WAS Science Nature
WASSN

ISSN: 2766 - 7715
WAS PRESS

Review

Insects as Medical Suppliers of Bioreactors: Mini Review

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Received: 12.01.2018; **Accepted:** 15.02.2018; **Published:** 20.02.2018

Abstract: Many have been published on therapeutic derivatives from living organisms including insects. More than 1100 therapeutic products were isolated from insects. Most people think in insects as enemies but my thought is that insects are our friends not our enemies as those see. Many beneficial insects rather than Honey Bees, Silk Worms and Shellac insect can be seen. Insects could be our MicroFactories, Biosensors or Bioreactors. InsectFarm is an amazing example of the applied research that transfers the insects from laboratory to market. Prof Mircea Ciuhrii (The founder of InsecFarm) is Italian Entomologist who worked for more than 23 years to derive therapeutics from insects. He derived more than 30 commercial medicines from insects (Imunomax, Noblesse, …etc.). Various approaches were followed in this regard. Many laboratories selected the biochemical approach to purify components of the innate immune system of insects and insect metabolites to be used in many therapeutic purposes. Others relied on the molecular proteomic approach to sequence and/ or synthesize components of the innate immune system of insects and to use these components in medicine. The approach of our laboratory is somehow different. We used different routes of administration to induce the insect immune system, then a transcriptomic study was done to discover the induced genes and to identify specific biomarkers that can help in drug discovery. Biomarkers play an important role in medicine and in drug discovery and development as well. Optimum biomarker development and application will require a team approach. because of the multifaceted nature of biomarker selection, validation, and application. The team members use different techniques such as pharmacoepidemiology, pharmacogenetics, pharmacogenomics, and functional proteomics; bioanalytical development and validation; disease process and therapeutic intervention assessments; and pharmacokinetic/ pharmacodynamic modeling and simulation to improve and refine drug development. The team approach will minimize the effect of such component on the rest of the process. Our Achievements included the discovery of three components of the innate immune system of the cotton leafworm, Spodoptera littoralis. These components were designated as SpliDef (defesin), SpliLec (lectin) and SpliCec (cecropin). SpliDef and SpliLec were confirmed as antimicrobial peptides, while SpliCec was additionally confirmed as anticancer peptide. More than 40 antimicrobial peptides have been isolated from different insects in our laboratory. Our current research is going on to achieve something in antioxidants and anticoagulants from insects. Our perspective is to achieve something in the mass production of prototypes of our products and to become commercially efficient. These achievements are the integrated contributions of everybody in my team staff.

Keywords: Insects; Entomotherapy; Honey bee; Venom; AMPs.

1. Introduction

Insects are considered the most successful and diverse group of animals. They constitute about three-fourths of the total number of living organisms present on the planet [1]. From the all known

animal species, approximately 80% of them are insects [2, 3]. Estimations of 200,000,000 insects per one human and 40,000.000 insects per one acre of land have been reported [4]. Tremendous biodiversity and numerous utilities of insects brought them in steady services of the mankind like other resources [4]. Concerning their utility, insects can be divided into 4 categories. First is the use of insects in industry which assures utilization of honeybee, silk worm, dye insect, lac insect and aesthetic insect. Second is therapeutic and nutritional utilization of insects. This includes usage of insects in traditional medicine and as food resource. Therapeutic application of honeybee products (honey, royal jelly, beeswax, bee venom, bee bread, pollens and propolis) has been reported to cure diseases like rheumatic pains, arthritis, back pains, cancers and other diseases. Edible insects like ants, grasshoppers, termites, grubs, crickets, moths, caterpillars and pupae are consumed by many peoples worldwide. Third is the applications of forensic entomology. By investigating the succession of insects and different stages of insect colonizers, a rough estimation of the postmortem intervals can be calculated. Fourth is the use of insects as ecofriendly component. Predators and parasites could be used as potential biocontrol agents of destructive pests. House fly larvae are used as biomass recycler. These decompose organic wastes to produce proteins and fats. Many insects are used as bioindicators to estimate the accumulation of pollutants and other environmental stressors [5]. Insects have been utilized by people in different ways around the world. Advancement of insect's utilization and industrialization has been reviewed by Zhang et al. [6]. Considering the above-mentioned facts, the present review threw the light on the most significant and recent developments in bioengineering natural products from insects with potential use in alternative medicine.

