Egypt. J. Chem. **Vol. 65,** No. 3 pp. 739 - 750 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Mechanism Of Antibacterial Effect For Different Venoms (*Naja Haje, Cerastes Cerastes, Apis Mellifera*) On *Staphylococcus Aureus*

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Abstract

The overuse and misuse of antibiotic without going to doctor leading to antibiotic resistant strains that represent a serious problem in the field of health protection, *Staphylococcus aureus* (*S. aureus)* remains one of the main causes for hospital infections specially in the intensive care unit (ICU), so that many researches directed to natural products as alternative antibiotic drug, nowadays special attention towards venoms as (elapids, viprides, honey bee venom, etc….) which contain molecules with antibacterial properties such as phospholipase A2 (PLA2), L-amino acids (LAAO) and melittin. The present study aimed to evaluate *in vitro* and *in vivo* antibacterial effect of three different venoms the Egyptian Cobra (*Naja haje*), the horned viper (*Cerastes cerastes*) and honey bee (*Apis mellifera*) on *S. aureus*. There were tested *in vitro* by Minimum inhibitory concentration (MIC) test and disc diffusion method. *In vivo* evaluation was performed by infecting groups of mice by *S. aureus*, then treated with these three venoms and standard antibiotic. The hematological studied showed that effect on Hb and RBCs not all groups like others compared to control. Biochemical analysis of mice sera, liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and kidney function (urea and creatinine) as well as cardiac enzyme creatinine phosphokinase (CPK) showed that all treated and untreated groups in biochemical studies recorded a significant increase in all liver, kidney and cardiac functions. Histological examination illustrated that liver and kidney tissue in untreated group with inflammatory cells infiltration, degree of inflammation decrease in both *N. haje* and *C. cerastes* groups, while *A. mellifera* and antibiotic groups approximately normal in both mice liver and kidney.

Keywords**:** *A. mellifera, N. haje, C. cerastes, S. aureus, In vitro, In vivo, Biochemical*

Introduction

One of the most important clinical problem in the field of microbiology today is the great growing resistance to antibiotics in bacteria. *S. aureus* is a gram-positive coccus binds to bone sialoproteins causing acute bacterial arthritis which is a glycoprotein found in joints [1] nasal and throat of healthy individuals [2], skin [3]. Invasive *S. aureus* infections are a leading cause of morbidity and mortality rates up to 30% in both hospital and community settings, especially with multi-drug resistant methicillin-resistant *S. aureus* strains MARSA [4] leading to sepsis. Due to antibiotic resistance crisis, an urgent need exists for new strategies to fight *S. aureus* resistance. Venoms from different animal sources such as bees, snakes, and scorpions have great biological importance in medical field and their peptides represent potent antimicrobial agents against various microbial pathogenesis [5]. Many studies found that snake venoms, the Egyptian cobra (*Naja haje*) and the horned viper (*Cerastes cerastes*) have molecules such as phospholipases A2 (PLA2) and L-amino acids oxidases (LAAO), have bactericidal properties [6]. As well the Egyptian honey bee (*Apis mellifera*) has a high biological and pharmacological activity and is the best form for potential antibacterial ability [7,8]. The antibacterial action of bee venom belongs to the main components, phospholipase A2, apitoxin, and melitin, alone and in a mixture, have been evaluated against several pathogens [9]. Antimicrobial peptides (AMPs) are attractive candidates as therapeutic agents, as they can kill a broad range of bacteria including antibiotic-resistant strains also do not tend to develop drug resistance [10], both melitin and phospholipase A2 serve as AMPs, that can replace divalent cations such

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Receive Date: 30 August 2021, Revise Date: 15 September 2021, Accept Date: 16 September 2021

DOI: 10.21608/EJCHEM.2021.93083.4402

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as Mg2+ and Ca2+ bound to lipopolysaccharide (LPS), leading to membrane disruption causing bacterial death [11] that can attack DNA and RNA to inhibit protein synthesis [12].

This study aimed to evaluate *in vitro* and *in vivo* antibacterial effect of three different venoms *(N. haje, C. cerastes* and *A. mellifera*) on *S. aureus.*

2. MATERIALS AND METHODS

Venom: Lyophilized crude venoms (Egyptian cobra (*N. haje*), the horned viper (*C. cerastes*) and honey bee venom (*A. mellifera*) were obtained from the Egyptian organization of biological products and vaccines (VACSERA), Doki, Giza. Venoms stored at 4˚C until used. LD_{50} of them were calculated according to Reed and Muench, [13]

ANTIMICROBIAL SUSCEPTIBILITY TEST (DISC DIFFUSION METHOD):

Antibacterial activities of tested venoms were evaluated using disc diffusion method [14] in compared to standard antibiotics as positive control. A loopful from the overnight growth of *S. aureus* (ATCC 6538) was transferred to 150 ml Mueller-Hinton Broth (MHB) medium incubated at 37°C with vigorous shaking (150 rpm) for 18 hr before adjusting concentration to 0.5 McFarland standards [15]. The surface of the plates was uniformly covered with 0.5 McFarland *S. aureus,* using sterile filter paper discs (7-mm diameter) were placed on the surface of the plates after loaded with 200µg\disc of each tested venoms (*N. haje, C. cerastes and A. mellferia*) venoms. Discs of standard antibiotics (Oxoid) like erythromycin and Ampicillin as positive drug control. The plates were incubated at 37°C for 18-24 hrs and examined for the presence of zone of inhibition for bacterial growth around the disc that were measured in millimeter. The experiments were performed at least five times. The means and standard deviations of the data were collected [16]

