IJP (2014), Vol. 1, Issue 1 **(Research Article)**

Received on 13 October 2013; received in revised form, 24 November 2013; accepted, 27 December 2013; published 01 January 2014

THE EFFECT OF CHLOROFORM EXTRACT OF GERMAN CHAMOMILE (*MATRICARIA RECUTITA*) ON *ESCHERICHIA COLI* INFECTED MICE

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Keywords:

Chamomile, BALB/c mice, *Escherichia coli*, *In-vivo*

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ABSTRACT: Emergence of new strains of *Escherichia coli* with the ability of resistance to a wide range of antibiotics necessitates the efforts to search alternatives for better treatments to deal with these infections. Chamomile is one of the most widely used herbs in the world. In this study, we investigated the potential therapeutic effects of chamomile chloroform extract on *E. coli* intraperitoneal (IP) infection of BALB/c mice. Female BALB/c with an average weight of 20 to 25 g, were divided into 8 groups. The lethal dose of the *E. coli* for mice has injected IP in test groups and treated with 5.25, 10.5 and 21 mg/mL of chamomile chloroform extract. The combined effect of the extract (50 µg/mg) with amikacin and amikacin alone were also examined. The highest number of survived mice at the dose of 10.5 mg/mL of extract and the highest death rate with 5.25 mg/mL of extract was observed. In the amikacin group, all infected animals were survived. The mortality rate in chamomile extract combination with amikacin is almost the same as amikacin alone. The significant lowest mortality rate was observed in some group which had been treated with a combination of extract and amikacin. The result of this study does not recommend in-vivo using of chloroform extracts of chamomile for *E. coli* infection *via* IP administration. In vivo chloroform extracts of chamomile, it seems not to have a strong antibacterial effect on *E. coli* IP infection of BALB/c mice.

INTRODUCTION: *Escherichia coli* were first isolated in 1885 by Theodor Escherich and are now one of the most widely studied bacteria in the world [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.1(1).39-44

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(1).39-44

Some strains of *E. coli* are considered the main pathogens of humans and animals [2]. Infections caused by pathogenic strains of *E. coli* can limit the colonization of the mucosa or spread throughout the body and cause urinary tract infections, sepsis, meningitis, and infections of the gastrointestinal tract indicate the importance of treating these infections [1, 3]. Also, the emergence of new strains of this organism with the ability to resist a wide range of antibiotics, especially beta-lactamase family (by producing ESBL or Extended Spectrum

Beta-Lactamase) and increasing of these strains in several reports requires serious efforts to search for better treatments and alternatives to deal with the related infections [4-6].

Since, the previous few decades, there has been much attention on searching and providing herbal extracts as an alternative or adjunctive therapy to enhance the effectiveness of antibiotics [7, 8]. Chamomile plant is one of the most widely used herbs in the world. The ancient Egyptians considered it as the holy gift from the sun god and used it for fever and sunstroke. In Europe, due to various medical applications, this plant is known as treating all (Cure-all) [9]. Its antimicrobial properties on a range of different organisms are well known *in-vitro* [10-13].

So, in this study, we used BALB/c mice model to investigate the potential therapeutic effects of chamomile extract on *E. coli* infections *via* intraperitoneal (IP) injection.

MATERIALS AND METHODS: The antibiotic used in this study was amikacin (EXIR - Iran) which was IP injected into laboratory animals. Also, the culture media used in this study was Muller Hinton broth (Oxoid - UK)

The bacterial strain used in this study was a confirmed isolated *E. coli* with some TCC.1789 that was isolated from a patient in Namazi Hospital in Shiraz, Iran.

Laboratory animals used were female BALB/c mice with an average weight of 20 to 25 grams; they were placed in individual cages with controlled temperature, humidity and light, and all experiments were performed by supervision and approval of the animal experimentation ethics committee of the Shiraz University of Medical Science.

The MIC and MBC of chamomile chloroformic extract were determined *in-vitro* in a separate study to be 2.63 and 5.25, respectively [14].

Chamomile chloroformic extract was prepared by a soaking method in Department of Pharmacology, Shiraz University of Medical Science.

The lethal dose of the bacteria was calculated $5x10^8$ CFU/mL through IP injection for mice which has been previously tested through a series of experiments (data not shown). Before the injection, *E. coli* was cultured for 24 h at 37 °C in Mueller Hinton broth, then centrifuged at 1,800 × g for 10 min, and washed with normal saline. The number of bacteria was adjusted at 10^9 CFU/mL in normal saline. 0.2 mL of this concentration was injected IP in the animals.

