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Effect of Antimicrobial Agents Extracted from American Cockroach Insect *Periplaneta Americana* L. on Some Species of Microbes

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Abstract

current study was conducted for the period of October 2017 until November 2018 to investigate the effect of antimicrobial material of American cockroach *Periplaneta americana* hemolymph against strains of pathogenic bacteria and yeast. The samples of adults American cockroach was collected and divided into three major groups, the first collected their haemolymph without injecting the cockroaches with bacteria (non-immunizator) the second collected their haemolymph after 12 hours of being injected with *E. coli* (immunizator 12h), and the third group after 24 hours of being injected with the same bacteria (immunizator 24h), then divide each totals above to two subgroups one by cooling centrifuge on 4ºC and the other by table centrifuge at room temperature (non-cooling). protein concentration had measured of all transactions and was the highest value is immunizator 12-hour by cooling centrifuges.

Keywords: *species of microbes ; Periplaneta americana L. ; Antimicrobial Agents*

Introduction

Excessive and non-organizer use of antibiotics contributed increased resistance of microbes to antibiotics and emergence of new strains more resistant than their predecessors, it becomes a great challenge for human health [1], that make various medical institutions looking for new sources instead of antibiotic, one of it the antimicrobial peptides symbolized AMPs for short, scientists and researchers have focused in the past on plants only to find antimicrobial articles, but as the plants produce those articles most insects produce these complex chemicals for various purposes including defense against microbes, mating, communication, and other operations that help bugs survive [2], it has been found that a wide variety of living organisms and especially insects produce AMPs as part of their defensive line, scientists have identified hundreds of these peptides and explained their significance in the innate immune system [3]. This AMPs are self- produces without induction or either after induction by some infectious or inflammatory stimuli like bacteria or bacterial molecules, a hypothesis developed that animals which live in unsanitary and contaminated conditions they evolution of ways to protect themselves from disease causing by microorganisms [4]. Toke [5] stressed that AMPs have a key role in the fight against invading pathogens in insects. The cockroaches are common household insects that live in human environments and feeding at random on a litter therefore various pathogens carried on their body [6], the ability of roaches flourish under such threats and prevent diseases causing because them body are a good source of antimicrobials [7]. So the aim of this study is to investigate f antimicrobial substances in the haemolymph of American cockroaches *Periplaneta americana* on some microbes.

Materials and Method

Types of microbes : In this study used microbes obtained from the microbiology laboratory at the University of Samarra College of Applied Sciences three gram negative bacteria were *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis*. and two gram positive bacteria which were: *Staphylococcus aureus* and *Staphylococcus epidermidis*, and one type of yeasts *C. albicans.*

Collection of cockroach samples

90 adult cockroaches were collected from different part of Samarra city from the gardens, houses, and

sewers manually included male and female, cockroaches diagnosis based on phenotypic characteristics in the natural history museum and research center at the University of Baghdad according to 601 system rankings and Q.S standards, The insects have putted in perforated plastic container to ensure ventilation feeding in laboratory conditions, haemolymph directly extracted [8].

immunization of *Periplaneta americana* and extraction

Immunization is injection a specific type of bacteria to stimulate insect immune system to production antimicrobial materials [9]. *E. coli* chosen to inject cockroaches as in [10] with dose 100 microliter contain almost CFU/ml had compared with McFarland standard 0.5 [11]. insects refrigerated for anesthetized putted on a sterile petri dish on dorsal part, with needle size 1 ml injection 100µ of bacterial suspended solution in phosphate buffer solution between the 4th and 5th abdominal ring slowly to prevent bleeding [12].

Collection of cockroaches Hemolymph

All samples were divided to three main groups first was non immunizator did not injected with bacteria their haemolymph had extracted immediately after anesthetized and injected 0.1 ml ringer solution for every insect then hind pair legs were cut with a sterile scissors in coxa membrane [13] the second their haemolymph had extracted after 12 hours of immunization and a third after 24 hours of immunization [14], after the completion of the time required, each major group was split into two subgroups, first extracted with table centrifuge at 1800 rpm and CFU 6000 g for 15 minutes [12] second extracted with cooling centrifuge (Sigma Germany) at temperature 4 ºC with 14000 rpm and accelerate17968 g for 15 min [15], Finally insects had threw extract stored in clean and chilled tubes with information at -20ºC until used [13]. sterilized all of previous extracts using Millipore filters 0.22mµ [7], this crude extract was used in all tests In this study.

