

IMMUNOLOGIC ADJUVANT ACTIVITY OF NEEM LEAF EXTRACT IN RATS WITH SPLENECTOMY

Barboza-Herrera Carolina¹, Castillo-Maldonado Irais¹, Delgadillo-Guzmán De almy², Vega-Menchaca María-del-Carmen³, Haro-Santa Cruz Jorge¹, Ramírez-Moreno Agustina⁴, Flores-Loyola Erika⁴, Avalos-Soto Joaquín⁵, Téllez-López Miguel Ángel⁵ & Pedroza-Escobar David*^{1,6}

¹Department of Biochemistry, Biomedical Research Centre, Faculty of Medicine, Universidad Autónoma de Coahuila Unidad Torreon, Torreon, Mexico

²Department of Pharmacology, Faculty of Medicine, Universidad Autónoma de Coahuila, Torreon, Mexico

³Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Mexico

⁴Faculty of Biological Sciences, Universidad Autónoma de Coahuila Unidad Torreon, Torreon, Mexico

⁵Cuerpo Académico Farmacia y Productos Naturales, Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Mexico

⁶Centro de Actividades Multidisciplinarias de Prevención CAMP A.C.
Email: dpedroza@uadec.edu.mx

Received-01.11.2021, Revised-19.11.2021, Accepted-25.11.2021

Abstract: Introduction: Immunologic adjuvants are substances of very varied chemical structure that are used to reinforce the immune response against an antigen administered simultaneously in immunization schemes when the antigen's immunogenicity is low. On the other hand, splenectomy is an intervention that consists in the total removal of the spleen with the intention of suppressing the immune response. So that, the aim of this work was to evaluate an immunosuppressant model of rats with splenectomy and the immunologic adjuvant activity of Neemaqueous leaf extract in that model. Material and methods: Cytotoxicity tests and phytochemical composition were performed on the extract to establish the extract concentrations to be tested. Later, 16 rats were immunized in a 30-day immunization scheme with bovine serum albumin as an antigen, and three Neem-based adjuvants (10, 100 and 1000 µg/mL). After doing the experiments, leukocytes were counted by manual method with Turk's liquid and Neubauer's chamber; and total proteins in serum were quantified by Bradford method as an indicative of immunoglobulin production among experimental groups. Hepatic enzymes were analyzed by automated biochemical analysis. Results: The biotoxicity assay of the extract showed a 1077 µg/mL concentration as a LD50. A significant increase in leukocyte counts and protein concentration was observed between the beginning and end of the experiments. While the hepatic enzymes showed a normal profile. Conclusions: Neem aqueous leaf extract at concentration of 10 µg/mL exhibits immunologic adjuvant activity by enhancing leukocyte counts.

Keywords: Immunologic adjuvant, Neem, *Azadirachta indica*, Splenectomy, Leukocytes

REFERENCES

Abbas, A.K. and Lichtman, A.H. (2003). Métodos de estudio de la activación de linfocitos T, In: *Inmunología celular y molecular. Spanish version of the 5th Edition in English "Cellular and molecular immunology"*, Edited by Elsevier Science, (Madrid, España), 166-167.

[[Google Scholar](#)]

Awah, F.M., Uzoegwu, P.N. and Ifeonu, P. (2011). In vitro anti-HIV and immunomodulatory potentials of *Azadirachta indica* (Meliaceae) leaf extract, *Afr J Pharm Pharmacol*, 5(11): 1353-1359.

[[Google Scholar](#)]

Balish, E., Yale, C.E. and Hong, R. (1972). Serum proteins of gnotobiotic rats, *Infection and Immunity*, 6(2):112-118.

[[Google Scholar](#)]

Batista-Duarte, A., Lastre, M. and Pérez, O. (2014). Adyuvantes inmunológicos. Determinantes en el balance eficacia-toxicidad de las vacunas

contemporáneas, *Enferm Infecc Microbiol Clin*, 32(2):106-114.

[[Google Scholar](#)]

Biswas, K; Chattopadhyay, I; Banerje, RK; Bandyopadhyay, U (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*), *CurrSci*, 82(11): 1336-1345.

[[Google Scholar](#)]

Blanco-Quirós, A. (2014). Update on vaccines and new perspectives, *An Real Acad Med Cir Vall*, 51(1):141-157.

[[Google Scholar](#)]

Braiman-Wiksman, L., Solomonik, I., Spira, R. and Tennenbaum, T. (2007). Novel insights into wound healing sequence of events, *Toxicol Pathol.*, 35(6):767-79.

