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Effect of dehydroleucodine (DhL) on the acrosome reaction in sperm of *Chinchilla lanigera*: signalling pathways involved

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Research Article

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Abstract

The secondary metabolites of several plant species, particularly sesquiterpenic lactones (SLs) have been studied by different research groups for over 30 years. This group of metabolites presents numerous biological activities such as antibacterial, antiviral, antiulcer, cell proliferation inhibitor, and oocyte activator with participation in exocytosis processes. This study aims to assess some sperm parameters in epididymal gametes of *Chichilla lanigera* exposed to increasing concentrations (0 to 2 mM) of DhL for various incubation times from 10 to 40 minutes. We determined the participation of different cell signalling pathways in the induced acrosome reaction. Our results showed an alteration in the progressive motility pattern and cell viability depending on DhL concentration and exposure time of gametes. When analyzing acrosomal status, higher percentages than the negative control were obtained in all tested doses. Both isolated and joint inhibition tests of PKA and phospholipases (PLC and PLA_2) showed a greater participation of PI-PLC. This is the first report concerning the effects of this lactone on the medium of sperm incubation. Consequently, further studies will be necessary to determine the molecular implications of this lactone on the fertilizing potential of the sperm.

Introduction

Sesquiterpene lactones (SLs) constitute one of the largest biogenetically homogeneous groups of known secondary metabolites (Heinrich *et al.*, 1998). These natural compounds have a broad spectrum of biological activities, which are related to the great structural diversity. Cytotoxic effects on animal cells have been attributed to these compounds (Lee *et al.*, 1971) as well as antimicrobial (Vega *et al.*, 2009; Mohamed *et al.*, 2017), antiamoebic (Rodríguez-Expósito *et al.*, 2021), antiviral (Li *et al.*, 2005; Ozçelik *et al.*, 2009) and antiallergic effect (Lee *et al.*, 2018). Hehner *et al.* (1998) even demonstrated an inhibitory effect on immune and inflammatory responses.

Dehydroleucodine (DhL) is a sesquiterpene lactone of the guaianolide type, featuring an alpha-methylene butyrogamma ring connected to a seven-atom ring, which is fused to another exocyclic alpha beta-unsaturated cyclopentenone ring (Priestap *et al.*, 2011) as shown in Figure 1. DhL was isolated from the aerial parts of *Artemisia douglasiana Besser*, a plant used in traditional medicine. Studies with DhL have demonstrated gastric cytoprotective and antiulcer effects (Giordano *et al.*, 1990; Guardia *et al.*, 1994; Penissi *et al.*, 1998), justifying its use in popular medicine as an infusion.

Assays on the cell cycle have shown that DhL causes an arrest in the G_2 phase in meristematic cells of *Allium cepa* (Lopez *et al.*, 2002), in myocytes from the blood vessels of cultured rats (Cruzado *et al.*, 2005; Polo *et al.*, 2007), in cancer cells (Siriwan *et al.*, 2011), and affects mitotic clonal expansion to inhibit preadipocyte differentiation at the G_0/G_1 phase (Abood *et al.*, 2018). Similarly, Sánchez Toranzo *et al.* (2007, 2009) recorded its inhibitory effect on the meiotic cell cycle in *Rhinella arenarum* oocytes.

Interestingly, it was demonstrated that the use of DhL in mature bovine oocytes induces their activation, while exocytosis of cortical granules was observed in amphibians (Medina *et al.*, 2014). Studies carried out in embryos obtained by intracytoplasmic sperm injection and somatic cell nuclear transfer, showed encouraging results when DhL was used in the activation of embryo development (Vichera *et al.*, 2010).

Assays by Suhaiman *et al.* (2011) administering DhL in the drinking water of male mice, did not show changes in the levels of testosterone or o-estradiol in plasma. However, capacitated

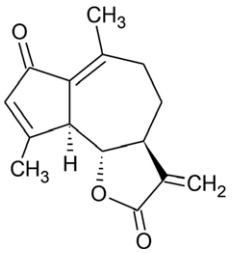

Figure 1. Structure of Dehydroleucodine (DhL). It belongs to the group of guaianolides and has an alphamethylenebutyro-gamma-lactone ring connected to a seven-membered ring fused to an exocyclic alpha, beta-unsaturated cyclopentenone ring.

epididymal sperm showed a higher percentage of binding to zone-free oocytes compared to controls.

