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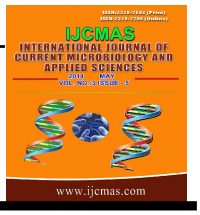

Original Research Article

Study the therapeutic role of Alcoholic Extract of *Artemisia* aganist infection with *Escherichia coli*

Aseel J. Mohammad*

Ministry of Education Baghdad.Iraq
Corresponding author

Keywords

Artemisia E.coli, gastro-intestinal infection, Mice

A B S T R A C T

The present study was carried out to investigate the antibacterial activity of alcoholic extracts of *Artemisia*leafes *in vitro* and *invivo* by inducing gastrointestinal infection in mice orally infected with *E.coli*isolated from patient suffering from diaraha. Extracts showed significant effect (P<0.05) on the inhibition of the growth of *E.coli in vitro* with the superiority of the concentration 150mg / ml of alcoholic extract with the mean of inhibition zone diameter 32 mm,while zone diameter was (23, 29) mm due to the concentration 50, 100mg/ml respectively. This study included the therapeutic role of 100 mg/kg . B.W. of 0.25ml daily orally of alcoholic extract dissolved in DMSO of *Artemisia*leaves in the pathogenesis of *E.coli* in mice by the orally infection (0.25 ml 0f *E.coli* 1.5 x 10^8 cfu/ml) in compared with the control positive group (mice injected with *E.coli* without treatments).The results of histopathological changes showed the role of *Artemisia* extract on the decreasing of pathological sings in liver tissue after 14 and 21 days and gave negative results by decrease congestion in the blood vessels of liver hemorrhage,in compared with the positive control which showed acute histopathological change.

Introduction

For a long time medicinal plants has been shown throughout the world because of the safe and effective constituents of plant products, the increasing demand for herbal medicines, both in the developing and developed countries (Ganesan and Bhatt, 2008).

Artemisia herbs have been used as tonics, antimalarials antihelmintics, and antidiabetics, and in t reating wounds, bronchitis,ulcers, and tuberculosis in traditional Anatolian medicine (*Uzun et al., 2004).*

There are also several reports conerning the antimalarial, antioxidant, cytotoxic,antipyretic, analgesic, antidiabetic, antimicrobial,and antifungal activities of different *Artemisia* species(Tan RX, Zheng et al., 1998).

E. coli are wide spread species and include different types ranging from highly pathogenic strains to avirulent strains (Schoenian, 2007; Kaper, 1994). It is a common cause of gastroenteritis and it accounts for 30% of the total number of pathogenic agent's diarrhea of some regions of the world (Alikhani *et al*., 2007).

The virulence factors of pathogenic strains of *E.coli* include capsule, endotoxin, structures responsible for colonization, enterotoxins and other secreted substances(Quinn,2006), lipopolysaccharide is the major molecule responsible for the pathogensis of infection caused by gram negative bacteria (Quinn,2006). This study was aimed to study the therapeutic role of *Artemisia* (alcoholic extrat) against infection with *E.coli*

Materials and Methods

Plant material

Plant were purchased from alocal market in Baghdad City. Later the leaves of the plant were washed under tap water and then dried in room temperature .the dried leaves were crushed to afine powder by an electrical grinder.

Preparation of crude organic solvent extract of *Artemisia* herba-alb

Organic solvent extract of the*Artemisia* was carried out by using ethanol (95% ethyl alcohol). This was done by using Soxhlet apparatus (*Effrainet al ,2000***)**.

Preparation of Different Concentration of plants Extract

Three concentrations 50,100,150 mg /ml of *Artemisia* leaves were prepared by suspending 0.5, 1, 1.5 gm respectively in 10 ml of Dimethylsulphoxide (DMSO). Each concentration was mixed then filtered through whatman (No.1) ,and kept in sterile test tube at 4 **C** until used .

Preparation of Bacteraial Suspention

*E.*coli isolates were obtained from patients suffering from diaraha in Baghdad city. Diagnosis of all these isolates were depended on the cultural and biochemical tests, then the diagnosis was confirmed by using API 20 system kit.

Invitro antbacterial activity of *Artemisia* herba-alba

Agar-well diffusion method was used to check the activity of plant extract *in vitro* against *E.coli* isolate (*Kavanagh et al*, 1972) to achieve this purpose, *E.coli* pure colonies were selected.