2. Natural Therapeutics

"BACK TO NATURE" is an acceptable invitation in a great welcome in most fields, all over the world in the past decades up till now. A growing branch of alternative medicine is glowing all the time, especially in South Eastern Asian countries (China, India, …etc.). Maggot therapy and ethnomedicine are very successful and growing therapies in many reported cases. The use of animal, insect, botanical, fungal, algal and bacterial extracts and/ or products is the main research subjects in many laboratories worldwide. The discovery of the nutritional, medicinal and therapeutic effects, the industrial and energetic power, the technological and ecological uses of natural products from these organisms will definitely introduce many added values and endless power to the technological revolution of the new world; this is "THE POWER OF NATURE" [7].

3. Insects are Our Possible Friends not Our Enemies

Laboratory and clinical investigations on the honey and other bee products have increased during the past decades. The most eminent discovery was their antibacterial activity. Several diseases are known to be treated by honey (e.g. healing of wounds). The use of honey as antiseptic agent has been replaced by antibiotic therapy. However, the increasing number of antibiotic-resistant bacteria has led to the re-examination of the former therapies. Honey, royal jelly, propolis and bee venom have proven their strong antibacterial activity [8-10]. Even antibiotic-resistant strains such as methicillin-resistant Staphylococcus aureus (MRSA) and Vancomycine-resistant Enterococcus (VRE) have exhibited its sensitivity to honey as compared to the antibiotic-sensitive strains [11-12]. Bee products displayed varied potency as antibacterial agents. Botanical origin of the product has a major role in its antibacterial activity [13]. The strongest antibacterial action has been reported for propolis. This is probably due to its richness in flavonoids. Still the most defying problem of using hive products for medical purposes are safety and dosage [13]. For instance, honey and royal jelly are usually not filtered when produced for food purpose. However, they are filtered to remove impurities and allergens in the case of use in wound care [13]. It is worthy mentioned that honey does not allow survival of vegetative bacteria and it does contain viable spores, including clostridia [13]. Clinical use of bee products is expected to increase as the

increased availability of licensed medical stuffs containing bee products. Only certified antibacterial agents which are safe should be used in professional care centers [13].

4. Many Beneficial Insects Rather Than Honey Bees And Silkworms

Lac is a natural, biodegradable and nontoxic resin produced by lac insects. Lac insects produce lac, dye, and wax. There are 9 genera and 87 species of lac insects worldwide. Out of which 19 species belonging to two genera are reported in India [15]. Lac resin has many applications in electrical, textile, food, surface-coating and pharmaceutical industries [16].

The demand for natural dye is directly proportional to sensibility of the public to synthetic dyes and associated problems [17]. The production of natural dyes from insects has been appraised by Prasad [18]. The natural red dye, carmine acid, has been extracted from the coccid, Dactylopius coccus (Hemiptera: Dactylopiidae). This acid is used in pharmaceutical, cosmetic and food industries [19]. Dried females of the coccid, Opuntia ficus indica, are a source of red dyes vastly used in textile, pharmaceutical and food industries [20]. Cochineal species produce orange, scarlet and red tints. Meanwhile, cochineals are characterized by high protein and mineral contents. Therefore, the residuals can be utilized as food additives for avian species or to produce fertilizers [21]. Methods of exploitation and production of a dye were studied by many researchers [22, 23]. The method by which insects are killed produces different colors. These result in diverse looks of commercial cochineal. In order to produce one kilogram of cochineal, it consumes about 155,000 insects [24]. Tannic acid, the main component of wool dyes and black hair colorants, is still used in tanning and dying leathers and in ink manufacture. Tannic acid is obtained from the Aleppo gall on oak trees which produce galls as a response to infestation of the tiny wasp larvae, Cynips gallae tinctoriae; Hymenoptera: Cynipidae [25].

The insect attractiveness is generally attributed to colors, beauty and mode of life. Colored elytra of some beetles are used in pottery, jewelry, embroidery and basket makings [26]. Butterflies have the most attractive colors among beautiful insects. To satiate the increasing demand on butterflies amongst the collectors, plentiful number of butterfly farms have been developed in Europe [27]. The butterflies trade in such farms is very profitable. The insects are massively reared for sale as adults. This trade yields approximately $100,000,000 a year [28].