MEASUREMENT OF THE MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC test was performed according to the National Committee for Clinical Laboratory Standards (NCCLS), 0.5 McFarland standards inoculated with equal volume of serial dilutions of the tested three venoms (*N. haje, C. cerastes and A. mellferia*) also antibiotic were prepared from 80µg venom /ml of saline to 1.25 µg venom /ml of saline into each well of a microtiter plate were added in triplicate [17], negative control wells were included. Plates were incubated at 37°C for 24hr. O.D was measured at 600 nm using ELISA reader [18]. MICs were taken as the lowest concentration of tested venoms that inhibited visible growth and then made sure the growth of bacteria to determine the final minimum bacterial concentration (MBC).

MICE AND PREPARATION OF BACTERIAL CELLS FOR INFECTION

Single colony of *S. aureus* bacteria was selected and incubated in MHB for 16 h in shaker incubator at 37°C. the proliferated bacteria were collected by centrifugation at 4000 g for 10 min. and the bacterial pellet washed three times with sterile phosphate-buffered saline (PBS). Then adjusted to 1.5×10^9mL CFUs at 600 nm using a spectrophotometer. The mice were injected intraperitoneally (i.p) with 1.5×10^8 mL CFUs of *S. aureus* [19].

Male BALB/c mice 6-10 wk-old and weigh 15-20gm were supplied by the animal facility of VACSERA and kept under aseptic conditions at 25˚ C, 12hr/day of light / day, controlled humidity (60–80%) and temperature (22 ± 1°C), sterilized pelleted food and acidified tap water, Proposal was approved by the Ethical authorities (CU-1-S-57-17 approved by Institutional Animal Care and use committee CU-IACUC in Cairo University). The safety and efficacy of the tested venom was determined by the recommended dose of (1/10 LD_{50}) [20-21], was 0.106 for *N. haje*, 0.535 for *C. cerastes* and 9.02 for *A. mellifera* µg/ Kg body weight of mouse. Tested BALB/c mice were divided into 6 groups 10 mice/group as following: the 1st group severed as control group, the 2nd group was infected with *S. aureus* bacteria and untreated [22], the 3rd group was infected and treated by *N. haje* venom, the 4th infected group treated by *C. cerastes* venom and the 5th infected group treated by *A. mellifera* venom. Treatment of the three venoms at 1/10 LD_{50} of each venom, the 6th infected group treated by cefotaxime as standard antibiotic treatment. Infection and treatment were done by i.p. injection, treatment repeated 3 times as 24hr interval. All the animals were bled by retro-orbital vein and blood samples were collected and divided into two volumes: EDTA blood samples were analyzed for complete blood picture and sera were separated by centrifugation at 3000 rpm for 15 min. The labeled sera samples were kept at -20˚C until used for biochemical analysis.

BIOCHEMICAL EXAMINATIONS: Renal function was investigated by quantitative estimation of creatinine and urea using commercial kit from Diamond Diagnostics, Egypt. according to Kaplan and Murray [23-24]. Liver functions were estimated by

serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities [25], also activity of serum creatinine phosphokinase (CPK total) [26] as cardiac function using Biosystems S.A. kit.

HEMATOLOGICAL EXAMINATION: blood samples with EDTA were used for quantitative hemoglobin using Diamond Diagnostics kit [27], manual erythrocytes count: hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) according to Cheesbrough [28]. Manual platelets and leukocytes count (WBCs) were measured according to Becton - Dickinson [29].

HISTOLOGICAL STUDIES: Mice (4/each group) livers and kidneys samples were collected and fixed in 10% neutral buffered formalin, washed in tap water then exposing to ascending concentrations of ethanol (70, 80, 90 and 100%) for dehydration. Specimens were cleared using xylene and embedded in paraffin. Tissues sections (4-5 µ thickness) using microtome. Tissue sections were deparaffinized, and stained with hematoxylin and eosin stain for subsequent histopathological examination **[30].**

STATISTICAL ANALYSIS:
All numerical values were expressed as mean ± Standard error (SE) of mean. They were calculated by the use of Microsoft Excel 2007 and comparisons between each group and control were done using Student's t-test values was performed using the statistical program SPSS version 17 and $P \leq 0.001$ is considered highly significant, $P \leq 0.05$ is considered significant and $P > 0.05$ is considered non-significant, *P* versus control.

1. RESULTS

3.1. DISC DIFFUSION METHOD: After testing purity as shown in figure (1). Figure (2) and table (1) illustrated the effect of *N. haje, C. Cerastes, A. mellifera* venoms and antibiotics (Ampicillin and Erythromycin) on *S. aureus* by disc diffusion method. The result showed Highly effective AMP (Ampicillin) and E (Erythromycin) antibiotic produced a wide ring of inhibition zone (27, 25 mm), while the *N. haje, C. Cerastes and A. mellifera* venoms, respectively recorded an intermediate inhibition zone (17,15 and 15 mm) on the *S. aureus* bacterial growth.

