

Effect of extract of punica granatum on experimentally induced salmonellosis

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Abstract:

minimal inhibitory concentration (MIC) of Kirby-Baur method was need to determine the activity of medicinal plants on induced salmonellosis in rabbits. The results revealed that aqueous extract of punica granatum had anti salmonella activity with MIC (250 µg/ml) when used at stock concentration of (2000 µg/ml). At the mean time a significant potentiation was obtained by double combination of chloramphenicol with punicagranatum MIC (31.25 µg/ml), on the other hand the triplecombination of chloramphenicol plus amoxicillin and punica granatum was more-evident with MIC (7.81µg/ml). The anti salmonella activity of puni ca granatum with antimicrobial drugs when used invivo in rabbits produced a significant relief to all the symptoms corre spording with laboratory findings which was equal to 100% after 2 weeks of therapy. The postmortem of rabbits had shown a prevention of organ complications which had been reduced from 66% for untreated (Group B) to 5% for treated (Group D&E)

Key words
salmonellosis

Punicagranatum,

MIC,

تأثير خلاصة حب الرمان على التايفونيد المحدث عملياً في الارانب

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استعملت طريقة كيربي باور _ التركيز المثبط الاصفر _ بالزجاج لتعيين فعالية الخلاصة المائية للنباتات الطبية على التايفونيد المحدث عملياً في الارانب. لقد كشفت النتائج ان الخلاصة المائية لبذور حب الرمان تملك فعالية مضادة للتايفونيد تعادل (٢٥٠) مايكرو غرام/مل حينما استعملت بتركيز قياسي (٢٠٠٠) مايكرو غرام/مل وبنفس الوقت تم الحصول على تقوية لفعل حب الرمان المضاد للتايفونيد عندما اعطى في اتحاد ثنائي مع الكلورامفينيكول وبجرع صغيرة حيث سجل التركيز المثبط الاصفر (٣١.٢٥) مايكرو غرام/مل يقابل ذلك الاتحاد الثلاثي بين حب الرمان مع الكلورامفينيكول والاموكسيسيلين وبجرع اصغر حيث كان التركيز المثبط الاصفر (٧.٨١) مايكرو غرام/مل ان استعمل حب الرمان مع المضادات الحيوية على الحيوان مباشرة وبجرع اصغر كان سبباً في ازالة كل الاعراض المرضية بصورة معتدة وارتباط ذلك بالمؤشرات المعتمدة وكذلك الفحوصات المختبرية بما يعادل ١٠٠% خلال فترة اسبوعين من التداوي. ان تشريح الارانب بعد الموت اظهر حالة منع لمضاعفات جسيمة في الاعضاء حيث كانت نسبتها ٦٦% في الارانب المعالجة في حين كانت نسبة المنع ٥% في الارانب غير المعالجة

الاستنتاج : امكانية استعمال بذور حب الرمان في تقليل المضاعفات الحاصلة في مرض التايفونيد

الارانب

التايفونيد

حب الرمان

الكلمة المفتاح

Introduction:

Salmonellosis is an infectious diseases in most developing countries caused by any strains of the genus salmonellae. The classical symptoms of the disease include fever, headache, myalgia, relative tachycardia, constipation or diarrhea, rose spots, cough & splenomegaly lead to coma & death if it is not treated⁽¹⁾. The salmonella pathogenicity / is land 2 (SPI2) was found to modify the defensive mechanism of host cell⁽²⁾. Rabbit is a good model for studying salmonellosis by producing many lesions in the intestine with edema, hemorrhage, ulceration & enlarged follicles⁽³⁾. It has been reported that the extract of *Allium sativum* had an effect against gram negative bacteria with anti brucella & possible anti salmonella activity⁽⁴⁾ and used in this study as control. The present study was performed to examine the possible anti salmonella activity of *punicagranatum* both *invivo* & *invitro* in experimental rabbit model in order to find a safe, effective & less cost remedy.

Materials & methods:

The anti salmonella activity of *punicagranatum* was determined by MIC (Kirby-Baur) method *invitro* & also by induction of salmonellosis in rabbit *invivo*. The selected antimicrobial drugs which was used in present study include Amoxicillin (pan pharma-France) ciprofloxacin (ciprodar-Kimadia-Jordan) chloramphenicol (miphenicol-misr) & Trimethoprim-sulfamethoxazole (methaprim SDI-Iraq).

Plant extraction:

The seed of punica granatum (pomegranate) was identified & authenticated by IRAQI national institute for Herbs. It was cleaned carefully & powdered with a nelectrical grinder then pass through a sieve to remove the debris. The sieved powder was stored in airtight container at room temperature. The aqueous extract was prepared by diluting one volume of powder to 10 volume of water at 80 °C in the stoppered flask. After shaking completely thus, it was allowed to stand for 10 minutes to be cooled and filtered for use. The aqueous extract must be used within 12 hours⁽⁵⁾.

