RESEARCH CENTER FOR THE NATURAL AND APPLIED SCIENCES
University of Santo Tomas, Manila, Philippines

CHECKLIST FOR NEW PROJECT PROPOSALS

1. THE PROJECT

a. The project proposal should be submitted in both hard and soft copies in accordance with the RCNAS Project Proposal Form 2 duly signed by proper authorities as indicated.

b. Annexes

   1. Annex "A" – Project Line Item Budget. detailed (Indicate in separate column proponent's counterpart and collaborating agency funds, if any)
   2. Annex "B" – Position description of project personnel

2. ATTACHMENTS

a. Curriculum vitae of Project Leader and co-Researcher
b. Certification of latest appointment of Project Leader and co-researcher

RESEARCH CENTER FOR THE NATURAL AND APPLIED SCIENCES
University of Santo Tomas, Manila, Philippines

AY 2021-2022

PART I. RESEARCH PROJECT PROPOSAL

1. PROJECT TITLE: Neuromodulatory Activity of Spider Venom Fractions

2. PROPONENT: Leonardo A. Guevarra Jr., MSc/ MS Biochemistry
Name and Degree

Business Address/Tel. No. Department of Biochemistry, Faculty of Pharmacy
Academic Rank Assistant Professor

3. Date of Birth 02/15/1979 Home Address / Tel No. Unit 1930 Avida Towers Intima Quirino Ext cor Zulueta St., Paco, Manila

3A. COOPERATING AGENCIES, if any on this project (College/Faculty Affiliation or DOST): Romblon State University

3B. IMPLEMENTING/COORDINATING AGENCY(ies): UST/RSU

4. OBJECTIVES: (State whether the project proposal is a new idea or a continuation of, or related to, previous research work(s) undertaken by the proponent's and what the proponent(s) aim to discover or established).

This proposal is a continuation of the spider venom project previously funded by CHED. The objective of this study is to elucidate the neurotoxicity of spider venom components by observing the swimming patterns and correlating it with the quantity of neurotransmitters in zebrafish models.

5. SIGNIFICANCE OF THE PROJECT: (Importance to science and/or technology. In what way would the research, if successful, contribute to the increase of national income and who, or what sectors, can possibly benefit from the research project when completed).

Understanding the mechanism of bioactivity is important in the evaluation of drug targets. It will help identify which molecules that can be developed into therapeutic agents that can be used to treat diseases.

This study on the mechanism of neuromodulation by spider venom fractions will allow us to identify molecules that are promising candidates for the treatment of neurological disorders including neuropathic pain. The mechanism of actions that will be elucidated in the neuromodulatory assays together with the peptide structure-activity relationship analysis information that can be acquired from molecular dynamics, a future research perspective, will provide the biochemical explanation of the venom peptides activity. The neuromodulatory data will provide an insight on the therapeutic window of characterized bioactive molecules.

6. PRESENT STATUS OF THE PROPOSED PROJECT:

6.1 Previous work done on this project. Describe briefly, any work you have done to date which is particularly pertinent.

We have, so far, identified, purified, and partially characterized toxic components of spider venom. One of the bioactivities we have reported are the paralytic and neurotoxicity of certain fractions of venom from spiders collected in Mindanao.

6.2 Result obtained by others. Summarize important results to date obtained by others on this problem, citing publications.

Spider venoms are known to contain biologically active low molecular weight peptides that can modulate cellular processes by binding to specific cell membrane receptors. These disulfide-rich peptides (DRP) which forms inhibitor cysteine knot (ICK) motifs selectively bind to a family or subclass of receptors and inhibit many biological activities such as ion channel polarization-depolarization process and induction of synthesis of neurotransmitters hence its neuromodulatory activity (Saez et al., 2010).

