Thi-Qar Medical Journal (TQMJ): Vol.(8), No.(1), 2014
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Evaluation of Cephalopoda extract against some nosocomial bacterial isolates

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ABSTRACT:

To evaluate the pathogenicity of *Staphylococcus aureus* and *Escherichia coli* in male mice and to compare between antibacterial activity Cefotaxime 250mg and cephalopoda extract (*Sepia* sp.) and showed the effect on some blood parameters in male mice. The mice were divided into seven groups (six mice for each) ,all treatments were given intraperitoneally to experimental mice. First group (control animals) were injected with 0.9 % normal saline (0.1 ml for each animal), second group were received i.p. single dose of *E. coli* (0.1×10^8 CFU), third group were injected with a single dose of *S. aureus* (0.1×10^8 CFU), fourth group were injected with a single dose of *E. coli* (0.1×10^8 CFU) then treated with (0.1 Cefotaxime 250mg) for 3 days fifth group were injected with a single dose of *S. aureus* (0.1×10^8 CFU) then treated with (0.1 Cefotaxime 250mg) for 3 days, sixth group were injected with a single dose of *Escherichia coli* (0.1×10^8 CFU) then injected with (0.1 of extract,720 µg for each animal) for 3 days, seventh group were injected i.p. with a single dose of *S. aureus* (0.1×10^8 CFU) then injected with (0.1 of extract, 720 µg for each animal) for 3 days. The results conducted that all infected mice were suffered from elevated in their body temperatures, while decline in their body weights and subsequently, changes in blood parameters compared with normal value. On the other hand, treated mice with Sepia extract show healthy and maintained their body temperatures and body weights as normal, in addition to blood parameters remained within normal ranges. These above results explained the role of Sepia extract as antimicrobial substance, acting against nosocomial bacterial isolates.

Introduction:

Escherichia coli is a member of the normal intestinal flora, but when transmitted from normal habitat will be considered as pathogens which caused several clinical important diseases, represented by diarrhea disease, septicemia and meningitis (Geo *et al*., 2004).*E. coli* have ability to invasive from natural habitat to different body sites and subsequent caused several medical important diseases, represent by urinary tract infection, diarrhea and septic conditions (Hashim, 2005). Most investigations explained the bacterial infections, which occurred due to nosocomial- acquired infections, especially Gram-negative and Gram-posative pathogens (Founior and Philpott, 2005).*Staphylococcus aureus* pathogen causing significant morbidity and mortality in both community and hospital acquired infections (Lowy, 1998). It causes a diverse array of infections from relativity minor skin and wound infections to more serious and life threatening disease such as pneumonia,endocarditis,osteomyeliti, arthritis and sepsis (Nilsson *et al* .,1998). On other hands, Methicillin resistance *S. aureus* (MRSA) is the major focus of public awareness of healthcare-associated infection (HCAI) problems in many countries (Petra *et al*.,2012). Most Gram-positive bacteria such as *Enterococcus* spp. ,*Staphylococcus aureus*, and *Streptococcus pyogenes* survive for months on dry surface, also many Gram-negative species such as *Acinetobacter* spp., *E.coli*, Klebsiella spp., *Pseudomonas aeruginosa*, *Serratia marcescens* and *Shigella* spp. can survive on inanimate surfaces even for month. These species found among the most frequent isolates from patients with nosocomial infections (Axel *et al*., 2006).

There is an everlasting need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to the alarming increase that has been witnessed in the incidence of both new and re-emerging infectious diseases. The

increasing resistance of antibiotics by the pathogenic microorganisms develops the demand for the isolation of novel alternative antimicrobial substances (Obeidat*et al*., 2012). A further big concern is the development of resistance to the antibiotics in current clinical use (Ilhan *et al*., 2007).The Class: Cephalopoda includes nautilus, cuttlefishes, squids and octopods which are exclusively marin e, varying in their form, size and nature (Worms, 1983). Marine invertebrates offer a good source of potential antimicrobial drugs (Mayer *et al*.,2007; Jayaraj *et al*., 2008). Many studies on bioactive compounds from molluscs exhibiting antibacterial, anti-leukemic and antiviral activities have been reported (Rajaganapathy *et al*.,2000). Proteins and glycoproteins with antibacterial activity have been demonstrated in the different organs of molluscs (Pakrashi, 2001).

` The antimicrobial activity of polysaccharides extracted from cephalopods such as *Sepia aculeate* and *Sepia brevimana* and heparin and heparin – like glycosaminoglycans (GAGs) from the cephalopod *Euprymnaberryi* was reported against the human pathogenic microorganism (Shanmugam *et al*., 2008a; Shanmugam *et al*., 2008b). The aim of this study to investigate the pathogenicity of *Staphylococcus aureus* and *Escherichia coli* in male mice and to compare of antibacterial activity between Cefotaxime 250mg and cephalopoda extract (*Sepia* sp.) and showed the effect on some blood parameters in male mice.