Determination of the toxic effect of the extract was performed by an IP injection of 0.3 mL of chamomile extract with the concentrations of 5.25 and 21 mg/mL to two groups of the mice and observation of injected animals for 48 hours to see if they show any sign of acute toxicity. At the first phase of this study, the mice were divided into 8 groups of 10. The amount of ip injection for each group is listed in **Table 1**.

TABLE 1: GROUPING OF THE ANIMALS AND INJECTIONS IN EACH GROUP

Groups	No. of mice	First injection	Second injection*
1	10	IP injection of *E. coli*	IP injection of 21 mg/ml extract
2	10	IP injection of *E. coli*	IP injection of 10.5 mg/ml extract
3	10	IP injection of *E. coli*	IP injection of 5.25 mg/ml extract
4	10	IP injection of 21 mg/ml extract	IP injection of *E. coli*
5	10	IP injection of 10.5 mg/ml extract	IP injection of *E. coli*
6	10	IP injection of 5.25 mg/ml extract	IP injection of *E. coli*
7	10	IP injection of *E. coli*	IP injection of normal saline
8	10	IP injection of *E. coli*	IP injection of *Amikacin* 500mg/ml

* Second injection after the half minute break

In the first three groups, injection of *E. coli* was done half an hour before extraction, and in the three next groups, it was reversed. After injections, the animals were returned to cages and monitored for 2 h by a trained expert. At the second phase, after the death of all the mice in the control group (group 7), that only had IP injection of bacteria with normal saline, a total count of the number of live mice at other groups was done. At the next step, the combined effect of chamomile extract (the highest dose of the extract had shown an antibacterial effect in the previous phase); the amikacin was examined at doses of 100, 50 and 25 and 12.5 mg/mL. For this purpose, the mice were divided into 9 groups of 10, and the amount of IP injection for each group is listed in **Table 3**. After injection, the mortality rate of mice was observed every 12 h.

Statistical Analysis: was performed using SPSS19.0 statistical software. Fisher's exact test was conducted to analyze survival rates in tested groups'. Values of $p<0.05$ were regarded as statistical significance.

RESULTS: As can be seen in the results shown in **Table 2**, the highest number of surviving mice at the targeted groups that had received chloroform chamomile extract was related to group 5 (60% survivor), and the highest death rate was observed in group 3 (no survivor). Also, a significant difference was seen in the survival rate of the three-second groups that received the extract before *E. coli* injection ($p<0.03$).

All mice at two separate group as well as considered individually for determining the potential of chamomile extract toxicities after 48 h had remained alive and no signs of acute toxicity were observed. Also, the groups with 8 mice that were treated with an injection of 500 mg/mL of amikacin stayed alive. The results of the extract injected combinations with amikacin to the animals are shown in **Table 3**. As can be seen, the mortality rate in chamomile extract combined with amikacin is almost the same when amikacin was administered alone. However, a significantly lower mortality rate was observed in group 3, which had experienced an injection of combined extract and amikacin with a dose of 50 mg/mL, than other groups receiving amikacin combined with the extract.

TABLE 2: SURVIVAL RATES IN DIFFERENT GROUPS OF BALB/c MICE AFTER THE DEATH OF ENTIRE CONTROL GROUP

Groups	No. of mice	No. of mice survival	p-value compared to the control group	p-value*	p-value**
1	10	1	> 1.00		
2	10	3	> 0.21	> 0.50	
3	10	0	NS		< 0.03
4	10	3	> 0.21	< 0.01	
5	10	6	< 0.01		
6	10	3	> 0.21		
7	10	0			
8	10	10			

* P-value when compared each 3 first and second groups with the control group
** P-value when 3 first and second groups compared with each other
*** Control group
NS; not significant

TABLE 3: GROUPING OF THE ANIMALS AND EFFECT OF EXTRACT IN COMBINATION WITH AMIKACIN

Group	Injection	Dose (mg/g)	No. of mice	No. after 12 h**	No. after 24 h	No. after 36 h	No. after 48 h
1	Amikacin + Chamomile extract	100	10	10*	6*	4	2