3.2. THE MINIMUM INHIBITORY CONCENTRATION (MIC):
As represented in table (2), the MIC of the *N. haje, C. cerastes and A. mellifera* venoms against *S. aureus* showed that MIC of *N. haje* (14.4), *C. cerastes* (62.5) and *A. mellifera* venoms (31.7) ug/ml, respectively which indicating that *N. haje* and *A. mellifera* are the most effectiveness against *S. aureus* strain than *C. cerastes* venom.

3.3. BIOCHEMICAL EXAMINATIONS:
This study indicated that all treated or untreated groups of mice showed a highly significant increase ($P \leq 0.001$) in the liver enzymes (ALT, AST) and cardiac enzyme (CPK). Also, kidney functions (Creatinine, Urea) levels recorded the same significant increase ($P \leq 0.001$) except *A. mellifera* treated group showed non-significant difference ($P > 0.05$) compared to control group as shown in table (3).

3.4. HEMATOLOGICAL EXAMINATIONS:
In the present study, Hb (g/dl) in untreated infected and *C. cerastes* showed high significant decrease, non-significant difference in *A. mellifera* treated group while high significant increase in both *N. haje* and Antibiotic treated group compared with control. RBCs count results showed significant decrease ($P \leq 0.05$) in *N. haje* and *A. mellifera* treated groups while untreated, *C. cerastes* treated group and Cefotaxime as antibiotic treated group showed highly significant decrease compared to control group. As well as, HCT (PCV), MCH, MCV and MCHC recorded the same results. Both WBCs and platelets showed high significant increase ($P < 0.001$) in all untreated and treated groups compared to control as shown in table (4).

3.5. HISTOLOGICAL STUDIES:
Figure 3: Mice liver showed inflammatory cells infiltration diffuse kupffer cells proliferation in between the hepatocytes in untreated group, while *N. haje* treated group showing some degenerative changes and some inflammatory cells in *C. cerastes* treated group, and minor changes in *A. mellifera* treated group, while antibiotic treated group no histological alteration.

Figure 4: Mice kidney showed inflammatory cells aggregation in the cortex in both untreated and *N. haje* groups, while both *C. cerastes* and *A. mellifera* treated groups showing minor changes as some focal inflammatory cells between the glomeruli and tubules and no histological alteration.

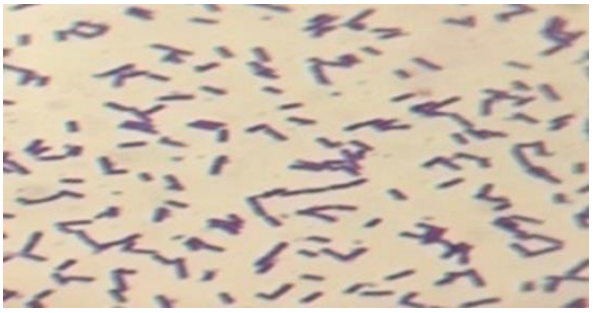

Figure 1: Pure gram (+ve) cocci *S. aureus*

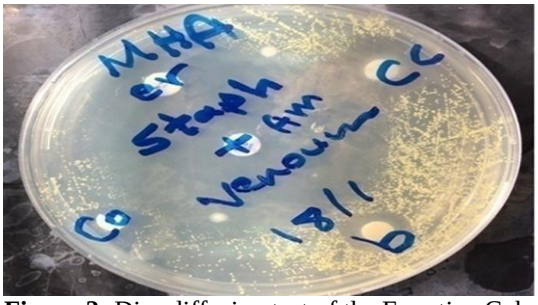

Figure 2: Disc diffusion test of the Egyptian Cobra *N. haje* (Co), the horned viper *C cerastes* (Cc), the honey bee *A. mellifera venoms* (b)

