters. Chani & Noor (2016) referred that *M. chamomilla* extract play important role against toxicity of methomyl induced hepatotoxicity. Mice administrated with methomyl showed significant increase in the levels of ALT and AST but after these mice treated with *M. chamomilla* extract showed significant decreased in the levels of ALT and AST and back to the normal ranges [29].

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