In previous studies the neuromodulatory activity of toxins in zebrafish can be assessed by observing their swimming patterns. Neurotoxic compounds alter the fish physiologic and behavioral swimming patters of zebrafish. For example, the pain and distress test innovated by Martins et al. (2016) provides a quick assessment of neurotoxicity of compounds in zebrafish based on erratic swimming motion and behavior and their response to stimulus (Martins et al., 2016). The Novel Tank Test, Fish Motion Test and the Mirror Biting Test which can be analyzed using motion tracking softwares idTracker and ImageJ evaluates the behavioral changes (van der Valk & van der Meijden, 2014; Ruszkiewicz et al., 2018; Recidoro et al., 2014). Measuring the transmitters related to behavioral patterns via RP-HPLC can evaluate and relate the neurologic activities of the spider venom toxins *in vivo* (Faria et al., 2018).

7. WORK PLAN

7.1 Procedure/Methodology in sufficient detail (if possible, present research design, schedules/questionnaires to be used, sampling procedures/techniques, etc.). Please utilize **GREEN Methods** (**Reduce, Replace, Reuse**). Reduce waste, eliminating costly wastes treatments, use safer products and reduce use of energy and resources

Neuromodulatory activity of the spider venom will be tested in zebrafish. Five microliters of peptide solution containing 1 micrograms of bioactive peptide will be introduced by injecting intramuscularly on the dorsal part of the body.

The Novel Tank Swim Test will be done according to methods by Audiria et al. (2018) with modification. Zebrafish injected with the bioactive peptide will be allowed to swim in a 28 cm (length) x 22 cm (depth) x 16 cm (height) tank containing 4 liters of room temperature water. The tank will be externally marked with a horizontal line at mid-depth. The behavioral responses that will be observed are: 1) average, maximum, and minimum motion speeds; 2) distance traveled; 3) freezing time percentage; 4) swimming time percentage; 5) rapid time movement percentage; 6) time spent at the top; and 7) the ratio of time spent at the top to time spent at the bottom. These behavioral responses will each be recorded for one minute at time intervals of 0, 5, 10, 15, 20, 25, and 30 minutes; and will be performed for 4 days, between 11:00 AM and 4:00 PM. After 4 days, garnered video data, captured by the camera, will be analyzed through the idTracker software, and calculated using the Microsoft Excel software (Audira et al, 2018).

For the 3D locomotion test, the video camera will be placed approximately five meters away from the observation tank and will be adjusted to minimize the visible gap borders present in the frame. An acrylic tank that is surrounded by non-transparent shields on both the left and right sides will be used to be the observation deck. The background of the tank will be a light-emitting diode (LED) platform to ensure a high contrast video with minimal background noise. A larger-sized mirror will be placed at a 45° angle on the top border of the observation tank to reflect all the images observed. Analysis of the video clips will be done using the idTracker while the analysis and plotting of the 3D trajectories will be analyzed in Origin 9.1 software (Audira, et al., 2018).

Aggression, boldness, anxiety, and sociability of zebrafish will be done through Mirror Biting Test as described by Pagliara et al. (2020). A zebrafish being tested will be placed in a 21 L and would be left undisturbed for 10 minutes. A mirror will be carefully positioned into the tank and the reaction of the fish against the image on the mirror will be recorded (Pham et al., 2012; Moretz et al., 2007).

After the behavioral tests, the zebrafish will be decapitated, and the brains will be dissected on ice. Collected brains will be weighed, macerated using a micropestle and centrifuged at 10,000 rpm at 4º C. The supernatant collected and subjected to quantitative analysis by HPLC as described by Zapata et al. (2009).

7.2 Timetable of research (Please give details by stating the duration of the project and estimated time to be spent for the various phases of the project, and description of each phase) Please complete the Gantt Chart)

Schedule Project Work Activities: Gantt Chart AY <u>2021-2022</u>

Activity	Output	Date		Gantt Chart											
		Start	*Completion*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*
1. Application of IACUC and IBS clearance	Approved IACUC and IBC Protocols	Aug	Oct												
2. Spider collection and identification	Identified spider species ready for venom extraction	Oct	Dec												
3. Venom extraction and fractionation	Lyophilized venom fractions	Nov	Mar												
4. Set up of aquarium for fish test		Jan	Jan												
4. Swim Test	Swim Test data	Feb	April												
5. Quantitative analysis of neurotransmitters	Quantitative analysis of neurotransmitters data														
6. Statistical Analysis	Analyzed data														
7. Preparation of report and writing of publication	Terminal report and draft of publication														

Note: Table may be adjusted to accommodate entries.