Grasshoppers, beetle larvae, caterpillars, crickets, termites and ants are amongst the most consumable insects worldwide. Some insects showed higher nutritive value than other eaten animals when reared in the same conditions and fed on equal-quality diets. For example, the house cricket, Acheta domesticus, showed nutritive value equal to six times higher than steer, twice higher than pigs and boiler chicks, and four times higher than sheep when losses in slaughtering are counted [29]. Thus production of insect protein for human nutrition would be ecologically preferable than vertebrate protein. The traditional entomophagy particularly locusts and grasshoppers is known to have a great importance from issues of nutritional and pest control, as well. The increase of the insect consumption has corresponded to decreased use of pesticides in Asia and Oceania [30]. Entomophagy is not regarded as emergency but as planned diet wherever and whenever obtainable. No significant health issues were associated with entomophagy. Moreover, many insects (e.g. Belostoma indica) are used as flavor additives [31-34]. Reducing the bias against entomophagy is hopeful, by supporting production of edible insects in the form of safe and stable diets. Despite the above mentioned benefits, some modernized populations refuse entomophagy without suggesting nutritional equivalent alternatives [35].

5. Insects Could Be Our Microfactories Or Bioreactors

In many parts of the southeastern Asia, medico-entomological medicines have been used in daily life of the society [26]. The term entomotherapy was suggested by Costo to describe the use of

insects in therapeutic applications [36]. Many other famous authors threw lights on the use of insects in therapeutic exercises [37-44]. Blow fly larvae are frequently used in medicinal trials. It was observed by the surgeons, during World War II, wounds infested by fly maggots became better than noninfested ones. Later on, the antiseptic chemical, allantoin, was discovered [42, 44].

Application of bee venom in traditional medicine has been documented. [9, 10, 45-47]. Eighteen bioactive compounds have been reported in bee venom. These include peptides, enzymes and biogenic amines. Melittin is the major peptide of bee venom. It is useful in relieving rheumatoid pains and multiple sclerosis. Melittin blocks the expression of genes encoding for inflammation [8]. Recently, melittin was reported to inhibit DNA-binding activity of the transcriptional factor, NF-kB, wich is known to regulate inflammatory gene expression [48]. In addition, anticancer activity of melittin has been determined for liver, renal, prostrate, bladder, lung, mammary and leukemia cancer cells [49-52]. Bee venom has been regarded as the coming generation of cancer combating drugs. Currently, pharmaceutical companies are extensively funding for research on anticancer activity of bee venom.

Furthermore, cantharidin of blister beetles was used as a diuretic and to alleviate epilepsy, to relief asthma, to treat rabies, and sterility [53]. Eggs of red ants are used to control malaria. Chronic diarrhoea and profuse menstruation are treated by cocoon extract of mulberry silkworm [17]. Pierisin from Pieris rapae pupae, larvae of P. brassicae and P. napi showed cytotoxic action against gastric cancer of human [54]. Many insect cell lines have been proved as expression hosts of many proteins and monoclonal antibodies [55]. Insect antimicrobial peptides (AMPs) have been proposed by Tang et al. as potential alternatives of traditional antibiotics [56].

Forensic entomology is the use of insects and other arthropods in forensic investigations has paid attention through the last years [57]. “The Manual of Forensic Entomology” by Smith [58], “The entomological review” by Catts and Goff [59] and the “International homepage of Forensic Entomology” [60] may be cited as examples. The postmortem interval (PMI) could be calculated by analysis of insect-succession, climatic conditions and stage corpse’s decay [57, 58, 62]. Flies, beetles, and other insects have been listed as feeders of corpses [61]. Forensic entomology, therefore, occupy a crucial position in the field of forensic sciences. Insects have a powerful role in this field. It could not be replaceable by any of the corpse feeding organisms [5].

6. Therapeutics From Insects

Documented folk healing worldwide utilized insects and insect-derived products. The promised products have been investigated experimentally. Immune response or immunity of insects against pathogens are the most exciting area to be studied for potential use in medical targets. As mentioned before, many insect-derived products have been discovered, including hive products, sericulture, maggot-therapy and allantoin, cantharidin, body extracts and blood-sucking arthropods [63]. Further benefits could be provided by mixing traditional medicine and insect-derived products. Bioengineered molecules have been proved to be very potent as pharmaceutics in modernized medicine. Forceful antimicrobial activity against broad spectrum of antibiotic-resistant bacteria and anti-HIV activity have been reported [64-68]. Additionally, anti-cancer [69-71], anti-angiogenesis [72-73], anti-inflammatory [74], antioxidants [75,76], anti-coagulant factors [77, 78] and wound healing agents [73] have been discovered from insects.