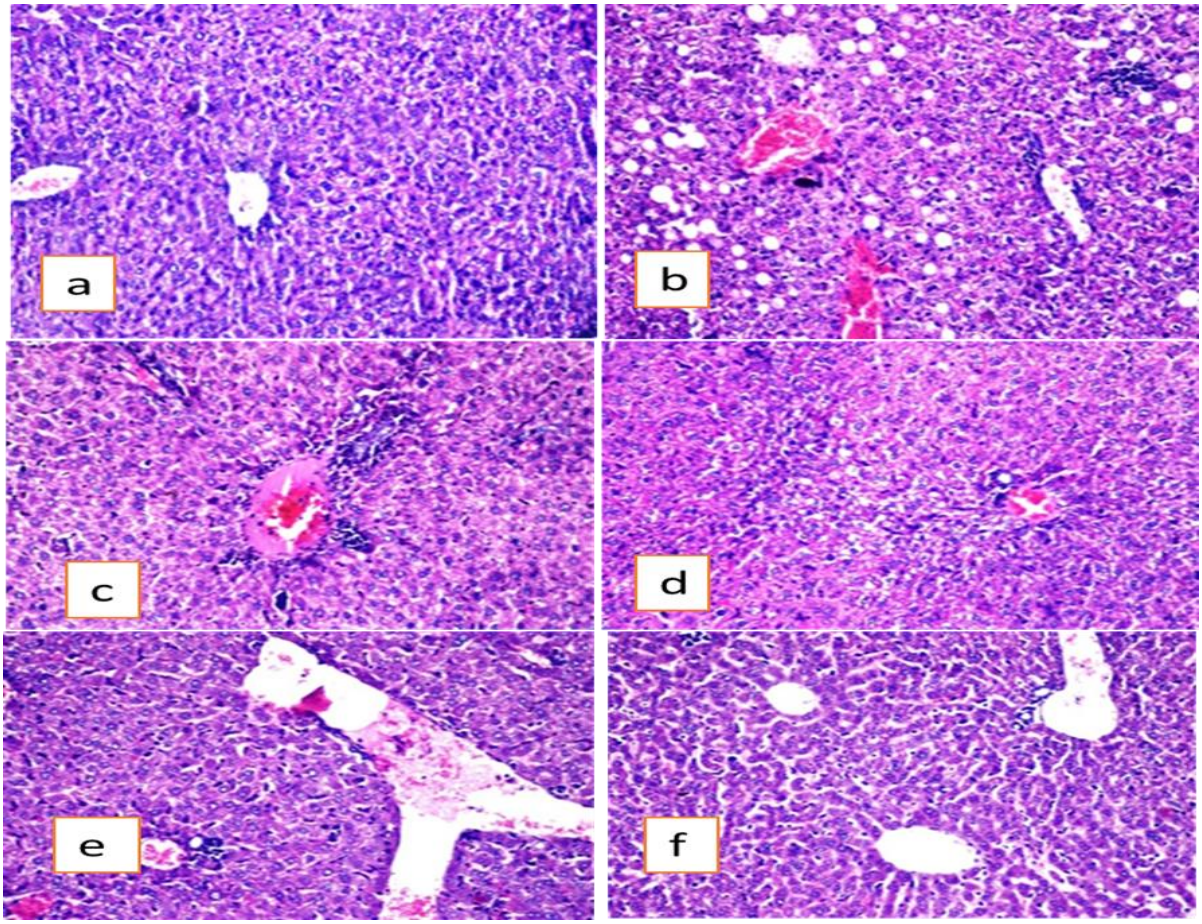

Figure 3: Histopathological observation of mice liver infected by *S. aureus* treated with *N. haje*, *C. cerastes*, *A. mellifera* venoms and antibiotic. **a:** Control mice showing normal histological structure **b:** untreated group showing inflammatory cells infiltration surrounding the congested central vein with diffuse kupffer cells proliferation in between the hepatocytes. **c:** treated group with *N. haje* venom showing degenerative change in some of the hepatocytes. **d:** treated group with *C. cerastes* showing that there was congestion in the portal vein with inflammatory cells infiltration at the portal area**. e:** treated group with *A. mellifera* venom showing dilatation in the central vein with diffuse kupffer cells proliferation in between the hepatocytes. **f:** treated group with Cefotaxime as standard antibiotics showing normal histological structure.

Table 1: Disc diffusion test of *N. haje, C cerastes, A. mellifera venoms*

Venoms	***N. haje***	***C. cerastes***	***A. mellifera***	***Ampicillin***	***Erythromycin***
S. aureus	(17± 0.14) mm	(15± 0.115) mm	(15±0.183) mm	(27± 0.7) mm	(25± 0.21) mm