8. FINANCIAL REQUIREMENTS:

	Details	Totals	Requested from UST	Proponent Contribution	Cooperating agencies counterpart
I	**PERSONNEL SERVICES:**				
	A. Salaries and Honorarium 1. Co-project leader	P 90,000.00	P	P	P 90,000.00
	B. Consultancy Services 1. Biostatistician	P 10,000.00	P	P	10,000.00
Total for Personal Services		P 100,000.00	P	P	P 100,000.00
II	**MAINTENANCE AND OTHER OPERATING EXPENSES:**				
	1. Travel * a. Transportation b. Accommodation	P 25,000.00 5,000.00	P	P	P 25,000.00 5,000.00
	2. Supplies and Materials ** a. Laboratory animals, housing and feeds b. Reagents c. Laboratory consumables d. Office supplies	P 15,000.00 100,000.00 140,000.00 20,000.00	P	P 40,000.00	P 15,000.00 100,000.00 100,000.00 20,000.00
	3. Meals	P 25,000.00	P	P	P 25,000.00
	4. Communication	10,000.00			10,000.00
	5. Printing and Publication	P 100,000.00			P 100,000.00
	6. Software Licenses	20,000.00			20,000.00
Total for Maintenance and Other Operating Exp.		P 430,000.00	P	P 40,000.00	P 390,000.00
III	**EQUIPMENT:**				
	(Include list of equipment and estimated cost and state if locally available or to be improved, justification for its use in the project if not available in the institute of agency	P	P	P	P
Total for Equipment		P	P	P	P
TOTAL COST OF PROJECT		**P 530,000.00**			

* Submit breakdown for requests exceeding P2,000.00. For travel expenses indicate place to be visited, frequency, tentative schedule, purpose and estimate cost of visit.

** Submit breakdown for request exceeding P1,000.00.

SUMMARY OF ANNUAL FINANCIAL PLAN

		Academic Year **2021-2022**
I.	Personnel Services	P 100,000.00
II.	Maintenance and Other Operating Expenses	P 430,000.00
III.	Equipment	P
	TOTAL COST OF PROJECT	**P 530,000.00**

NOTE: Yearly itemized financial breakdown of budget should be submitted.

PART II. ADDITIONAL INFORMATION

9. RESEARCH EXPERIENCE

Statement as to other research activities of the proponent. Indicate in what capacity; state whether receiving grants from other agencies; if so, give title of said research project or projects, and amount of grant(s) including honorarium.

The proponent have been working on peptide for application in diagnostics and therapeutics. The proponent served as project leader and co-leader in several CHED-funded research conducted at the Research Center for Natural and Applied Sciences (RCNAS) and UP-NIH-funded research at UP College of Medicine. The proponent is one of the project leaders in the CHED-Spider Venom Project where he was able to publish several papers on cytotoxicity and neurotoxicity of spider venom peptides in local and international peer-reviewed journals.

10. REFERENCES (literature cited in this proposal)

Audira, G., Sampurna, B. P., Juniardi, S., Liang, S. T., Lai, Y. H., & Hsiao, C. D. (2018). A simple setup to perform 3D locomotion tracking in zebrafish by using a single camera. Inventions, 3(1). https://doi.org/10.3390/inventions3010011

Faria M, Ziv T, Gómez-Canela C, Ben-Lulu S, Prats E, Novoa-Luna KA, Admon A, Piña B, Tauler R, Gómez-Oliván LM, Raldúa D. (2018). Acrylamide acute neurotoxicity in adult zebrafish. *Sci Rep, 8*(1):7918. doi: 10.1038/s41598-018-26343-2.