7. Insectfarm Is an Amazing Example

"Insect Farm" is an Applicative Scientific Center that produces medicines and cosmetics on the basis of active biological substances (ABS) extracted from certain species of insects. The substances are extracted in a sterile environment, then they are mixed with specific adjuvants. The products produced by "Insect Farm" are unique and they can be found only in this company system. One of

the most required products is HEPATITO-LIZ, that has a curative effect for the hepatic cells and stops the multiplication of viruses that cause hepatitis of the type A, B, C, and D. This product includes four types of ABS extracted from different types of insects administrated according to the instructions. GEL PENTRU INGRIJIREA CAVITATII BUCALE and CREMA SPECIALA MASAJ CORPORAL are viral products used for the treatment of the tissue, in the case of herpes-viruses. After the treatment the viruses are completely eliminated from the body.

Another series of products have curative effect on keratosis CREMA PENTRU INGRIJIREA PIELII and can absorb completely skin diseases of the type of moles, maculas etc. This type of products do not cause pains and do not leave marks on the skin. The products with antibacterial effect, CREMA PENTRU ADOLESCENTI, GEL PENTRU INGRIJIREA CAVITATII BUCALE, GEL PENTRU INGRIJIREA DINTILOR SI GINGIILOR, have a germicide action on staphylococcus and streptococcus, which have lately become resistant to the action of antibiotics. The products having antifungal action LIPOGEL PENTRU UNGHII totally eliminate infections of nails, skin, mouth cavity and vagina candida, with no secondary effects. Another type of products are those used for arteriosclerosis, piles etc. CREMA PENTRU INGRIJIREA PICIOARELOR, CREMA PENTRU INGRIJIREA GLEZNELOR, INTIM GEL H, which are very efficient. CREMA MASAJ CORPORAL eliminates the causes of rheumatism, using specific ointments with ABS extracted from insects. The products with anti-tumoral effect IMUNO-MAX SUPOZITOARE si CREMA PENTRU INGRIJIREA DECOLTEULUI can eliminate the tumor with no pain or secondary effects. The HAIR LOTION and BALM produced by INSECT FARM have become famous in Romania and they have a benefic action against hair-loss.

8. Other Approaches

There are two major approaches in discovery of therapeutic proteins from insects worldwide. Both approaches rely on the induction of insect immune system firstly. Then one approach selects to fractionate and purify the induced peptides using the available biochemical technologies like gel filtration and HPLC. The second targeted deeper molecular approach, including genomic, transcriptomic and peptidomic studies. Genes or peptides are the main target of the second approach. After picking up the mature active peptide, it could be cloned in suitable expression vector. Then the peptide could be produced at mini, medi, and maxi-scales. Biochemical purification of the protein is the last step to obtain extra-pure peptides. These peptides should be investigated for its activities at laboratory scale, at experimental model organisms scale, and finally at clinical trials. Besides, studies on side effects, antigenicity and allergy reactions are required. After finishing the research scale, prototype and product production are the finalizing steps. Formulation, packaging and obtaining license of the product are required before it is released for market use.

9. The Approach of Our Laboratory

Our team members have added their special fingerprint at all phases of the research. First we studied different methods of immunization and different inducers (different Gram's bacteria, chemicals, mechanical injuries and radiations) using different insect models. Second we studied the best route of immunization (topical application, pinning, feeding, injection, …). Third we investigated immune response in each case using native and SDS-PAGE, in Gel-antimicrobial assay, DD-PCR, ….. etc. Fourth we went in deep using transcriptomic approach. Herein, a gene-fishing strategy was applied and a simple, faster, cheaper, and effective technique like was modified to pick-up the target gene and sent to sequencing. Sequence analyses of the gene revealed if it is full-length or truncated gene. In case of it is full-length, sequence analyses were carried out to determine the mature peptide and specific primers were designed for full-length and mature peptides. In case of it is truncated gene, 5` race, 3` race or both were performed to obtain the full-length gene, sequence analyses were carried out and specific primers were designed for full-length and mature peptides. Sequence corresponding to the mature peptide was cloned in

expression vector, mature peptide was obtained and purified. Proper bioassays of the target activity were carried out at laboratory scale, using positive and negative controls.