InVITRO study:

The aqueous extract of punica granatum was diluted so that a stock concentration of 2000µg/ml was obtained and then serially diluted with two-fold dilution for 7 sequenced dilution with Muller-Hinton broth. Equal dose of 0.1ml of *S. typhi* wae added to tubes and mixed well followed by cultivation for 24h at 37°C in incubator. The inoculum was performed according to Macfrland international standard in order to obtain the turbidity required. The obtained results were collected for analysis and assessment.

InVivo study: Fifty healthy domestic rabbits of both sexes (900-1000g) were supplied by animal house of

Al-Nahrain college of Medicine they were housed in separated cages which were provided with a wire mesh floor at a controlled temperatue of 28°C with 12-hour light/dark cycle. They were fed standard oxoid pellets and water adlibitum. The study was conducted according to animale thics committee of Al-Nahrain college of Medicine. The animals wre randomly allocated to five groups of ten animals each. All the groups except (Group A) had been experimentally induced salmonellosis and they were treated in the following way for two weeks.

Group-A (normal control) received distilled water 0.2ml l-ml 3 times daily.

Group-B (diseased control) received distilled water 0.2 ml I-m 3 times daily.

Group-C (treated control) received 15mg/kg I-m chloramphenicol singly 3 times daily.

Group-D received double combination of 7.5mg/kg Im chloramphenicol plus 50mg/kg I.m amoxicillin 3times daily.

Group-E received triple combination of 50µg/kg Im chloramphenicol plus amoxicillin 25 mg orally plus *P. granatum* 50 mg orally 3 times daily. The clinical features of the rabbits were examined using the following parameters, fever, anorexia, weight reduction and dehydration in addition to laboratory findings WBC, RBC count, HB and PCV levels, blood, urine and stool cultures also Widal test were monitored through two weeks course of triple combination therapy as compared with single drug therapy. Post mortum studies were performed to follow up the histopathological changes 3 weeks after starting therapy.

RESULTS AND DISCUSSION

The diagnosis of salmonellosis confirmed by eliciting positive samples of blood, urine or stool cultures⁽⁶⁾. These results are compatible with our results in (Tables 4 and 5). However Widal test of increasing titer over a week was also significant⁽⁷⁾, in addition to the changes in the clinical features. *Salmonellae* are theoretically susceptible to antimicrobial drugs that show a cidal effect against gram-negative bacteria such as chloramphenicol⁽⁸⁾.

In deed, the antisalmoenella effect of *p.granatum* showed no significant difference over the results 75% which was related to its inhibitory action on gram-negative bacteria⁽⁹⁾ at $p<0.05$

The results of the present study revealed that aqueous extract of *p.granatum* had potent inhibitory action against *s.typhi* with MIC (250µg/ml) (Table 1).

Table 1

The anti salmonella MIC values of medicinal plants

Medicinal plants inhibitory activity against
salmonella

	Medicinal part	Number of tube	1	2	3	4	5	6	7
		dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128
		Concentration µg/ml	1000	500	250	125	62.5	31.25	15.62
Distal water	control		-	-	-	-	-	-	-
Allium sativum (garlic)	Dried bulb (powder)		+	+	+	-	-	-	-
Punicagranatum (pomegranate)	Dried seed (powder)		+	+	+	-	-	-	-

The MIC test confirmed the corresponding antimicrobials such as amoxicillin, ciprofloxacin and trimethoprim-sulfamethoxazole (TSM)⁽¹⁰⁾. In double combination trials, a marked inhibition of *S.typhi* had been obtained with *p.granatum* at a stock concentration of 1000µg/ml as well. The high potency was recorded for combination of *p.granatum* extract with chloramphenicol MIC (31.25mg/ml) (Table 2) compared with *p.granatum* alone MIC (250µg/ml). At the sametime combination of the extract of *p.granatum* with amoxicillin, TSM, or ciprofolxacillin showed potentiation of antisalmonella activity with MIC (62.5µg/ml, 62.5µg/ml and 125µg/ml respectively (Table 2).

Table 2

The anti salmonella MIC values of double combination of medicinal plants extract and antimicrobial drugs

Combined agent **inhibitory activity against salmonella**

Anti microbial	stock	Medicinal plant	stock	No. of tube	1	2	3	4	5	6	7
	Halved class		Halved class	Dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128
	µg/ml		µg/ml	Concentration µg/ml	1000	500	250	125	625	31.25	15.62
Amoxicillin	1000	Allium salivum	1000		+	+	+	+	+		
Amoxicillin	1000	Punica granatum	1000		+	+	+	+	+		
chloramphenicol	1000	Punica granatum	1000		+	+	+	+	+	+	
Trimethoprim	1000	Punica granatum	1000		+	+	+	+	+		
ciprofloxacin	1000	Punica granatum	1000		+	+	+	+			

The antisalmonella synergism that have been observed after combination could be attributed to a special mechanism of action other than the common mechanism of synergism. This point could be promising to minimize the rate of drug resistance.