[[Google Scholar](#)]

Chandrashekar, P.M., Prashanth, K.V.H. and Venkatesh, Y.P. (2011). Isolation structural elucidation and immunomodulatory activity of fructans from aged garlic extract, *Phytochemistry*, 72(2-3):255-264.

[[Google Scholar](#)]

*Corresponding Author

- Chandrashekar, PM. and Venkatesh, YP.** (2009). Identification of the protein components displaying immunomodulatory activity in aged garlic extract, *J Ethnopharmacol*, 124(3):384-390. [[Google Scholar](#)]
- Chandrashekar, PM. and Venkatesh, YP.** (2016). Immunostimulatory properties of fructans derived from raw garlic (*Allium sativum* L.), *Bioactive carbohydrates and dietary fibre*, 8(2):65-70. [[Google Scholar](#)]
- Clement, F. and Venkatesh, YP** (2010). Dietary garlic (*Allium SATIVUM*) lectins, ASA I and ASA II, are highly stable and immunogenic, *International immunopharmacology*, 10(10):1161-1169. [[Google Scholar](#)]
- El Bagir, NM, El Amin, RTH, Alhaidary, A., Mohamed, HE and Beynen, AC** (2010). Serum protein levels and hematological values in rats fed a diet containing black cumin seed, *Journal of Animal and Veterinary Advances*, 9(20):2604-2607. [[Google Scholar](#)]
- Flint, SJ., Enquist, LW., Krug, RM, Racaniello, VR. and Skalka, AM.** (2000). Preparation of vaccines, Chapter 19 Prevention and control of viral diseases, In "*Principles of Virology Molecular biology, pathogenesis and control*"; edited by ASM PRESS, (Washington DC), 820 pp. [[Google Scholar](#)]
- Ghosh, S., Sarkar, M., Ghosh, T., Guha, I., Bhuniya, A., Saha, A., Dasgupta, S., Barik, S., Bose, A. and Baral, R.** (2016). Neem leaf glycoprotein promotes dual generation of central and effector memory CD8(+) T cells against sarcoma antigen vaccine to induce protective anti-tumor immunity. *Mol Immunol*. 71:42-53. [[Google Scholar](#)]
- Gupta, R.K.** (1998). Aluminium compounds as vaccine adjuvants, *Adv Drug Deliv Rev*, 32(3):155-172. [[Google Scholar](#)]
- Hallday, R. and Kekwick, RA.** (1957). Electrophoretic analysis of the sera of young rats, *Proc R Soc Lond B Biol Sci.*, 146(924):431-437. [[Google Scholar](#)]
- Irais, CM, María-de-la-Luz, SG., Dealmy, DG., Agustina, RM, Nidia, CH, Mario-Alberto, RG., Luis-Benjamín, SG., María-del-Carmen, VM and David, PE.** (2020). Plant Phenolics as Pathogen-Carrier Immunogenicity Modulator Haptens. *Curr Pharm Biotechnol*, 20(15): 1236-1243. [[Google Scholar](#)]
- Kumar, VP. and Venkatesh, YP.** (2016). Alleviation of cyclophosphamide-induced immunosuppression in Wistar rats by onion lectin (*Allium cepa* agglutinin), *J Ethnopharmacol*, 186:280-288. [[Google Scholar](#)]
- Kumar, VS. and Navaratnam, V.** (2013). Neem (*Azadirachta indica*): prehistory to contemporary medicinal uses to humankind, *Asian Pac J Trop Biomed*, 3(7): 505-514. [[Google Scholar](#)]
- Leung, MYK, Liu, C., Koon, JCM and Fung, KP.** (2006). Polysaccharide biological response modifiers, *Immunol Lett.*, 105(2):101-114. [[Google Scholar](#)]
- Li, P. and Wang, F.** (2015). Polysaccharides: Candidates of promising vaccine adjuvants, *Drug Discov Ther.*, 9(2):88-93. [[Google Scholar](#)]
- Licciardi, PV. and Underwood, JR.** (2011). Plant-derived medicines: A novel class of immunological adjuvants, *Int Immunopharmacol.*, 11(3):390-398. [[Google Scholar](#)]
- Lindblad, EB. and Schonberg, C.** (2010). Chapter 4: Aluminium adjuvants: preparation, application, dosage, and formulation with antigen, in "*Vaccine adjuvants, methods in molecular biology*", edited by G Davies, Humana Press, (Washington DC), 314 pp. [[Google Scholar](#)]
- Maruyama, H., Sperlagh, M., Zaloudik, J., Liang, S., Mizuki, K., Molthoff, C. and Herlyn, D.** (2002). Immunization procedures for anti-idiotypic antibody induction in mice and rats, *J Immunol Methods*, 264(1-2):121-133. [[Google Scholar](#)]
- Patwardhan, B. and Gautam, M.** (2005). Botanical immuno drugs scope and opportunities, *Drug discovery today*, 10(7):495-502. [[Google Scholar](#)]
- Pedroza-Escobar, D., Serrano-Gallardo, LB., Escobar-Ávila, EAD., Nava-Hernández, MP. and Vega-Menchaca, MDC.** (2017). HIV-1 infection inhibition by neem (*Azadirachta indica* A. Juss.) leaf extracts and Azadirachtin, *Indian j. tradit. knowl.*, 16(3):437-441. [[Google Scholar](#)]
- Pedroza-Escobar, D., Serrano-Gallardo, LB., Escobar-Ávila, EAD. and Sevilla-González, MDLL.** (2016). Using of medicinal plants among people living with HIV, *J. plant dev. sci.*, 8(7):311-314. [[Google Scholar](#)]
- Pedroza-Escobar, D., Serrano-Gallardo, LB., Sevilla-González, MDLL., López-García, S., Escobar-Ávila, EAD., Luna-Herrera, J. and Vega-Menchaca, MDC.** (2016). Effect of neem (*Azadirachta indica* A. Juss.) leaf extracts in human T lymphocytes, *Indian j. tradit. knowl.*, 15(2): 219-222. [[Google Scholar](#)]
- Qiao, N., Liu, Q., Meng, H. and Zhao, D.** (2014). Haemolytic activity and adjuvant effect of soyasaponins and some of their derivatives on the immune responses to ovalbumin in mice, *Int Immunopharmacol.*, 18(2):333-339. [[Google Scholar](#)]
- Reyna-Margarita, HR., Irais, CM, Mario-Alberto, RG., Agustina, RM, Luis-Benjamín, SG.**