Chinchillas are an endangered species, in their natural habitat and, in captivity, they present a low reproductive rate. Knowledge of their reproductive biology is essential to develop effective conservation strategies and improve reproductive protocols. While DhL has shown promising effects on several animal models by influencing exocytosis processes and oocyte activation, its impact on chinchilla reproduction could offer new possibilities for improving reproductive efficiency, which is essential for the preservation of this species in both natural habitats and captivity.

The present study constitutes the first on the effect of a sesquiterpene lactone on the physiology of the male gamete, showing an induction of the acrosome reaction (AR) in *Chinchilla lanigera* epididymal sperm. The results presented contribute to a better understanding of the molecular mechanisms involved in this process.

Materials and methods

Animals

The animals used in this study were provided by a commercial hatchery authorized for skin production. We selected 6 sexually mature male specimens, 10 to 12 months of age, with a body mass of 550–650 g. All animals were housed under a 12-h dark-light cycle at a constant temperature of 22 ± 1 °C and received water and food *ad libitum*. The slaughtered animals were sacrificed by cervical dislocation and then immediately transported to the laboratory in thermal containers to maintain a constant temperature.

Reagents and incubation media

PBS 1X (Dulbecco's phosphate-buffered saline solution) Irvine Scientific_ (Santa Ana, CA, USA). Dimethyl sulfoxide (DMSO), N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H89 dihydrochloride hydrate), quinacrine, 1-[6-[((17β)-3-Methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1H-pyrrole-2,5-dione (U73122 hydrate), dehydroleucodine (DhL) from Sigma Aldrich. Co. (St. Louis, MO, USA). DhL was used from a stock solution of 10 mM in DMSO. Coomassie Brilliant Blue G250 from Biopack® (CABA, Argentina).

Modified HTF medium (human tubal fluid)

To 750 ml of purified water, 5.931 g of NaCl, 0.35 g of potassium chloride (KCl), 0.05 g of magnesium sulphate heptahydrate (MgSO4.7H2O), 0.05 g of potassium dihydrogen phosphate (KH_2PO_4), 0.366 g of sodium bicarbonate (NaHCO3), 0.5 g of d-glucose, 0.036 g of sodium pyruvate, 0.3 g of calcium chloridedihydrate ($CaCl_2.2H_2O$) and 4.0 g of sodium dl-lactate (60% (v/v) syrup) were added. Ten µg of phenol red, 100 UI penicillin and 50 µg streptomycin sulphate were added to 1 ml of the above medium. pH was then adjusted to 7.4 with 1 mol/l hydrochloric acid (HCl).

Collection and preparation of spermatozoa

Immediately after slaughtering, spermatozoa were collected by puncture from the cauda epididymis. The samples were washed twice in PBS, centrifuged at 1000 × *g* for 10 minutes, and the pellet was resuspended with HTFm. All materials used for the sample collection and subsequent trials were under strict temperature control (37 ± 0.5 °C). We only worked with samples with a vitality percentage equal to or >80%, using the eosin test 0.5% (w/v) with an inverted microscopy at 400x. Counts were performed on 200 cells per replicate. Eosin enters only dead cells, staining them a pink colour, while in living cells, the plasma membrane prevents its penetration.

To assess motility patterns, we placed 10 µl of sperm suspensions on a clean and dry slide, covered with a 22 mm × 22 mm coverslip to ensure a 20.7 µm chamber depth, allowing free movement of the sperm. Observations were made on 200 cells in duplicate, with microscope of phase contrast at 400x.

Capacitation of spermatozoa: assay of DhL inducer effect

The washed spermatozoa were separated into aliquots (final concentration 3–5 × 10^6 cells/ml) and incubated with HTFm supplemented with 2.5 g/l albumin fraction V for 2.5 h at 37 °C in an atmosphere containing 95% relative humidity and 5% CO_2. Previously capacitated sperm suspensions were treated with different concentrations (0–2 mM) of DhL for different incubation times (0–40 minutes). Reactions were stopped with buffered formalin 4% final concentration, and samples were processed for cytological staining and scanning electron microscopy.