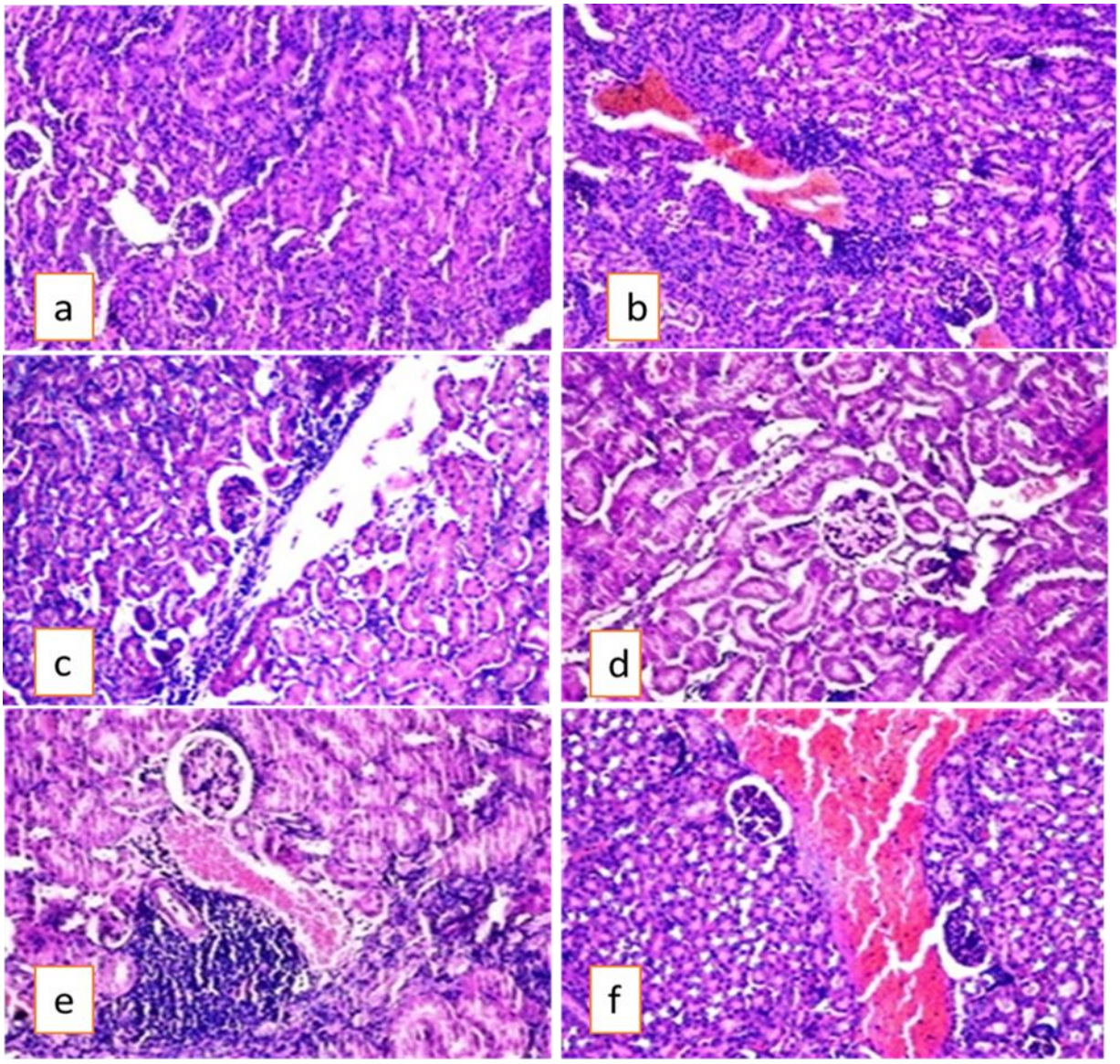

Figure 4: Histopathological observation of mice kidney infected by *S. aureus* treated with *N. haje*, *C. cerastes*, *A. mellifera* venoms and antibiotic **a:** Control mice showing normal histological structure **b:** untreated group showing focal perivascular inflammatory cells aggregation was observed surrounding the congested blood vessels at the cortex. **c:** treated group with *N. haje* venom showing inflammatory cells aggregation in the cortical portion. **d-** treated group with *C. cerastes* venom showing that few inflammatory cells infiltration was detected in focal manner between the tubules and glomeruli at the cortex **e:** treated group with *A. mellifera* venom showing focal inflammatory cells aggregation between the glomeruli and tubules with congestion in the cortical blood vessels. **f:** treated group with cefotaxime as standard antibiotics showing congestion was observed in the cortical blood vessels.

Table 2: The minimum inhibitory concentration of *N. haje, C. cerastes and A. mellifera* venoms

S. aureus	***N. haje***	***C. cerastes***	***A. mellifera***	**Cefatroxon**
MIC	14.4 µg/ ml	62.5 µg/ ml	31.7 µg/ ml	10µg/ ml
MBC	3.6 µg/ ml	31.25 µg/ ml	15.8 µg/ ml	5 µg/ ml

Table 3: Biochemical analysis (ALT, AST, creatinine, urea, CPK) of different mice groups

Type of Treatment	Control	Untreated		*N. haje*		*C. cerastes*		*A. mellifera*		Antibiotic	
Parameters	**m±SE**	**m±SE**	**%change**	**m±SE**	**%change**	**m±SE**	**%change**	**m±SE**	**%change**	**m±SE**	**%change**
ALT U/L	26.8 ± 0.19	79.2±0.39 ***	195%↑	44.8±0.16 ***	67.2%↑	56.8±0.54 ***	112%↑	30.4±0.49 ***	13.4%↑	38.2±0.44 ***	42.5%↓
AST U/L)	37.4 ± 0.28	179.9±0.52 ***	381%↑	99.7±0.35 ***	166.5%↑	131.37±0.25 ***	251%↑	46.53± 0.53 ***	24.4%↑	67.77±0.76 ***	81.2%↑
Urea mg/dL	45.64±0.27	92.84±0.84 ***	103%↑	84.9±0.35 ***	86%↑	64.1±1.23 ***	40.45%↑	46.11±0.36 *	1%↑	47.97±0.51 **	5%↑
Creatinine mg/dL	0.62± 0.01	1.54±0.03 ***	150.2%↑	1.57±0.02 ***	156%↑	1.39±0.04 ***	127%↑	0.62 ±0.01 *	2%↑	1.05±0.04 ***	71.2%↑
CPK U/L	71.7±1.59	253.2±2.17 ***	253%↑	204.1±3.15 ***	184.6%↑	184.7±2.86 ***	157.6%↑	102.9±2.96 ***	43.5%↑	170.5±1.38 ***	137.7%↑