10. Our Achievements

In 2009, prof. Seufi concentrated research in the field of biomedical science and thus he originated a laboratory to work on the discovery of therapeutics from insects in Cairo Univ., Egypt. He was the principal investigator (PI) of a project entitled: "Production of Antibacterial Peptides from Insects to Improve Human and Animal Health" which was funded by the University of Cairo. Six M.Sc. and two Ph.D. students were team members of this project. All the team members worked on antimicrobial peptides (AMPs) and we got more than 40 AMPs from different insects [e.g. 79-83]. In addition, we discovered three components of the innate immune system of the cotton leafworm, Spodoptera littoralis. These components were designated as SpliDef (defesin) [84], SpliLec (lectin) [85] and SpliCec (cecropin) [82]. SpliDef and SpliLec were confirmed as antimicrobial peptides, whilst SpliCec was additionally confirmed as anticancer peptide. Another line in Seufi's laboratory is antioxidants and we have got three antioxidant peptides from insects. The third line is anticancer and we have had at least one anticancer peptide (other peptides are under investigations). The fourth line is anticoagulants and we have isolated four anticoagulant peptides. In addition prof. Seufi registered a patent at the Egyptian Scientific Academy entitled: "antibiotic from cotton leafworm" at 4/ 11/ 2011. If we succeeded to share enthusiastic and ambitious partner(s), we are expecting to develop and extend our work to have some prototypes in the next few years. It is worthy mentioned that our achievements are the integrated contributions of everybody in my team staff.

Supplementary Materials: All materials have been included in the article.

Author Contributions: All authors contributed equally in this research.

Funding: This research received no external funding.

Acknowledgments: My team leader and Deputy PI is prof. Fatma H. Galal, a professor of medical entomology at Dept of Entomology, Fac of Science, Cairo University, Egypt. She is currently working at Biology Dept., College of Science, Jouf University, KSA. Dr. Shaymaa H. Mahmoud, lecturer of entomology at Dept of Zoology, Fac of Science, Menofyia University, Egypt. She is working on AMPs from the house fly, *Musca domestica*. She supervised two M. Sc. students in the team. Dr. Mohamed Gamal a lecturer of microbiology at the Dept of Botany, Fac of Science, Cairo University, Egypt. He was working on the expression of Asparginase B gene in *E. coli* expression system. Dr. Tamy Samir is a lecturer of entomology at the Dept of Biology, Atomic Energy Commission, Egypt. She was working on DNA-repair genes. Dr. Yussuf Abdel Aziz is a lecturer of entomology at Dept of Entomology, Fac of Science, Cairo University, Egypt. He is working on classification of insects by using molecular markers. Mrs. Soaad Ali Hassan is Assistant lecturer of entomology at Dept of Entomology, Fac of Science, Cairo University, Egypt. She is working on AntiCoagulant genes from mosquito species. Mr. Rami Essa is Assistant lecturer of entomology at Dept of Entomology, Fac of Science, AlAzhar University, Egypt. He is working on AntiCoagulant genes from mosquito species. Mr. Mohamed Said is Assistant lecturer of entomology at Dept of Entomology, Fac of Science, Benha University, Egypt. He is working on AMPs from the honeybee, *Apis mellifera*. Mrs. Omaima Zaki is M.Sc. student graduated from Helwan University. Dept. of Botany and Microbiology. She is working on Bacterial and fungal Isolation from mosquito developmental stages. Mrs. Lina Mohamed is Assistant lecturer of entomology at Dept of Entomology, Fac of Science, AlAzhar University, Egypt. She is working on AntiOxidants from insects. Ms. Mona M. Yussuf is M.Sc. student graduated from Alexandria University. Dept. of Biochemistry. She is working on AMPs from *Drosophila*. Mr. Ahmed Abdel Raouf is Assistant lecturer of entomology at Dept of Entomology, Fac of Science, Benha University, Egypt. He is working on Forensic Entomology. Ms. Dina Amro is Assistant lecturer of entomology at Dept of Chemistry, Fac of Science, Cairo University, Egypt. She is working on increasing bacterial transformation efficiency by using Nanotechnology. Ms. Zynab ElGammal is Assistant lecturer of Chemistry at Dept of Chemistry, Zowail University, Egypt. She is working on cardiac disease treatment by Stem cells. Mrs. Mona Hanafy is M.Sc. student graduated from Cairo University. Dept. of Entomology. She is working on AMPs from *Sarcophaga*.

Conflicts of Interest: The authors declare no conflict of interest.

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