Materials and methods:

Bacterial isolates:

Tested bacteria were obtained from Al-Hussain Teaching Hospital ,and they identified by API System (Steve and Dennis 2001).

Bacterial infectious doses: The infections doses of *Staphylococcus aureus* and *Escherichia coli* were 10^8 CFU (Nathan *et al*., 2000; Victor *et al*.,2007 ; Shigenobu *et al*., 2012). Isolated bacterial were inoculated in nutrient broth for 48 hours at 37° c, the centrifuged (2500 rpm\5 minutes), then comparing turbidity of the test suspension with (0.5×10^8 CFU) standard tube (Anandia and Juncarb, 2009).

Animals :

White albino male mice (25-35 grams) were obtained from the animal house at the Science college in Thi-Qar University. The mice were housed in standard metal cages (6 mice/cage).

Experimental design:

The mice were divided into seven groups comprising six animals in each group. All treatments were given intraperitoneally to experimental mice.

1-First group (control group)were injected with 0.9 %normal saline (0.1ml for each animal).

2-Second group were received i.p. single dose of *Escherichia coli* (0.1for each animal).

3-Third group were injected i.p. with a single dose of *S. aureus* (0.1for each animal).

4-Fourth group were injected i.p. with a single dose of *Escherichia coli* (0.1for each animal) then treated i.p. with (0.1 Cefotaxime 250mg) for 3 days.

5-Fifth group were injected i.p. with a single dose of *S. aureus* (0.1for each animal) then treated i.p. with (0.1 Cefotaxime 250mg) for 3 days.

6-Sixth group were injected i.p. with a single dose of *Escherichia coli* (0.1for each animal) then injected i.p. with (0.1 of extract 720 µg for each animal) for 3 days.

7-Seventh group were injected i.p. with a single dose of *S. aureus* (0.1for each animal) then injected i.p. with (0.1 of extract 720 µg for each animal) for 3 days.

The effective dose of *Sepia* sp. extract (720 µg) was determined according to (Litchfield and Wilcoxon,1949).

Collection of Blood: Blood was collected from heart from each animal after three and seven days post-treatment in tubes with EDTA then used for the determination many blood parameters: white blood cells number, HCT,PDW, MCH,RDW, MPV,PCT,MCV, platelet.

Results:

The results showed mice injected with pathogenic bacteria have a significant decrease in their body weight compared to the control, especially infected by *E.coli* (P: 0.0008),while there was no significant change in the body weight of mice

that treated with extract and antibiotic (Table 1).

Table 1: Average weight of an experimental mice before and after infection

Animal groups	Average weight/gm/ Before	Average weight/gm/ after
Control	**27.8**	**28.1**
***E. coli*+ antibiotic**	**27.6**	**27.3**
***S. aureus*+ antibiotic**	**27.8**	**27**
S. aureus	**29.9**	**24**
E. coli*	**29.5**	**23**
***E.coli*+ extract**	**29.5**	**29.4**
***S. aureus*+ extract**	**27.9**	**27**

Mice infected by E.coli p value: 0.0008 (body weight)

From table 2 showed increases in the body temperature of infected mice groups from 37˚C into 38.3- 38.5 C˚, whereas noted animals treated with extract and antibiotic have normal temperature.

Table 2:Average temperatureof an experimental micebefore and after infection.

Average temperature	Control	*S. aureus*	*E. coli*	*E. coli*+antibiotic	*S. aureus*+antibiotic	*E.coli*+extract	*S. aureus*+extract
Before	37̊c	37̊c	37̊c	37̊c	37̊c	37̊c	37̊c
After	37̊c	38.5 ̊c	38.3 ̊c	37̊c	37.2̊c	37̊c	37̊c

Re-isolation of *E. coli* and *S. aureus* from an experimental mice

Table 3 showed re-isolation of *E. coli* and *S. aureus* after 3 and 7 days from liver and spleen of control and infected groups. The re-isolation percentages of tested bacteria appeared as 100% in liver and spleen after 3 days while the percentage decrease to 25% in liver and spleen after 7 days.

Table 3: Re-isolation of *E. coli* and *S. aureus* from infected mice organs of *E. coli* and *S. aureus* after 3 and 7 days of infections

organs	Control	*S. aureus* after 3 days	*E. coli* after 3 days	*S. aureus* after 7 days	*E. coli* after 7 days
Liver 1	-	+ (100%)	+ (100%)	+ (25%)	+ (25%)
Spleen 1	-	+ (100%)	+ (100%)	+ (25%)	+ (25%)
Liver 2	-	+ (100%)	+ (100%)	+ (25%)	+ (25%)
Spleen 2	-	+ (100%)	+ (100%)	+ (25%)	+ (25%)
Liver 3	-	+ (100%)	+ (100%)	+ (25%)	+ (25%)
Spleen 3	-	+ (100%)	+ (100%)	+ (25%)	+ (25%)

The re-isolation percentage of *E. coli* and *S. aureus* after 3 days from liver and spleen of groups that received antibiotic no detectable growth except animal 2 and 3 that have percentage 25% (Table 4). On the other hand the same table explain, groups were received *Sepia* sp. extract have no detectable growth of *E. coli* and *S. aureus*.