Determination of signalling pathways involved in DhL-induced acrosome reaction

In order to determine the participation of cAMP-dependent PKA, Phospholipase A2 (PLA_2) and Phospholipase C (PLC) in the AR induced by DhL, we used capacitated spermatozoa pretreated with various pharmacological inhibitors of these key enzymes: H89 (30 µM) – a PKA inhibitor, for 30 min; quinacrine (5 µM) – a PLA2 specific inhibitors, for 15 min; and U73122 (20 µM) – a PLC inhibitors, for 30 min. After incubation with the inhibitors, the AR was induced with 0.5 mM DhL.

When the protocol included the addition of two or more drugs, the sperm was washed in HTFm and centrifuged before addition of the next drug. All control experiments were performed under identical conditions. Negative controls were treated with the corresponding solvent. The doses and incubation times used in this study were selected as described in our previous study (Gramajo-Bühler *et al.*, 2016) and the same final concentrations were consistently used.

Evaluation of acrosomal status by Coomassie Blue staining

In all incubations, reactions were stopped by adding buffered formalin (final concentration 4%). Samples were fixed at 4°C for 10 minutes, then washed twice with a 0.1 M ammonium acetate solution at pH 9, followed by centrifuging for 5 minutes at 1200 × *g*. The smears were air-dried, hydrated in distilled water for

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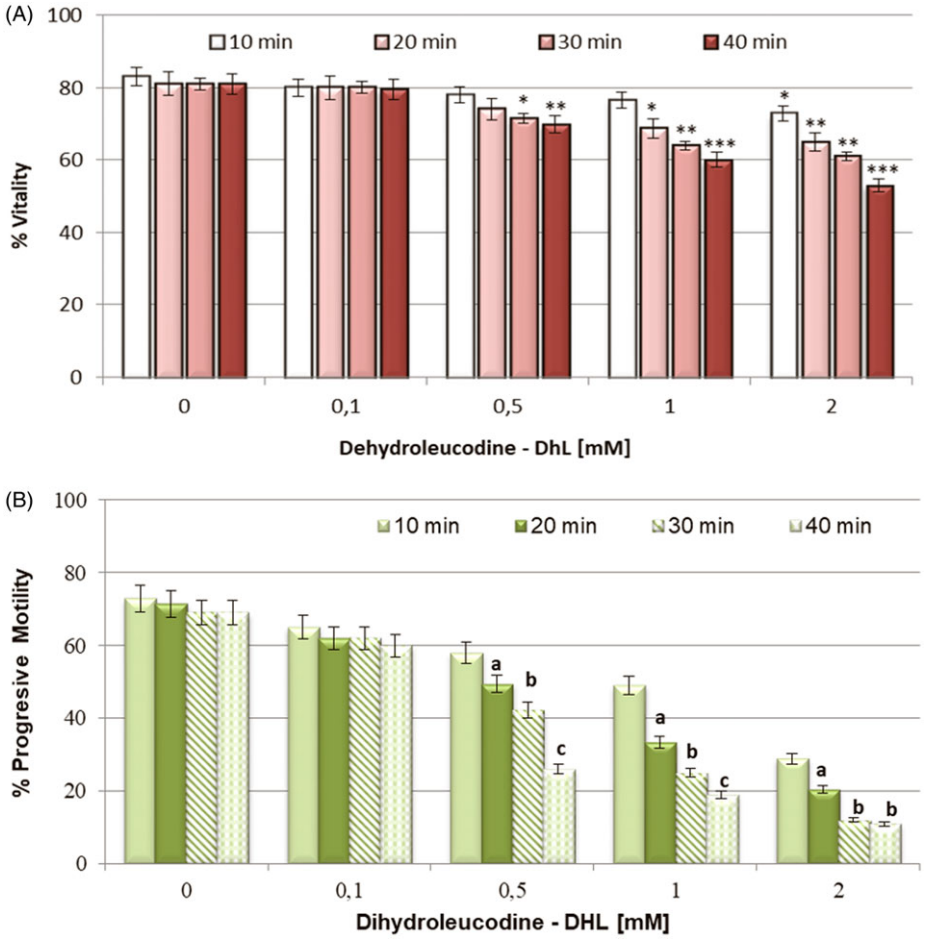