In triple combination trials the p.granatum extract at $1/3^{\text{rd}}$ of the original stock concentration plus another two antimicrobials like chloramphenicol, amoxicillin, TSM, or ciprofloxacin. The inhibitory activity inVitro had revealed (15.63µg/ml) in a 100% of the above

triple antisalmonella combination which was highly significant at $p < 0.05$ (Table 3). Compared with the effect of double combined agents in which 83/ had MIC (62.5 μ g/ml) (Table 2). This result confirmed the phenomena of antisalmonella synergism between the drugs.

Table 3

The anti salmonella MIC values of triple combination between antimicrobial drugs & medicinal plant extract

triple combined agents					inhibitory activity against salmonella										
Anti microbial 1	1/3 stock dose	Anti microbial 2	1/3 stock dose	Medicinal plant 3	1/3 stock dose	No. of tube	1	2	3	4	5	6	7	8	9
	µg/ml		µg/ml	3	µg/ml	Dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
						Concentration µg/ml	1000	500	250	125	62.5	31.25	15.62	7.81	3.96
Amoxicillin		TSM		Allium sativum			+	+	+	+	+	+	+	+	
Amoxicillin		ciprofloxacin		Allium sativum			+	+	+	+	+	+	+	+	
Amoxicillin		ciprofloxacin		Punica granalum			+	+	+	+	+	+	+	+	
Amoxicillin		chloramphenicol		Punica granalum			+	+	+	+	+	+	+	+	+
TSM		ciprofloxacin		Allium sativum			+	+	+	+	+	+	+	+	
TSM		ciprofloxacin		Punica granalum			+	+	+	+	+	+	+	+	

There was a highly significant potentiation by triple combination at $p < 0.05$ in comparison with double combination

InVivo study had shown that all the clinical features and symptoms of the rabbits had a significant response to therapy and 100% of the rabbits showed improvement at the end of the second week (Tables 4,5). Different studies had documented that defervescence upon chloramphenicol therapy occurred between 2-7 days of enteric fever by lysis⁽¹¹⁾⁽¹²⁾.

(Table 4)

Shows the daily percentage of diarrhea along the treatment course.

Days	Percentage of diarrhea %				
	A	B	C	D	E
1	0	100	100	100	100
2	0	100	90	80	40
3	0	100	80	40	20
4	0	80	60	20	10
5	0	60	30	10	0
6	0	40	20	0	0
7	0	40	20	0	0
8	0	30	10	0	0
9	0	30	0	0	0
10	0	30	0	0	0
11	0	20	0	0	0
12	0	20	0	0	0
13	0	20	0	0	0
14	0	10	0	0	0
15	0	10	0	0	0

(Table 5)

Shows the daily percentage of fever along the treatment course.

Days	Percentage of fever %				
	A	B	C	D	E
1	0	100	100	100	100
2	0	100	100	80	90
3	0	100	90	40	60
4	0	100	60	20	40
5	0	100	40	10	10
6	0	100	20	0	0
7	0	100	10	0	0
8	0	100	0	0	0
9	0	90	0	0	0
10	0	90	0	0	0
11	0	80	0	0	0
12	0	80	0	0	0
13	0	90	0	0	0
14	0	80	0	0	0
15	0	80	0	0	0
16	0	90	0	0	0
17	0	90	0	0	0

The combination of chloramphenicol with amoxicillin and p.granatum extract (Group 6) showed 100% cessation of the complaints compared with 70% improvement in chloramphenicol alone (Group c) after the end of the second week.

The laboratory findings of salmonellosis induced rabbits had shown a decrease in their HB levels in 80% of them due to the consumption of salmonella to the iron. In general there was minor improvement in the blood hemoglobin level after the end of the second week of therapy. Rgarding the WBC count after induction of salmonellosis the picture in the present study does not differ from that commonly seen in

typhoid fever of human in producing leucopenia⁽¹³⁾. The negativity of blood culture at the end of the second week, in response to therapy was 100% in (Groups C,D,E) as compared with 50% negative blood cultures in (Group B) (Table 4). However there was no significant difference between double combined treatment (Group D) and triple combined treatment (Group E) in blood picture findings since rabbits showed negative Widal test prior to induction of salmonellosis which changed to positive titer 1:160⁽¹⁴⁾ later. Urine cultures were negative from the start of the induction throughout the two weeks while human urine cultures changed to 30% positive⁽¹⁵⁾.

The postmortem autopsy (Group B) had shown different complications include hemorrhages, perforation, bone and joint infection, cholecystitis, myocarditis, meningitis and nephritis which equal to 66% while the treated (Group E,D) complications reached 5% .

Finally using p.granatum extract combined with antimicrobial drugs can further give more protection against systemic and organic complication of salmonellosis.

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