OPTIMIZATION OF A HEMOLYMPH PROTEIN EXTRACTION METHOD FROM NATIVE POLYACRYLAMIDE GEL

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INTRODUCTION:

Although there are indications that insect-based proteins may have potential biomedical applications (anticancer and antimicrobial), as well as in cellular agriculture (food and feed), they have not been sufficiently investigated. The hemolymph of insect larvae is protein-rich, particularly in storage proteins that are involved in amino acid metabolism and protein synthesis. In order to characterize these proteins, the first step is their successful isolation. Using diapausing 5th instar larvae of the economically important European corn borer moth (ECB) *Ostrinia nubilalis* (Hbn.) as a model system, in this study we optimized a method for isolating individual native hemolymph proteins from polyacrylamide gels and we performed initial bioactivity tests of isolated proteins.

OBJECTIVES:

The main objective in this study was to optimize an easy and affordable method for isolation of individual hemolymph proteins in the native state, without the use of chemicals that would affect their structure and function. This allows further testing of these proteins for biomedical uses and application in cellular agriculture, as well as further work with isolated proteins in downstream *in vitro* proteome research, which will bring new knowledge and directions for different *in silico* proteome research.

METHOD / DESIGN:

Figure 1 shows the experimental design that involved:

- Collection of hemolymph from diapausing 5th instar ECB larvae (**Fig. 2**) and removal of hemocytes from the hemolymph;
- Separation of hemolymph proteins by native PAGE;
- Determination of protein fractions positions on the gel after electrophoresis;
- Elution of proteins from the gel;
- Determination of eluted protein concentrations;
- Confirmation of successful protein isolation by native PAGE;
- Effect of three different protein concentrations (9.3, 3.7 and 1.86 ng/µL, respectively) on MRC-5 cell viability determined with MTT assay.

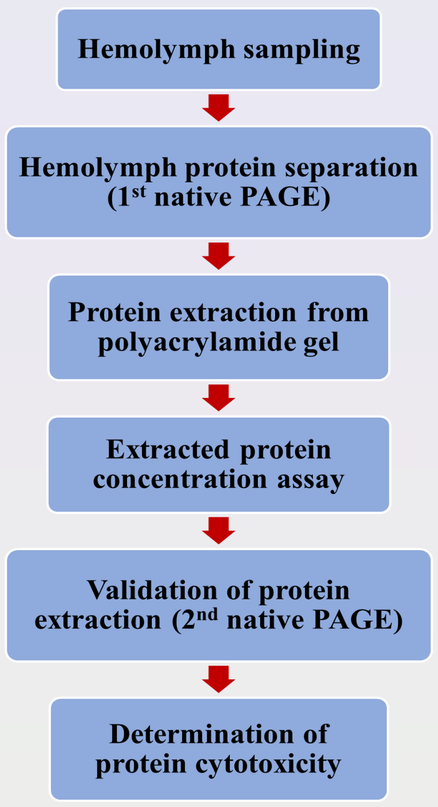

Fig 1. Experimental design.

Fig 2. European corn borer (ECB), *Ostrinia nubilalis* (Hbn.), 5th instar larvae.

RESULTS:

- ❑ Five distinct protein fractions were detected after the 1st native PAGE (P1-P5). After elution from the gel, these fractions and the method for their isolation were validated with a 2nd native PAGE (**Fig. 3**);
- ❑ The results of the MTT assay indicate an antiproliferative effect of all five protein fractions at each tested concentration to a greater or lesser extent, especially in the P4 fraction at a concentration of 1.86 ng/µL (**Fig. 4**).

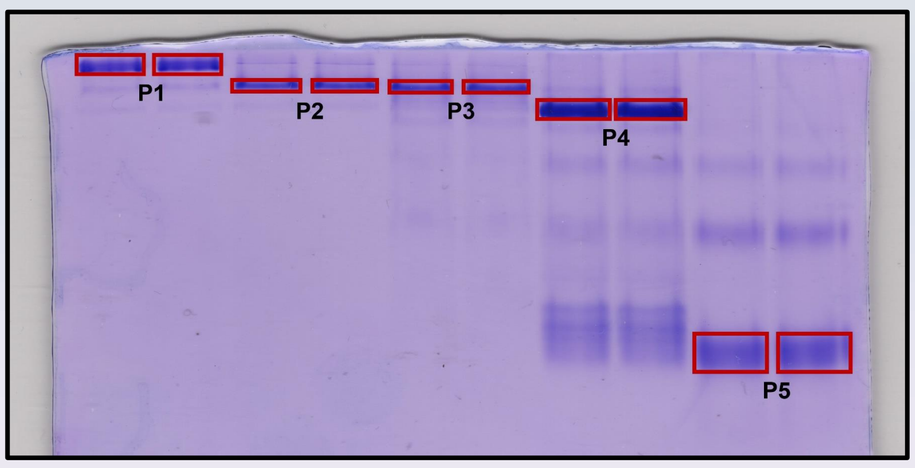

Fig. 3 Five protein fractions visible on polyacrylamide gel after 2nd PAGE.