	(10.5 mg/mL)						
2	#	50	10	10*	8*	5*	3
3	#	25	10	10*	6*	3	2
4	#	12.5	10	8*	4	2	0
5	Amikacin	100	10	10	6	5	3
6	#	50	10	10	8	6	3
7	#	25	10	10	6	4	2
8	#	12.5	10	10	5	2	0
9	*E. coli* + normal saline	-	10	0	-	-	-

* P-value were significant ($p<0.05$) compared to control group
**No. of survival mice 12 h after each injection

DISCUSSION: To our knowledge in this study, the antibacterial effect of German chamomile chloroform extract on *E. coli* infection in laboratory BALB/c mice for the first time was examined. Numerous studies exhibited the antibacterial effect of essential oil extracted from chamomile on several ranges of bacteria at *in-vitro* condition [10, 12, 15]. Also, the effect of the esters and lactones compounds from chamomile on *Mycobacteria* has been seen [16]. Compounds in chamomile are thought to have antimicrobial properties including alpha-bisabolol, luteolin, quercetin, and apigenin. Herniarin may have anti-bacterial properties in the presence of UV light [17]. The high alpha-bisabolol content in chamomile oil is credited for providing main anti-bacterial properties by antiseptic action against Gram-positive and Gram-negative bacteria [18].

As can be inferred from the results the most antibacterial effect of chloroform extract has been seen in the injection dose of 10.5 mg/mL ($p<0.01$). Also; it was observed that the antibacterial effect of the chloroform extract as the preventative, when that injected before bacteria inoculation would be better effective ($p<0.03$). As can be seen in Table 2 that total surviving rats at groups 4-6 is more than the other groups that experienced chamomile chloroform extract ($p<0.01$).

According to the results shown in **Table 3**, the best combination effect of chamomile extract with amikacin can be seen at a dose of 50 mg/mL. A mortality rate at this dose is significantly ($p<0.05$) lower than other injections. As observed in **Table 3**; antibacterial effect of chamomile extracts in some groups, when administered with amikacin, is less than when amikacin is injected alone. This has also been observed in some studies that combinations of antibiotic-extract could not control the induced infection in the tested animals, also increasing the concentration of the extract in the combination led to only a reduction of its efficacy [19].

However, as can be seen in **Table 2**, chloroform extracts of chamomile itself do not infer a strong antibacterial power, and it has only been able to delay the proliferation of *E. coli* and reach death time in laboratory animals. Probably may due to the inhibitory effect of flavonoid compounds of chamomile extract on mice immunity; especially apigenin on COX-2 and iNOS activities in LPS activated macrophages through suppressing the activation of NF-kB [20]. Also, it may occur because the low absorption of the extract is *via* the IP injection. It could also be due to the lower effect of plant compounds on Gram-negative bacteria, which has been shown at previous studies [17].

Anyway, although chamomile extract's antibacterial effect shown on different bacteria *in-vitro* [10, 15], the results of this study do not recommend its therapeutic effect at *in-vivo* conditions for *E. coli* infection *via* IP administration, of course, the antibacterial effect of the chamomile extracts might be found in other research through the absorption of other pathways investigated on laboratory animals or *via* IP administration with a higher dose of the extract.

Since, that has been reported particular toxicity study using rabbit models determined the acute oral LD_{50} and acute dermal LD_{50} to be more than 5 g/kg

body weight [21]. Chamomile extract is mentioned on the FDA's as (generally recognized as safe) GRAS [22].

CONCLUSION: Despite the mild effect of chamomile chloroform extract in a finding of the current study, potential therapeutic effects of chamomile for gram-negative infection suggest future studies.

According to the various properties of chamomile, compounds are recommended in future effect of these compounds on gram-negative infection studied individually to find the best treatment option. As well as finding the best way to absorb the extract must be a priority.

ACKNOWLEDGMENT: The authors would like to thank Ms. S. Gorji, Ms. N. Pirbonyeh, Mr. P. Talezadeh and Mr. M. Hosseini Farzad for their technical assistance. This study was supported by Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran with grant No. 5580.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Motamedifar M, Sedigh H, Mansury D and Rajabi A: The effect of chloroform extract of german chamomile (*Matricaria recutita*) on *Escherichia coli* infected mice. Int J Pharmacognosy, 2014; 1(1): 39-44. doi: 10.13040/IJPSR.0975-8232.1(1).39-44.