Four wells (6mm in diameter) were made in nutrient agar plates using a sterile cork borer , 200 Micro liters, of different concentrations of plant extract (100,150,200 mg /ml) were poured in each wells, other well were filled with 0.1 ml of DMSO as a control, the plates were incubated up down at 37°**C** for 24hr. Three replicates were carried out for each concentration extract and the diameter of inhibition zone was measured. The results and standard errors means values were tabulated (Mahmood *et al*., 1989).

Experimental Design

Group (1): (Control positive):mice were infected with 0.25 ml/mice which contain 1.5 x 10^8 cfu/ml of *E.coli* orally and left without treatment (treatment begin after 48 hrs. after inducing infection).

Group (2): mice were dosed 0.25ml of DMSO orally for 14 and 21 days which represented as control negative.

Group (3): mice infected (as in group 1) and treated orally with 100mg/kg B.W of alcoholic extract of *Artemisia* herba-alba for 14days.

Group (4): mice infected (as in group 1) and treated orally with 100mg/kg B.W of alcoholic extract of*Artemisia* herba-alba for 21days.

Results and Discussion

The results showed the superiority of the concentration 150mg /ml and this may be due to the solubility of high amount of active ingredient which inhibited the bacterial growth ,these results come in agreement with that mentioned byAlfahmi, (2007). Naili et al (2010) found that *Artemisia*have a good cure because it contain tunisian essential oil and they showed astrong effect of this extract against *E.coli* growth ,while *S.aureus* was the mast sensitive bacteria in one study

The current study agree with Al-Ukaily (2009)) who reported that infected mice as animal model with *E. coli* cause hemorrhagic in the interstitial tissue in intestine Odema and congestion blood vessel, Enteropathogenic *Escherichia coli* strains attached intimately to the intestinal epithelium and efface the absorptive microvilli, and efface initiating acomplex signaling cascade that ultimately leads to diarrhea by mechanisms that are only partially understood (Clarke et al , 2003).

The histopathological changes in liver tissue after 2 days from challenged by *E.coli* isolate in positive control group without treatment were showed lesion represented by multiple foci of MNCS infiltration in liver parenchyma & congestion blood vessels were seen in addition to and sinysaidal there lumen were seen in addition to vacular degeneration of some hypocyte accoapanied with appearance of number of apoptotic cellfigure (3), in spleen tissue after 2 days from challenged by *E.coli* isolate in positive control group without treatment were showed lesion represented by destraction in splenic parenchyma with lymphoid atrophy of white pulp figuere (4), as compared with the negative control group which showed normal structure of liver and spleen Figure (5),(6). *E.coli* was detected by cultural and biochemical tests listed in the following table(1).

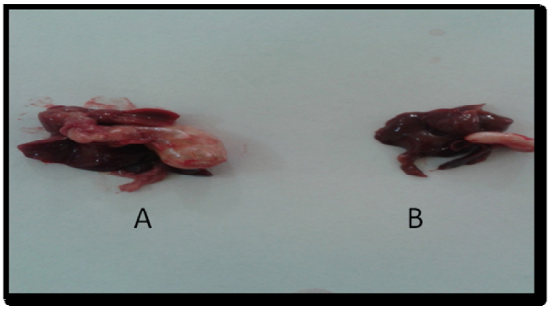

Figure.1 Pathological gross examination in infected (A) and normal viscera(B)

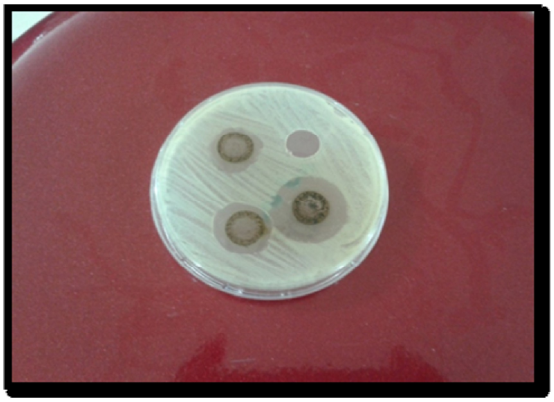

Figure.2 Effect of alcoholic extract of *Artemisia* herba-alba against *E.coli.* (mg/ml).