Figure 2. (A) Effect of Dehydroleucodine on the viability of sperm incubated for different time periods at 37 °C. The results are expressed as the mean ± SEM of five experiments performed in duplicate with different animals. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs control group (0 mM DhL). (B) Effect of Dehydroleucodine on the progressive motility of sperm. Samples were incubated with increasing concentrations of DhL at different times. The results are expressed as the mean ± SEM of four experiments performed in duplicate with different animals. Significant differences at the same concentrations for different incubation periods (a, b, c).

5 minutes, stained with Coomassie Blue G250 solution, and finally washed with distilled water for 5 minutes.

A total of 200 cells were analyzed by light microscopy at 1000x. A positive response was defined as the absence of acrosome, while a negative response was indicated by the presence of the acrosomal vesicle, visible as a brilliant blue band in the anterior region of the head.

Scanning electron microscopy (SEM)

Sperm cells were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 for 2 h at 4 °C. Subsequently, samples were post-fixed in 1% osmium tetroxide in phosphate buffer overnight, dehydrated in a graded series of alcohol solutions and 100% acetone, and finally desiccated using the critical point drying technique with CO_2. After dehydration, samples were mounted on specimen holders for SEM (2 cm diameter metal cylinders) and coated with gold. Observations were made with a JEOL 35CF SEM.

Statistical analysis

All experiments were performed independently in duplicate. Means and standard errors were calculated for all data sets. Differences between groups were evaluated using two-way analysis of variance followed by Fisher's exact test. A p value of <0.05 was considered statistically significant.

Results

Assay of the effect of dehydroleucodine on sperm

Given that previous studies have shown that this lactone decreases viability in tumour cells, we analyzed its effect on sperm viability and motility. Gametes were exposed for different time periods (0–40 minutes) to increasing concentrations of DhL (0–2 mM). The sperm suspensions incubated for 10 minutes with concentrations of up to 1 mM presented similar vitality percentages to the control. In contrast, gametes exposed to concentrations of 0.5 and 2 mM for longer times (20–40 minutes) showed a significant decrease in viable cell percentages (Figure 2A). Vitality percentages higher than 65% were recorded up to 20 minutes of incubation at all concentrations tested.

Sperm motility showed a significant decrease compared to the control at all concentrations tested. However, the suspensions incubated with 0.1 mM DhL presented similar values at all tested times. At higher DhL concentrations, lower percentages of cells with progressive motility were observed as a function of incubation time (Figure 2B).

Inducer effect of the acrosomal reaction

Taking into account the results of sperm viability, the samples were exposed to different concentrations of DhL (0–2 mM) for 10 and 20 minutes of incubation. The negative control (DMSO) triggered

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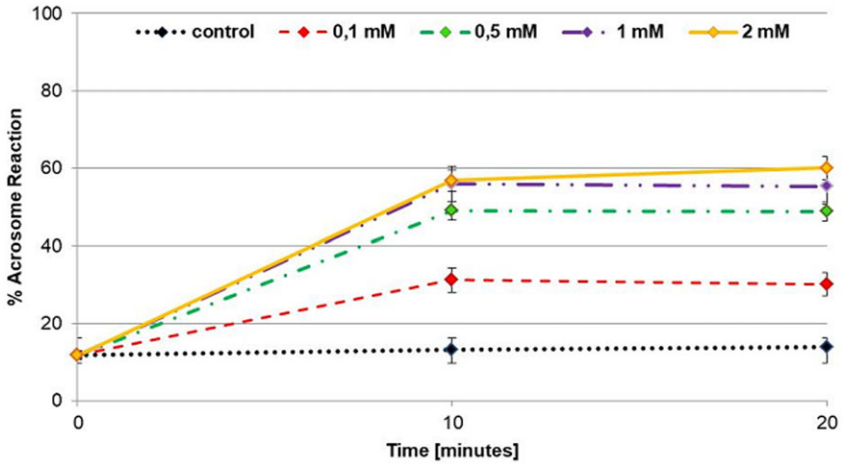

Figure 3. Effect of different concentrations of dehydroleucodine (DHL) in capacitated epididymal sperm, incubated at different times at 37 °C. The control presents the same volume of DMSO. The values recorded at time 0 correspond to the initial SAR. The results are expressed as the average ± SEM of five experiments carried out in duplicate with different animals.