$P \leq 0.001$ is considered highly significant *(***), $P \leq 0.05$* is considered significant *(**), $P > 0.05$* is considered non-significant *(*), P* versus control

Table 4: Blood profile of *S. aureus* infected mice groups treated by $^{1}/_{10}$ LD_{50} *N. haje*, *C. cerastes*, *A. mellifera* venoms and Cefotaxime as standard antibiotic**.**

Type of Treatment	Control	Untreated		*N. haje*		*C. cerastes*		*A. mellifera*		Antibiotic	
Parameters	**m±SE**	**m±SE**	**%change**	**m±SE**	**%change**	**m±SE**	**%change**	**m±SE**	**%change**	**m±SE**	**%change**
RBCs ($x10^6$/cmm)	7.05 ± 0.14	6.14±0.09 ***	12.9%↓	6.59±0.11 **	6.5%↓	5.40±0.096 ***	23.4%↓	5.93±0.02 **	14.1%↓	6.12±0.01 **	11.3%↓
Hb (g/dl)	11.04 ± 0.07	9.08±0.05 ***	17.8%↓	12.10±0.09 ***	9.6%↑	7.26±0.16 ***	34.2%↓	11.07± 0.015 *	0.27%↓	12.40±0.051 ***	11.7%↑
PCV (%)	35.44±0.29	33.82±0.22 **	3.2%↑	40.90±0.35 ***	15.4%↑	30.46±0.32 ***	14.05%↓	31.40±0.13 ***	8.85%↓	33.82±0.05 *	1.83%↑
MCV (Fl)	50.30± 0.55	59.60±1.56 ***	18.5%↑	62.44±0.97 ***	23.26%↑	56.30±1.48 ***	11.93%↑	52.93±0.26 ***	6.07%↑	55.30±0.51 ***	10.8%↑
MCH (Pg)	15.66±0.15	14.80±0.03 **	5.5%↓	18.47±0.39 ***	17.94%↑	13.40±0.41 ***	14.4%↓	18.66±0.053 ***	14.5%↑	20.29±0.03 ***	24.7%↑
MCHC (g/dl)	31.15±0.32	24.80±0.026 ***	20.3%↓	29.60±0.75 ***	4.97%↓	23.80±2.29 ***	23.6%↓	35.29±0.06 ***	9.6%↑	36.70±0.026 ***	13.48%↑
Platelets ($x10^3$/cmm)	279.30±4.8	877.30±10.9 ***	214%↑	409.70±9.33 ***	46.7%↑	235.60±4.98 ***	15.6%↓	448±3.36 ***	15.8%↑	765±5.59 ***	97.7%↑
WBCs ($x10^3$/cmm)	6.75±0.068	10.79±0.19 ***	59.9%↑	6.80±0.1 ***	0.74%↑	7.35 ±0.13 ***	8.9%↑	4.55±0.02 ***	39.1%↓	6.85±0.027 *	1.15%↓

P ≤ 0.001 is considered highly significant (***), ***P*** ≤ 0.05 is considered significant (**), ***P*** > 0.05 is considered non-significant (*), ***P*** versus control.

4. DISCUSSION

Extensive exposure to antibiotics has rapidly increased the propagation of multidrug resistance, and is now recognized by the World Health Organization (WHO) as a major emerging problem of global significance **[31]** *S. aureus*, is ranked second among the causes of hospital infections and is one of the three main causes of food poisoning. It is estimated that more than 90% of *S. aureus* strains are resistant to β. Lactam antibiotics **[6]** that become very worrisome. So, searching for alternative natural antibiotic fighting this problem, naturally occurring alternatives are the most common such as antimicrobial peptides (AMPs) that have great attention in recent years. The main advantages of AMPs with respect to other natural alternatives are their broad spectrum activity and lack of susceptibility to resistance development **[32-33].** Melittin is one of *A. mellifera* components with low molecular weight about 3kDa also represents about 50% from total dry weight considered good example for AMP, also *N. haje* venom appears to be relatively more efficient as antimicrobial agents than viper venoms **[34],** unluckily, the therapeutic use of venoms as drugs is problematic, as a result of their low bioavailability through the oral route, metabolic inactivation, poor permeability, the danger of proteolysis or enzymatic degradation and finally toxicity of these venoms **[35].** We can overcome these disadvantages by which, for example, by using antimicrobial, chitosan carriers which enhance bioavailability and reduce toxicity effect [**6, 36, 37]** or detoxification of venoms by different methods as γ irradiation method **[38].**

Cefotaxime is a third-generation of Cephalosporins, broad-spectrum antibiotic with activity against numerous gram-positive and Gram-negative bacteria **[39].**

The aim of present study to evaluate *in vitro* and *in vivo* the antibacterial effect of Egyptian cobra *(N. haje)*, the horned viper (*C. cerastes*), *and* Egyptian honey bee (*A. mellifera*) venoms as natural antibacterial products after safety and efficacy of this

selected venoms against *S. aureus*. It is shown that the significant antibacterial effect of *N. haje* on the *S. aureus* (Gram-positive bacteria) recorded highest antibacterial effect while *C. cerastes* and *A. mellifera* venom show equal antibacterial effect which are less than *N. haje* venom. Ampicillin has a highly antibacterial effect than Erythromycin. These results are in agreement with Abdul Hakim and Reza **[40].** Boda and his coworkers **[41]** reported that *N. haje* crude venom stop the growth of *S. aureus* (Gram-positive bacteria), dependent on the dose manner, while Sudarshan and Dhananjaya **[42]** and Bitar *et al.* **[43]** investigated that the effect of phospholipase A2 enzyme that found in all venoms has more antibacterial effect on the gram positive *S. aureus* Vidal *et al.* **[44]** reported that LAAO from snake venom has antibacterial effect.