Table.1 Cultural and biochemical tests of *E.coli*

Bacteria	Morphological examination		Biochemical tests	Bacteria
E. coli	Gram stain	-	Indole	+
	Blood agar	Hemolysis	Simon Citrate	-
	MacConkey agar culture	Rosy colonies lactose fermentation	Catalase	+
	EosinMethylene Blue agar	metalic sheen colonies	Voges proskauer	+

Table.2 In-vitro antibacterial activity of different concentrations of *Artemisia* herba-albaextract against *E.coli* growth

Concentration mg/ml of *Artemisia* L.	***E.coli* (inhibition zone-mm) (Mean ±SE)**
50	23 ±0.54C
100	29 ±0.47 **B**
150	32 ±0.47 **A**
90% DMSO	0.00±0.00 D

Values represent mean ±S.E

Different capital letters mean significant (P<0.05) results between different concentrations

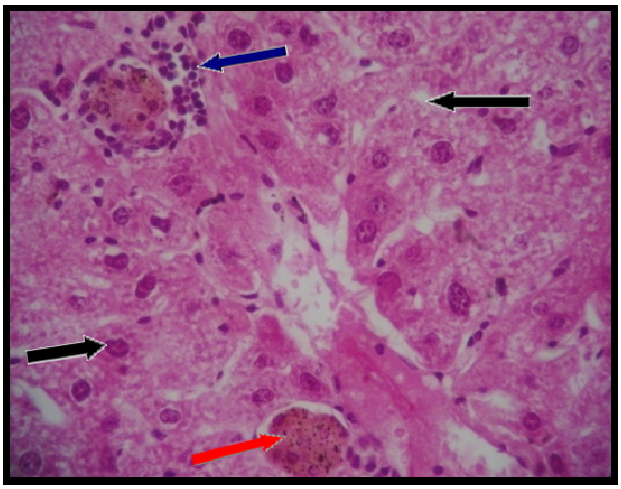

Figure (3). Histological section in liver of one animals at 2 days post infected with*E.coli* (G1) shows multiple foci of MNCS infiltration in liver parenchyma mainly aroud blood vessels inaddation blood vessels congestion and sinysaidal there lumen wer seen together with vacular degeneration of some hypocyte accoapanied with appearance of number of apoptotic cell *(H&E stain 40X)

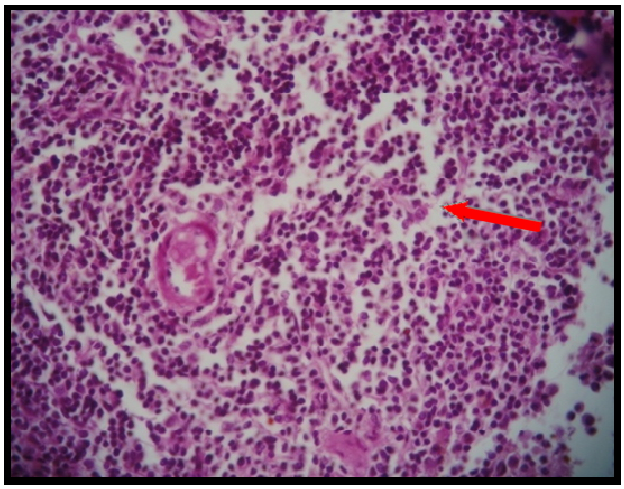

Figure (4). Histological section in Spleen of one animals at 2 days post infected with*E.coli* (G1) shows destraction in splenic parenchyma with lymphoid atrophy of white pulp *(H&E stain 40X).

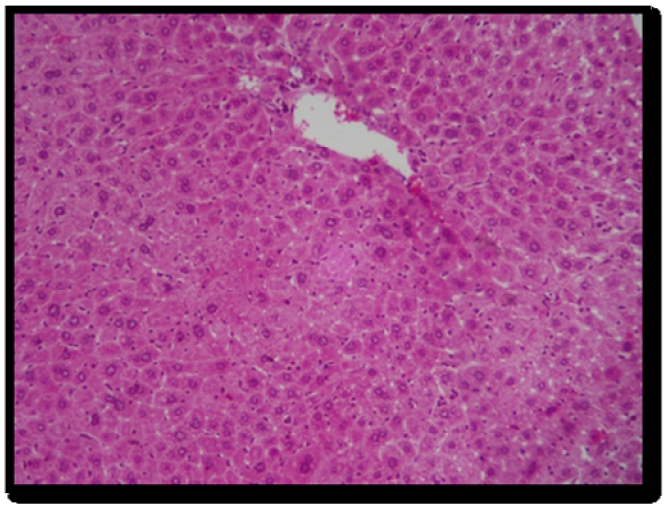

Figure (5): Hisological section in liver of normal animal showed normal structure of liver (H&E stain 40X)