and David, PE. (2019). Plant Phenolics and Lectins as Vaccine Adjuvants. *Curr Pharm Biotechnol*, 20(15): 1236-1243.

[[Google Scholar](#)]

Schepetkin, IA. and Quinn, MT. (2003). Botanical polysaccharides macrophage immunomodulation and therapeutic potential, *IntImmunopharmacol.*, 6(3):317-333.

[[Google Scholar](#)]

Sierra-González, G. and Tamargo-Santos, B. (2011). Adyuvantes inmunológicos para vacunas humanas: estado actual, tendencias mundiales y en cuba, *An. de la Acad.*, 1(2):1-32.

[[Google Scholar](#)]

Singh, D., Tanwar, H., Jayashankar, B., Sharma, J., Murthy, S., Chanda, S., Singh, SB. and Ganju, L. (2017). Quercetin exhibits adjuvant activity by enhancing Th2 immune response in ovalbumin immunized mice, *Biomed Pharmacother*, 90:354-360.

[[Google Scholar](#)]

Sun, HX. (2006). Haemolytic activities and adjuvant effect of Bupleurum chinense saponins on the immune responses to ovalbumin in mice, *Vaccine*, 24(9):1324-31.

[[Google Scholar](#)]

Sun, HX. and Pan, HJ. (2006). Immunological adjuvant effect of Glycyrrhiza uralensis saponins on the immune responses to ovalbumin in mice, *Vaccine*, 24(11):1914-1920.

[[Google Scholar](#)]

Sun, HX., Xie, Y. and Ye, YP. (2009). Advances in saponin-based adjuvants, *Vaccine*, 27(12):1787-1796.

[[Google Scholar](#)]

Tomar, A. and Singh, H. (2005). Folk medicinal uses of some indigenous plants of Baghpat district of Uttar Pradesh, India. *Journal of Non-Timber Forest Products*, Vol. 12 (3): 167-170.

[[Google Scholar](#)]

Tomar, A. (2007). Folk medicinal uses of some indigenous plants of Hastinapur block in Meerut District, (Uttar Pradesh) India. *Journal of Medicinal and Aromatic Plant Sciences*, Vol. 29 (4): 186-190.

[[Google Scholar](#)]

Tomar, A. (2008). Folk medicinal plants in Muzaffarnagar district of Western Uttar Pradesh, India. *Journal of Indian Botanical Society*, Vol. 87 (3 & 4): 200-208.

[[Google Scholar](#)]

Wasser, S.P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides, *App Microbiol Biotechnol*, 60(3):258-274.

[[Google Scholar](#)]

Zaias, J., Mineau, M., Cray, C., Yoon, D. and Altman, NH. (2009). Reference values for serum proteins of common laboratory rodent strains, *J Am Assoc Lab Anim Sci.*, 48(4):387-390.

[[Google Scholar](#)]