The antibacterial results of *A. mellifera* venom agreed with Zolfagharian *et al.* **[16]** and Tanuwidjaja *et al.* **[45]** which reported that the antimicrobial effect of honey BV returned to the presence of many components, such as melittin, apamin, adolapin, mast cell-degranulating peptides, enzymes, biologically-active amines and non-peptide components. Also, results agreed with Abdul-Hafeez **[46]** who reported that melittin (cationic peptides - AMPs), the major active peptide of *A. mellifera* has antimicrobial activity affecting on the bacterial cell wall (outer membrane proteins and lipopolysaccharides **[47-48].**

This *in vitro* study showed that the minimum inhibitory concentration (MIC) of *N. haje* venom determined on *S. aureus* compared to MIC values of *C. cerastes* which are the maximum values referred to the values of standard antibiotic ampicillin. These results match with the results obtained by lamas *et al.* **[34]** where venoms have been shown to be composed of great pharmacological interest, such as antimicrobial peptides, with high inhibitory activity **[49-50].** We conclude that MIC values of *N. haje* and *A. mellifera* venoms are very close to its values of used antibiotic (Cefotaxime) on gram positive bacteria (*S. aureus*).

In this study, groups of mice were infected i.p. by *S. aureus* then treated with these selected venoms (*N. haje, C. cerastes* and *A. mellifera*) with 1/10 LD_{50} dose (safety studied dose) accepted with Ramos *et al.* **[20]** and El Amir *et al.* **[21].** Some hematological, biochemical parameters and some histological examinations were estimated after infection and treated with 1/10 LD_{50} of the previous venoms after applying safety studied on them.

Normally every organ can elicit a specific pattern of enzyme release, specifically, above-normal enzyme level, was considered as diagnostic features for several diseases **[51].** AST is found in many organs as liver, cardiac muscle, skeletal muscle, etc., while ALT is found only in the liver **[52],** the elevation in serum AST, ALT which is a most sensitive marker in the diagnosis of liver damage **[53].**

In this study, the concentration of both enzymes ALT and AST showed high significant elevation in untreated infected groups with *S. aureus* which agreed with the documented data of Minemura *et al.* **[54]** that refer to liver function test abnormalities frequently accompany a variety of bacterial infections, especially sepsis. The level of the liver enzymes decreased in all treated groups compared to untreated group illustrating the antibacterial effect of this venoms which accepted by Riaz *et al.* **[55].** While the 1/10 LD_{50} dose of *A. mellifera* treated group is more safe and effective so the level of liver enzymes was near to the control group.

Kidney is a suitable home for colonization of *S. aureus* also resisting the host immune response as 20% of the cardiac output flow to renal through blood **[56].** Thus, *S. aureus* invade kidney tissue during blood filtration and allow to colonize **[57].** The study show that the kidney functions were severely affected during infection also the level of urea and creatinine still high in the groups that treated with *N. haje* venom which supported by Dkhil *et al.* **[58]**. There are no significant differences in antibiotic and *A. mellifera* treated groups compared to control illustrated that its safe antibacterial properties.

We observed in the present study a highly significant increase in the levels of cardiac enzymes CPK in untreated infected groups. This result applied with Georgieva *et al.* **[59]**. Also, Harris *et al.* **[60]** reported that CPK elevations ranged between 1½ and 6 times in infected groups more than normal group. While in the group treated with *C. cerastes* venom Oukkache *et al.* **[61]** estimated CPK serum concentration increased about 3 times after induction of *C. cerastes* envenomation, a concentration of cardiac enzyme that is dependent on the venom dose. While in treatment with *N. haje* increased the kidney urea and creatinine compared to control. Its' increase is returning presence of PLA2 and other cardio-toxin(s) that are found in most venoms as *N. haje* venom that are responsible for cellular necrosis and cytotoxicity **[62]** in addition to increase due to the effect of infection.

Infection with *S. aureus* bacteria produce unfavorable hematological parameters, including the RBCs, Hb, PCV, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) also WBC and platelets.

The untreated infected group showed significant decrease in RBCs and Hb may be returned to sepsis but showed high significant increase in Hb in *N. haje* treated group compared to rest groups. These results

were accepted with by Riaz *et al.* **[55]** who reported that 2 hr post envenomation with *N. naja* venom, all the erythrocytes' indices were higher and there was significant increase in RBCs count and Hb values. Also, in agreement with Abdou and Ibrahim **[63]** and Gabra *et al.* **[39]** who reported that there was a significant increase in RBCs count, Hb concentration and HCT values in animals envenomed with *N. haje* venom at 4 times of LD_{50} dose, they illustrated that it could be increased attributed to a physiological mechanism attempting to restore the normal blood composition and counteract hypoxia caused by the venom initial.