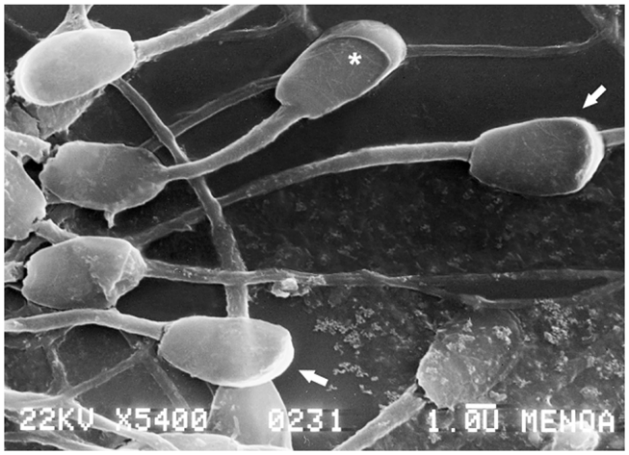

Figure 4. Electronic microphotography of epididymal sperm exposed to DhL. The acrosome is presented as a very noticeable structure (asterisk). Sperm with arrows have undergone complete exocytosis of their acrosomes.

a spontaneous acrosomal reaction of 9%, and this percentage did not show significant variation in the trials.

When analyzing the percentage of acrosomal reaction (AR) as a function of incubation time, significant increases are observed at 10 minutes in all tested concentrations compared to the control. These values remained constant for up to 20 minutes of treatment (Figure 3).

Suspensions treated with a concentration of 2 mM DhL recorded the highest AR percentages at both time periods (56.8% and 60.1%, respectively). However, there are no significant differences with the samples treated with 0.5 and 1 mM.

It is interesting to note that at all tested concentrations, AR percentages significantly higher than the negative control were recorded at all exposure times to DhL. Aliquots of the different treatments were processed for observation under electron microscopy to rule out false positives due to acrosome rupture or damage (Figure 4).

Determination of signalling pathways activated with DhL

To determine the involvement of cAMP-dependent PKA and phospholipases (PLs) in the acrosome reaction (AR) induced by dehydroleucodine (DhL), we designed various protocols (see Materials and Methods) using DhL as an inducer. Capacitated spermatozoa were exposed to specific enzyme inhibitors before DhL treatment to identify the signalling pathways involved.

In the PKA inhibition assays with H89, AR percentages decreased to 49.9% compared to the DhL control (61.4%). Gametes treated with quinacrine showed AR percentages of 53.6%. However, significant differences were observed with the PI-PLC inhibitor, U73122, where AR percentages were 26.5% compared to the positive controls. Sperm suspensions sequentially exposed to all inhibitors before the addition of DhL exhibited acrosomal exocytosis percentages of 27.4%, with no significant differences compared to assays with U73122 alone (Figure 5).

Discussion

Our results indicated that dehydroleucodine (DhL) is capable of inducing the acrosome reaction, altering the sperm motility pattern, and exerting cytotoxic effects in a dose- and time-dependent manner. The cytotoxicity of different sesquiterpene lactones (SLs) is known to depend on the presence of a lactone ring, with the unsaturated alpha-exo-methylene-gamma-lactone ring playing a crucial role, as observed in vitro.

Sperm suspensions incubated for 10 minutes with concentrations up to 1 mM showed viability percentages similar to those of the controls. However, extending the exposure time to 20–40 minutes and increasing the concentration to 2 mM resulted in a significant decrease in viable cells. Similar effects were observed in Rhinella arenarum embryos, where DhL concentrations of 0.5–2 mM led to 93% mortality (Moreno *et al.*, 2012).

The assessment of motility patterns revealed a detrimental dose- and time-dependent effect, with significant decreases at all concentrations tested. Notably, samples incubated with 0.5, 1, and 2 mM DhL showed significant differences within groups exposed to the same concentration at different times. These findings suggest that DhL has a direct negative impact on cell motility, which does not equally affect sperm viability.