Table4: Re-isolation of *E. coli* and *S. aureus* from infected mice organs of *E. coli*, S. aureus+ antibiotic and *E. coli*, *S. aureus* + extract groups

organs	*E. coli*+ antibiotic	*S. aureus*+ antibiotic	*E.coli*+ extract	*S. aureus*+ extract
Liver 1	-	+ (25%)	-	-
Spleen 1	-	+ (50%)	-	-
Liver 2	-	+ (25%)	-	-
Spleen 2	+ (25%)	+ (50%)	-	-
Liver 3	+ (25%)	+ (25%)	-	-
Spleen 3	+ (25%)	+ (25%)	-	-
Liver 4	-	-	-	-
Spleen 4	-	-	-	-
Liver 5	-	-	-	-
Spleen 5	-	-	-	-
Liver 6	-	-	-	-
Spleen 6	-	-	-	-

Blood parameters:

The results showed no a significant difference in the hematological composition of the blood parameters between the control group and groups treated with extract and antibiotic (Table 5), in the same table showed the groups that injected with pathogenic bacteria have increased of white blood cells specially lymphocytes and less number of monocytes and granulocytes ,and showed the same groups have decreasing in level of HCT,PDW, MCH. On the other hand there is increasing in level of RDW, MPV,PCT,MCV, platelet numbers.

Table (5): Effect of *Sepia* sp. extract on some blood parameter in male mice.

	Control	*E.coli*	*S. aureus*	*E.coli* +Extract	*S. aureus* +extract	*E.coli* +antibiotic	*S. aureus* +antibiotic
WBC	4.8	13.5	12.47	5.1	4.1	5	5.6
Lym	1.80	12	11	4.2	3	4	4.1
Mon	0.20	0.55	0.62	0.46	0.5	0.4	0.55
Gran	0.40	0.95	0.85	0.46	0.6		
HCT	36.4	58.7	59	35.4	36.5	38	39
MCV	50	67	62	52.3	50	55	51
MCH	15.4	11.3	12.6	18	15	16	15.9
MCHC	31	28.9	28.9	31.4	30		
RDW	17.6	24	20.2	16.1	15.7	16.2	17
Plt	620	898	1172	567	527	520	511
MPV	7.6	16	18.9	6.9	7	7.4	7.1
PCT	0.47	0.77	0.83	0.39	0.5	0.4	0.32
Pdw	13	2.7	6.4	12.4	11.1	13	12

Discussion:

In this study, there are statistically significant correlation between body temperature, body weight and bacterial infection. Elevated body temperature could be considered as one of the index parameter for the evaluation at bacterial infection (Deirdre *et al*.,2006). In addition to that, there is detectable of body weight loss before and after experimental infection, the same finding were recorded by other researchers (Lars *et al*.,2005).

The re-isolation percentages of tested bacteria appeared as 100% in liver and spleen after 3 days whereas reduced to 25% after 7 days. Present results, revealed a scientific fact that immune response formed against the bacterial inoculate, and this immune state reduce the risk of septicemia and death. This results was consistent with other results recorded by Cryz*et al*., (1983); Hassan, (2008) and Manati *et al*.,(2009).The animals that treated with extract have no detectable growth in these groups, the antibacterial activity of extract due to the extract rich with protein an amino acid specially prolein (Degaim, 2009).Degaim and Abbas, (2010) showed the antibacterial activity of *Sepia* sp. extract *in-vitro* on human pathogenic bacteria. The comparison between the effect of extract and antibiotic on re-isolation pathogenic bacteria showed the extract has best effect than antibiotic.

The results were agreement with Patterson Edward and Murugan (2000) and Patil*et al*.,(2001) reported that the ink extracts showed antibacterial activity, and showed the maximum antibacterial activity of ink extract against *Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *E.coli.*Antibacterial activity has previously been described in a wide range of molluscan species such as muricid mollusks(*Dicathaisorbita*) and sea hare (*Dolabellaauricularia*) (Andersonand Beaven, 2001; Benkendorff*et al*., 2001). In most ofthe species studied, the haemolymph, egg masses or the whole body have been tested for activity. Antimicrobial peptides have been isolated and characterized from

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the haemocytes of *Mytilusedulis* (Mitta*et al*., 2000a) and *M. galloprovincialis* (Mitta *et al*., 2000b), and from these a hare *Dolabella auricularia* (Iijima*et al*., 2003). (Vairamani*et al*., 2012) noted antimicrobial activity of Cuttlebone of *Sepiellainermis* on many pathogenic bacteria.Mean values of MCV, MCH and MCHC decreased with respect to time but this reduction was relatively more in mean MCV as compared to others.