Hyaluronidase from hymenoptera venom acts as a spreading factor that allows the toxic substances to infiltrate the tissues and rupture the blood cells. It leads to consequent loss of intracellular potassium and accumulation of sodium within the cytoplasm, high lipid peroxidation and oxidative damage. From damaged RBCs and due to loss of parts of the membrane more Hb comes out in the serum. The maximum increase in the PCV was obtained 2.5–2.55 times at 10 hr of treatment of 40% and 80% of 24-hr **[43].** In the present study, we treated *S. aureus* infected group by1/10 LD_{50} 3 times 24 hr intervals.

During this study untreated *S. aureus* showed high significant increase in platelets count, these results were accepted with Jensen *et al.* **[64]** who reported that *S. aureus* infected group increase 2.5 times of circulating platelets unbound to leukocytes compared to control, while *N. haje* treated group showed platelet count increase, this results coordinate with Salama and AL-Sadoon **[65]** who reported that large numbers of snaclecs (Snake venom C-type lectins) from different snake species have been reported to activate platelets, while *C. cerastes* venom show platelet count decrease which illustrated by Al-Sadoon *et al*. **[66]** who observed that platelets count was found to be increased at 1hr then began to be decreased to the control after 6hr and 72hr and still decreasing below the control at the 7th day. A decrease in RBC and platelets counts during this study indicated that the process of clotting by platelets arose to resist bleeding or haemorrhage and then declined parallel to RBCs. Snake venom metalloproteinases (SVMPs) affect the hemostatic system, as prothrombin or coagulation factor X activation, exhibiting activities of fibrinolytic and fibrinogenolytic, or inhibiting aggregation of platelets **[67].** Increase in platelet count in Cefotaxime antibiotic treat group despite of many research illustrated the destructive effect of the antibiotics on platelets and induced immune thrombocytopenia **[68].**

In the present study, a highly significant increase in WBCs count in all infected mice except antibiotic treated group compared to control, all *S. aureus* treated groups recorded highly significant increase. Leukocytosis enhanced by *C. cerastes* venom could be due to inflammation resulting from toxicity of venom components on the kidneys **[69].**

Pathological study illustrated that both untreated infected group and the effect of different venoms treatment could significantly affect the histopathological pattern. Our study illustrated the histological alteration of liver and kidney organs, it is observed that untreated *S. aureus*-infected groups, liver showing inflammatory cells infiltration surrounding the congested central vein with diffuse kupffer cells proliferation in between the hepatocytes. Also, fatty change in some of hepatocytes is found, and kidney showing focal perivascular inflammatory cells aggregation surrounding the congested blood vessels at the cortex. These results were accepted with Pollitt *et al.* **[70].** *S. aureus*-infected group treated by antibiotic, liver showed normal histological structure and mice kidney showing some congestion in cortical blood vessels as a result of the strength of blood flow inside blood vessels, while *S. aureus*-infected groups treated by *C. cerastes* venom, liver showed congestion in portal vein with inflammatory cells infiltration at the portal area. Albuquerque *et al.* **[71]** reported that snake venoms as a general effect on the kidney tissue are tubular degradation, mononuclear cell infiltration and congestion which were also detected in our study that mice kidney showing few inflammatory cells infiltration in focal manner between the tubules and glomeruli at the cortex. This minor effect as a result of venom treatment with 3 times of 1/10 LD_{50} dose each 24 hr intervals which is exposed to low dose. While the mice treated by *A. mellifera* venom after infection with *S. aureus*, the liver showed dilation of central vein with diffuse kupffer cells proliferation in between the hepatocytes while kidney showed normal histological structure. This normal structure as a result of the less toxicity of *A. mellifera* venom when compared with other venoms. Also, more potent antibacterial effect which the signs of the infection didn't appear on the tissue also on biochemical kidney function. *S. aureus* group treated by *N haje* venom, liver showed degeneration in some of the hepatocytes and kidney showed focal inflammatory cells aggregation in the cortical portion as discussed previously.

5. CONCLUSIONS

The study concludes that, Egyptian cobra *(N. haje)*, the horned *C. cerastes* and *A. mellifera* crude venoms has an antibacterial effect on some strains of bacteria so, it can be used as natural therapeutic against some bacterial diseases to overcome the resulted resistance coming from uncontrolled using of synthetic antibiotics. On the other hand, it induced significant

pathophysiological and histological alterations in liver and kidney in both *N. haje* and *C. cerastes* at 1/10 LD_{50} that they need further studies to use as safe natural drugs, while *A. mellifera* has lesser effect on tissues and consider more safe in using as natural crude therapeutic drug. All of the studied venoms give more effect as antibacterial natural therapeutics.

Conflicts of interest

There are no conflicts to declare

funding

There are no funding sources.

ACKNOWLEDGMENTS

The authors are grateful thanks to the valuable support from faculty of Science, Cairo University and VACSERA authorities for supporting and facilitating this work.

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