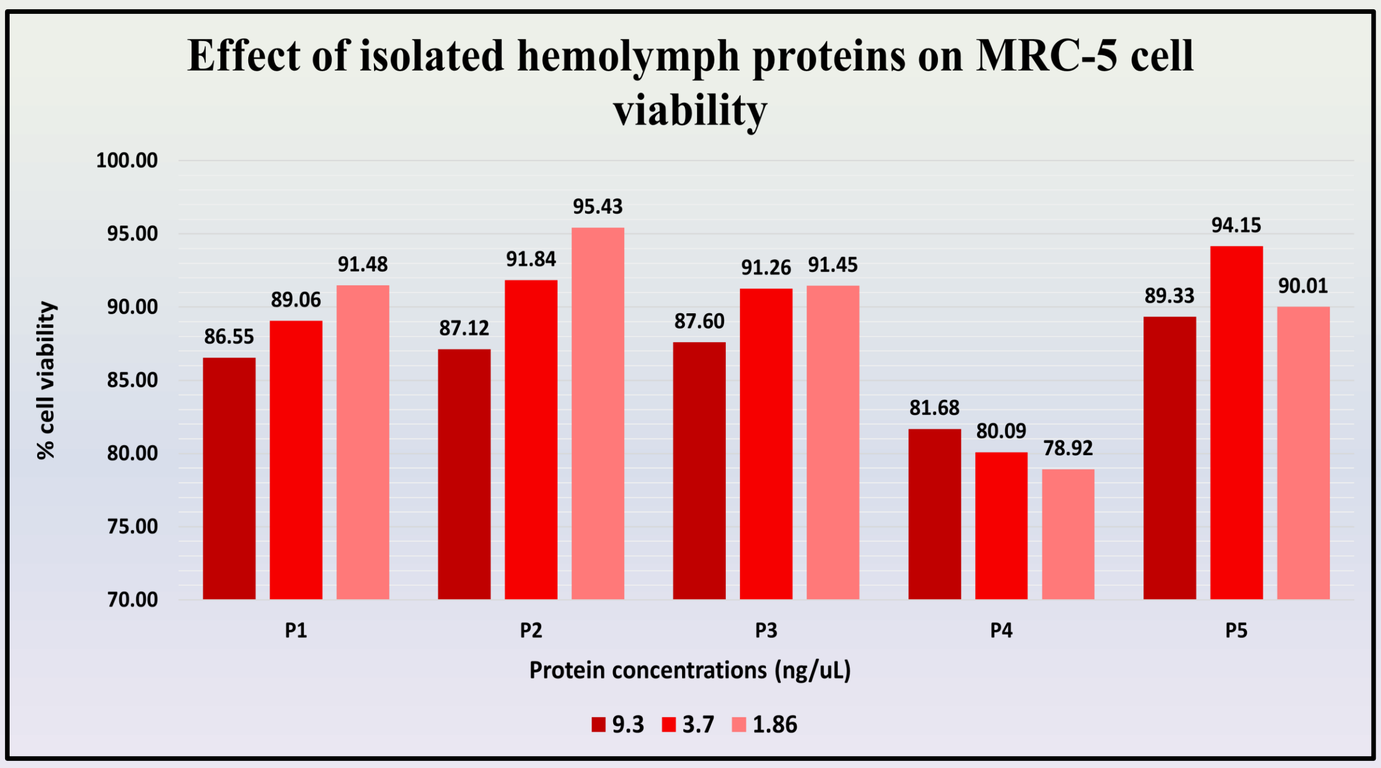

Fig. 4 Effect of three different concentrations (9.3, 3.7 and 1.86 ng/µL, respectively) of isolated hemolymph proteins (P1-P5) on MRC-5 cell viability.

CONCLUSIONS AND NEXT STEPS:

The insect hemolymph protein extraction method optimized in this study proved to be simple and successful and could potentially be applied to other insect species as well. Also, the structure and function of the proteins remained intact during the isolation process, which allows further use of the isolated proteins in downstream *in vitro* proteome research, the results of which will contribute to protein identification and *in silico* proteome research based on different bioinformatics tools. Finally, since the isolated proteins showed antiproliferative effects on the selected cell line, their cytotoxicity at higher concentrations will be further tested, as well as their anticancer and antimicrobial activity.

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