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Thi-Qar Medical Journal (TQMJ):Vol(8) No(1) 2014(25-38)

تقيم خلاصة قدمية الرأس ضد بعض العزولات البكتيرية المسببة لأمراض المستشفيات

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الخلاصة

لتقيم خلاصة قدمية الرأس ضد بعض العزولات البكتيرية مثل الأشريشيا القولونية والمكورات العنقودية الذهبية والتي عزلت من مستشفى الحسين التعليمي. حيث تم دراسة الفعالية الدوائية للمضاد الحيوي cefotaxime

وخلاصة قدمية الرأس وأيضا تم دراسة هاتين المادتين على بعض معايير الدم.لهذا الغرض حقنت ذكور الفئران في البريتون وقد قسمت الى سبعة مجاميع(ستة فئران لكل مجموعة). المجموعة الأولى وهي مجموعة السيطرة حيث حقنت بمحلول الملح الفسيولوجي وبجرعة 0.1ml . المجموعة الثانية حقنت ببكتريا الأشريشيا القولونية وبجرعة ($0.1\ ml \times 10^8$ CFU) .المجموعة الثالثة حقنت ببكتريا المكورات العنقودية وبجرعة $0.1\ ml \times 10^8$ (CFU) . اما المجموعة الرابعة فقد حقنت ببكتريا الأشريشيا القولونية وبنفس الجرعة ثم عولجت بالمضاد الحيوي cefotaxime وبجرعة 0.1 ml ولمدة ثلاثة أيام. المجموعة الخامسة فقد حقنت ببكتريا المكورات العنقودية الذهبية وبنفس الجرعة السابقة ثم عولجت المجموعة بنفس المضاد الحيوي لمدة ثلاثة أيام متتالية. اما المجموعة السادسة فقد حقنت ببكتريا الأشريشيا القولونية المذكورة اعلاه ومن ثم حقنت بمستخلص قدمية الراس وبمقدار 0.1 ml (720 µg) ولمدة ثلاثة ايام. المجموعة الأخيرة حقنت ببكتريا المكورات العنقودية وبجرعة $0.1\ ml \times 10^8$ CFU ومن ثم حقنت بمستخلص قدمية الراس وبمقدار 0.1 ml (720 µg) ولمدة ثلاثة ايام.لقد أظهرت النتائج ان جميع الحيوانات المصابة بالبكتريا المرضية قد عانت من ارتفاع في درجات حرارة اجسامها من ناحية اخرى هناك نقص في اوزانها ومن ثم حصلت تغيرات في بعض معايير الدم. اما الفئران الواتي حقنت بمستخلص قدمية الرأس كانت سليمة حيث حافظت على درجات حرارة اجسامها واوزانها كذلك بقيت قيم المعايير الدموية ضمن مستوى القيم الطبيعية . ومن خلال هذة النتائج تم الاستدلال على الدور الدوائي والفعالية الدوائية لمستخلص قدمية الرأس ضد العزولات البكتيرية المسببة لأمراض المستشفيات.

Evaluation of Cephalopoda extract against some nosocomial bacterial isolates

Average temperature	Control	*S. aureus*	*E. coli*	*E. coli*+antibiotic	*S.aureus*+antibiotic	*E.coli*+extract	*S. aureus*+extract
Before	37; 37;37.3	37 ; 37 ; 37	37 ; 37 ;	37 ; 37 ; 37	37ċ	37 ; 37 ; 37	37 ; 37 ; 37

			37				
After	37; 37 ; 37.2(NS)	38; 38.5; 39.5(ns)	38 ; 39 ; 38	37, 37.5, 37(ns)	37.3; 37.2; 37	37 ; 37 ; 37.2(ns)	37 ; 37 ; 37(ns)

Animal groups	Average weight/gm/ Before	Average weight/gm/ after
Control	**27.8; 27; 28.2**	**28; 29; 27.7p**=0.3437 (ns)
***E. coli*+ antibiotic**	**27; 28;28**	**27.3; 27; 27.7p**=0.4411 (ns)
***S. aureus*+ antibiotic**	**27.2; 28.8; 26**	**27, 28; 26p**=0.7546 (ns)
S. aureus	**30; 31; 29.9**	**24**
E. coli	**29.5; 30.5; 29**	**23,22,24p**= 0.0008Yes
***E.coli*+ extract**	**29.5; 30; 29.6**	**29; 30; 29.8p**=0.5780(ns)
***S. aureus*+ extract**	**27; 28; 27.8**	**27; 27; 27.2p**=0.5780(ns)