The protein concentration assay

Determined concentration of proteins for all transactions by Biuret test [16]. according to [17]. The total protein concentration was calculated for each sample by equation:

$$\text{total protein concentration}\ \frac{\text{g}}{\text{100ml}} = \frac{\text{sample absorbancy}}{\text{standard aborbency}} \times \text{standard concentration}$$

Antimicrobial assay

Antimicrobial assay was done by Wells Agar diffusion method in Muller Hinton Agar, 20 µl of crud extract putted in every well after spreading 0.1 ml of microbial suspended, incubated plates at 37ºC for 24 hours, measuring Inhibition Zone as in [18].

The toxicity assay on human Polymorphonuclear cell PMNs

PMNs human cells were isolated as in [19] followed method in [20] to measure the viability of PMNs, cells accounted by Haemocytometer under microscope as dyed cells, dead cells that didn't take the dye are living cells, calculating the percentage by: percentage of cells viability= number of living cells \ total x100

Static analysis

All the results of the current study were analyzed by analysis of variance test ANOVA of factorial treatments, at implemented $P< 0.05$ by SPSS software.

Results

The protein concentration of samples

All value of protein content of haemolymph in table 1, statistical analysis showed high moral differences of protein content of haemolymph immunizator 12-hour cooling centrifuge at $P > 0.05$ symbol a followed by of non- immunizator cooling centrifuge symbol b followed by the haemolymph immunizator 24-hour cooling centrifuge symbol c, the remaining transactions that symbol typefaces d didn't show any moral differences at $P > 0.05$.

Table 1 values of protein content of American cockroach extract

Type of centrifugation immunization period	Cooling g/100 ml	Static symbol	Non-cooling g/100 ml	Static symbol
Non-Immunizator	1.82	b	0.21	d
Immunizator 12h	3.182	a	0.225	d
Immunizator 24h	1.32	c	0.092	d

Antimicrobial activity of American cockroaches extract

All inhibition zone of haemolymph extracted for all transactions are in figure 1. Statistical analysis had moral differences to activity of Immunizator 12-hour cooling centrifuge on *E. coil* with all transactions to level P> 0.05 with 24 mm inhibition diameter symbol **. And moral differences Immunizator 24 hours cooling centrifuge on *E. coil* also inhibit diameter 23 mm compared to the rest of the microbes moral level P > 0.05 symbol * and effect of immunizator 24 hours cooling centrifuge on yeast *C. albicans* with 21.7 mm diameter with the rest of the microbes moral level P > 0.05 symbol * .

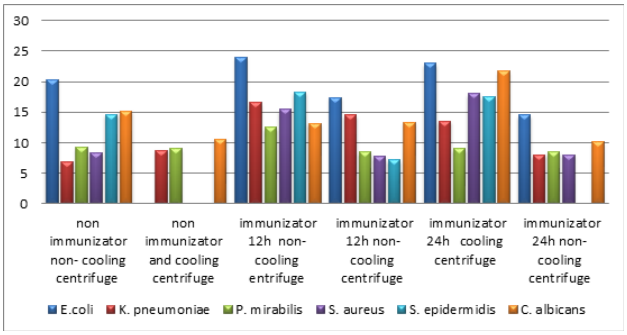

Figure 1 Antimicrobial activity of crude haemolymph extracted from *Periplaneta americana*.

Effect of extracted haemolymph on Polymorphonuclear leukocytes cell viability

The extracts Immunizator 12 hour and Immunizator 24 hour both with cooling centrifuge were selected to test their effect on availability of human Polymorphonuclear cell PMNs because their top antimicrobial activity in this study compared to other extract, PMNs cells-viability in table 2, Statistical analysis has shown that control symbol a the highest moral differences that haven't treated followed by treatment with Immunizator 24-hour cooling centrifuge symbol b decreased PMNs cells-viability because of extract's toxicity, The latest treatment with Immunizator 12 hours cooling centrifuge moral difference with the first and second transactions moral level P > 0.05 symbol c this means PMNs cells-viability decreases due to the increased of extract toxicity.