Martins T, Valentim AM, and Pereira N, Antunes LM. (2016). Anaesthesia and analgesia in laboratory adult zebrafish: a question of refinement. *Lab Anim*, *50*(6):476-488. https://doi.org/10.1177/0023677216670686.

Moretz JA, Martins EP, Robinson BD (2007) The effects of early and adult social environment on boldness and aggression in zebrafish (Danio rerio). Exp Biol Fishes 80(1):91–101

Pham, M., Raymond, J., Hester, J., Kyzar, E., Gaikwad, S., Bruce, I., Fryar, C., Chanin, S., Enriquez, J., Bagawandoss, S., Zapolsky, I., Green, J., Stewart, A. M., Robison, B. D., & Kalueff, A. V. (2012). Assessing Social Behavior Phenotypes in Adult Zebrafish: Shoaling, Social Preference, and Mirror Biting Tests. Neuromethods, 231–246. https://doi.org/10.1007/978-1-61779-597-8_17

Recidoro, A. M., Roof, A. C., Schmitt, M., Worton, L. E., Petrie, T., Strand, N., Ausk, B. J., Srinivasan, S., Moon, R. T., Gardiner, E. M., Kaminsky, W., Bain, S. D., Allan, C. H., Gross, T. S., & Kwon, R. Y. (2014). Botulinum toxin induces muscle paralysis and inhibits bone regeneration in zebrafish. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, *29*(11), 2346–2356. https://doi.org/10.1002/jbmr.2274

Ruszkiewicz JA, Pinkas A, Miah MR, Weitz RL, Lawes MJA, Akinyemi AJ, Ijomone OM, Aschner M. (2018). C. elegans as a model in developmental neurotoxicology. *Toxicol Appl Pharmacol*, 354:126-135. doi: 10.1016/j.taap.2018.03.016. Epub 2018 Mar 14. PMID: 29550512

Saez, N. J., Senff, S., Jensen, J. E., Er, S. Y., Herzig, V., Rash, L. D., & King, G. F. (2010). Spider-venom peptides as therapeutics. *Toxins*, *2*(12), 2851–2871. https://doi.org/10.3390/toxins2122851.

van der Valk T and van der Meijden A. (2014). Toxicity of scorpion venom in chick embryo and mealworm assay depending on the use of the soluble fraction versus the whole venom. *Toxicon*, 88:38-43. doi:10.1016/j.toxicon.2014.06.007.

Zapata, A., Chefer, V. I., Shippenberg, T. S., & Denoroy, L. (2009). Detection and Quantification of Neurotransmitters in Dialysates. Current Protocols in Neuroscience, 48(1). https://doi.org/10.1002/0471142301.ns0704s48.

PART III. ACCEPTANCE OF CONDITIONS

CONDITIONS:

1. Proponent shall not leave the country during the duration of the contract of project without approval of UST.

2. He/She shall submit periodic reports and/or pertinent reports when requested.

3. Any publication or news release regarding the project shall state that the project has been supported financially by the UST Research Center for the Natural and Applied Sciences.

I agree to accept and abide by all terms and conditions which the UST Research Center for the Natural and Applied Sciences may stipulate in respect to the grant of assistance to my project proposal and which shall be embodied in a Memorandum of Agreement to be executed by me and the UST Research Center for the Natural and Applied Sciences.

Asst. Prof. Leonardo A. Guevarra Jr., MSc
Name and Signature of Researcher

ENDORSED BY:

Prof. Aleth Therese L. Dacanay, Ph.D.
Dean
Faculty of Pharmacy

APPROVED BY:

Prof. Bernard John V. Tongol, Ph.D.
Director
Research Center for the Natural and Applied Sciences

Prof. Maribel G. Nonato, Ph.D.
Vice Rector for Research and Innovation
Office of the Vice Rector for Research and Innovation