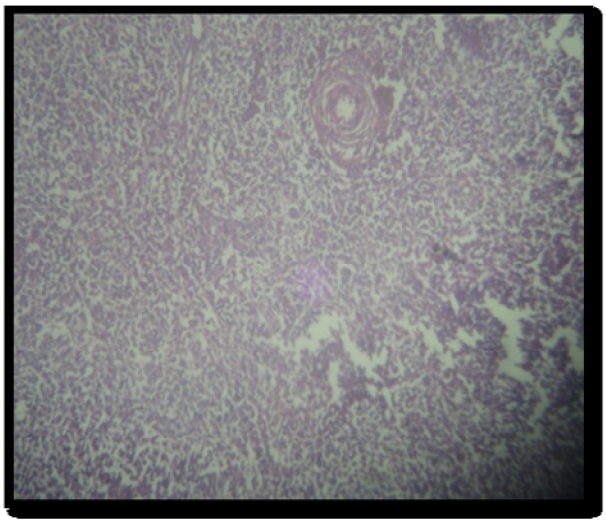

Figure (6): Hisological section in liver of normal animal showed normal structure of Spleen (H&E stain 40X)

Enterobacteria can cause infection depending on a number of factors. The most important factors are motility, colonization factors, endotoxin, and enterotoxin (Narins, 2003). *E. coli*, endotoxin translocation might play an important role in the development of inflammatory bowel disease, which may lead to accumulation of endotoxins in tissues. Endotoxin levels in hepatic homogenate are firstly increased. The liver is the most important organ for endotoxin accumulation after translocation because the portal circulation is the prominent route for endotoxin in the intestine to enter the body after infection and because the liver is the largest organ that has monocyte/macrophage system, (El-Garawany *et al*., 2005) and this fact is supported by a study done by Borissov and Andonova (2000) who found the same results.

After 14 days from injection by *E.coli* (0.25ml) isolate &treatment showed moderated MNC infiltration in liver parenchyma consist of &macrophage with sinusoidal dilation &congestion togther with slight hypertrophy of some hepatocyte figere(7) ,while in spleen showed red pulp congestion and increased number of megakaryocyte figure(8).

Group foure which treated with alcoholic extract of *Artemisia* leaves (after 21 days from treatment) showed no clear pathogenic lesion except MNC aggregation in peripontial area with slight dilation of bile duct in liver figuer(9),as well as in spleen shows slight thickening of splenic blood vessels wall associated with slight frbroplsia inaddation to moderated lymphoid hyperplasia in white pulp figuer(10), this may due to the role of this extract in killing of bacterial cells and repaired of tissue because this extract contain active ingredient which may act as antibacterial agent such as terpenoids and flavonoids.

Flavonoids activity is probably due to their ability to complex with intracellular soluble proteins and also with bacterial cell wall. (Chabot *et al*., 1992).). Flavonoids were widely found in *Artemisia* leaves and posses many function including anti-allergic,anti-inflammatory and anti-tumor activities (*Buhler et al*., 2000).

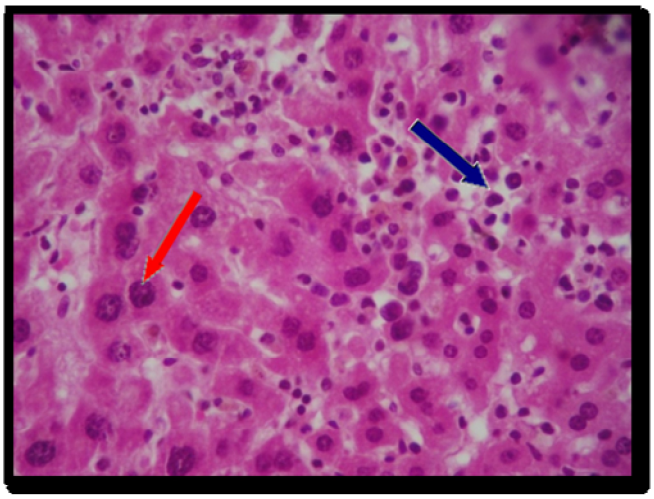

Figure (7): Histological section in liver at 14 days post infected with *E.coli* and treatment with alcoholic extract of *Artemisia* herba-alba showes moderated MNC infiltration in liver parenchyma consist of &macrophage with sinusoidal dilation &congestion togther with slight hypertrophy of some hepatocyte ((H&E40X)