Table 2: Effect of American cockroach haemolymph on Polymorphonuclear leukocytes cell viability

treatment	cells-viability percentage	Static symbols
control	91.3%	a
Immunizator 24-h cooling centrifuge	80.4%	b
Immunizator 12-h cooling centrifuge	74.5%	c

Discussion

Cooling centrifugation at temperature 4 ºC gave higher levels of protein content compared to the non-cooling for all transactions may reason is the difference in speed between the two devices, the more Speed and power increased the efficiency of the device unplug macromolecules including peptides [21], with terms of immunization**,** the Immunizator 12-hour extract was the highest protein content due to stimulate their immune system to synthesis peptides after being injected with bacteria, protein content decrease until less than the normal rate after 24 hours of Immunization with bacteria which shows weakness period of insects from bleeding gets, either bugs that didn't immunized has protein content too because exposure to pathogens, In addition to the natural proteins in their body.

The study found that cockroaches injected with bacteria effective against microbes higher than others did not injected especially on microbe used in immunization *E. Coli*, duration left after injection until extraction was affected too, which was 12 and 24 hours, as well as the centrifuge device used had impact on antimicrobial activity. the haemolymph non- immunizator by cooling centrifuge was affecting on *E. coli*, *C. albicans* and *S. Epidermidis* might because live in environments filled with these microbes [22], cockroaches use similar immune response in vertebrates to face a life-threatening [23]. **Seraj** et.al. (2003) has an explanation for the effectiveness of the haemolymph non- immunizator by non-cooling centrifuge called him non induced might revert to having a basic AMPs effectively against *E. coli* [14], but the on *S. aureus*, and other types of microbes have been weak because their high resist than the activity of extract. Study did not agree with [24] about

non immunizator haemolymph stating that no effected against both *E. coli* and *S. aureus* may because he used a pathogenic bacteria of nosocomial hospital, there an activity of extract although its protein content indicating that mean presence anther antimicrobial compounds which may fats or proteins associated with lipids or with carbohydrates, like AMP lectins a carbohydrate-binding proteins as in [13]. Some research findings supported the idea of a peptide natively in cockroaches by intestinal and exoskeleton extracts against *S. aureus* and *E. coli* he [2]. The activity of Immunizator 12-hours non-cooling centrifuge decreased for all microbes by changing the centrifuge device to non-cooling despite the stability of the duration of immunization, but had effect on *C. albicans* may target on yeast different from on bacteria. One reason to changes antimicrobial activity is fragmentation haemolymph contents and became useful for bacteria as food or lose activity due to braking of the disulfide bond and Hydrogen bond by heat of centrifuge device which led to change alpha helix or beta sheet shape which help penetrate microbe's membrane [25]. all of the positive charge and hydrophobicity and the length of peptide's chain were important to activity [26], so may heat of centrifugation had change this properties lowering activity. Our results have agreed with respect to immunization period with [14] that highest effective between 9 to 12 hours after injection then declined after 24 hours. inhibition diameters decreased in the non-cooling centrifuge for most microbial species**,** Either the immunizator 24 hours cooling centrifuge had decreased antimicrobial ability on most microbes but still highly effective on *E. coli* and was higher effect on *C. albicans* that showed the persistence of antimicrobial activity against microbes even after 24 hours of exposure to microbes. While the antimicrobial activity of immunizator 24-hours non-cooling centrifuge decreased significantly in our study results came in line with [14]. effectiveness of AMPs depend on microbial plasma membrane but some AMPs show broad spectrum activity [27]. differences between study and others due to differences in feeding cockroaches, environmental qualities and humidity factors morph type of species used, and dangerous of bacteria injection [24].

Conclusion

Results show that antimicrobial activity of haemolymph extract on microbes with Well diffusion agar was higher inhibiting to immunizator 12 hours by cooling centrifuges on *E. coli* with 24 mm. Also Determined effect of the extract on a viability of polymorphonuclear leukocytes of human.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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