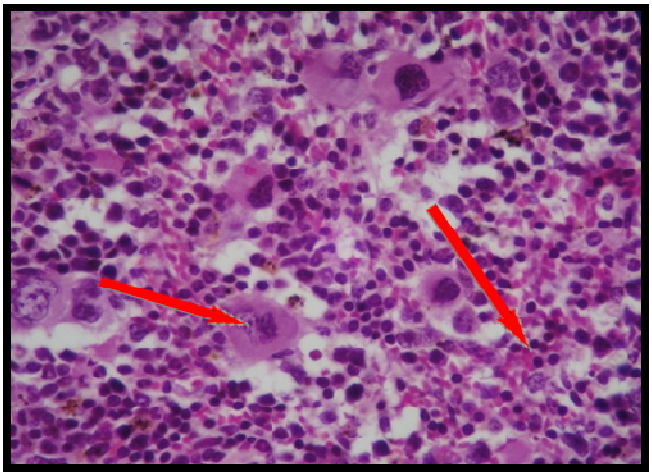

Figure (8). Histological section in Spleen of one animals at 14 days post infected with*E.coli* (G3)and treatment with alcoholic extract of *Artemisia* herba-alba shows red pulp congestion and increased number of megakaryocyte *(H&E stain 40X).

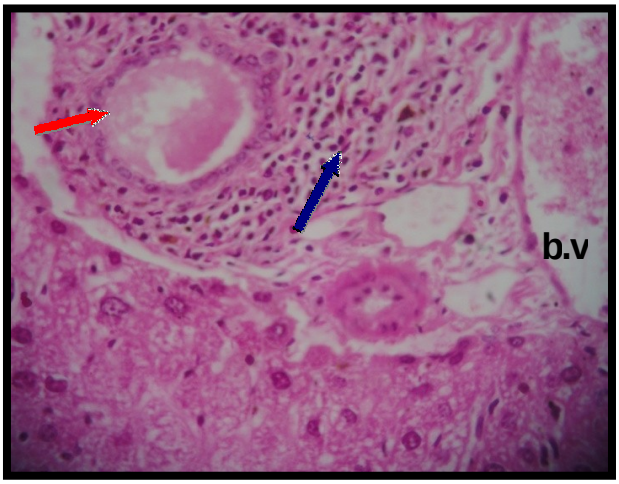

Figure (9). Histological section in liver of one animals at 21 days post infected with*E.coli* (G4) shows MNC aggregation in peripontial area with slight dilation of bile duct *(H&E stain 40X).

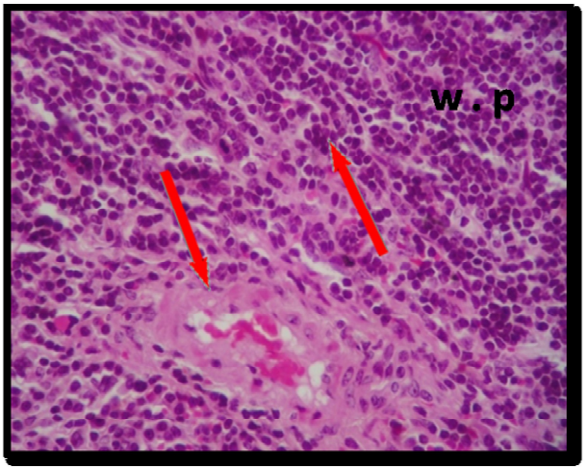

Figure (10). Histological section in spleen of one animals at 21 days post infected with*E.coli* (G4) and treatment with alcoholic extract of *Artemisia* herba-albashows slight thickening of splenic blood vessels wall associated with slight frbroplsia inaddation to moderated lymphoid hyperplasia in white pulp *(H&E stain 40X).

It is well known that there is a relationship between antioxidant activity and the phenolic content of the plant extracts. (Albayrak 2010) . Antioxidant properties of the methanolic extracts can be attributed to the phenolic and flavonoid contents of the *Artemisia* species.

Therapeutic value of plants used in trade medicine derives from the presence of phytochemicals principles (secondary metabolites) which are found in all parts of plant such as alkaloids, tannins, flavonoid, saponins and phenols (Ayodele, 2003).

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