Introducing a sesquiterpene lactone like DhL into the incubation medium of male gametes offers a valuable opportunity to study its effects under *in vitro* conditions. The ability to induce acrosomal exocytosis and identify the involved signal transduction pathways is crucial for evaluating sperm quality. Experimental designs limited to 20 minutes of exposure aimed to mitigate cytotoxic effects on cell viability while demonstrating that DhL induces the acrosome reaction at all tested concentrations.

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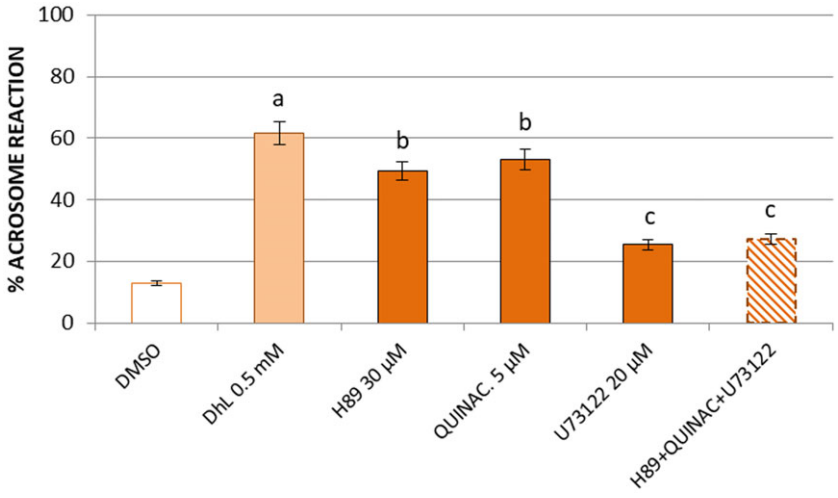

Figure 5. Assessment of PKA/cAMP, PLA_2 and PLC participation in the acrosomal reaction induced by Dehydroleucodine. Sperm suspensions were treated with inhibitors of signalling pathways (H89, Quinacrine and U73122) alone or in combination, prior to the addition of DhL. Negative control: DMSO. Positive control: DhL. The values recorded with the negative control represent the spontaneous acrosome reaction (SAR). The results are expressed as the mean ± SEM of five experiments performed in duplicate with different animals.

When analyzing the results of DhL as an inducer, we observed significant decreases in AR percentages in samples treated with each inhibitor alone compared to the positive control. This indicates a minimal involvement of these signalling pathways. Evaluating the significance among different inhibitors, the inhibition percentages recorded with H89 and quinacrine did not show significant differences, whereas the inhibition of PLC with U73122 led to a marked decrease in AR percentage. Although similar studies in male gametes are lacking, analogous results were obtained in Rhinella arenarum oocytes (Zapata-Martínez *et al.*, 2016). The authors suggested that DhL may activate IP-PLC, resulting in the production of IP3 and DAG, which mobilize calcium ions from cytoplasmic reserves. However, based on our studies, we cannot definitively confirm the same mechanism in sperm.

In summary, DhL demonstrates a significant potential to induce the acrosome reaction in epididymal sperm of *Chinchilla lanigera* and affect sperm motility in a dose- and time-dependent manner. While it directly impacts motility, its effect on viability is less pronounced. Further research is required to fully elucidate the mechanisms involved and their implications for male fertility. DhL's ability to influence acrosomal exocytosis and signalling pathways makes it a valuable tool for in vitro studies of male gametes.

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Competing interests. The authors declare none.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Abood, S., Veisaga, M.L., López, L.A. and Barbieri, M.A. (2018) Dehydroleucodine inhibits mitotic clonal expansion during adipogenesis through cell cycle arrest. *Phytotherapy Research: PTR*, **32**(8), 1583–1592. https://doi.org/10.1002/ptr.6089

Cruzado, M., Castro, C., Fernandez, D., Gomez, L., Roque, M. and Giordano, O.E. (2005) Dehydroleucodine inhibits vascular smooth muscle cell proliferation in G2 phase. *Cellular and Molecular Biology (Noisy-legrand)* **51**, 525–30.

Giordano, O.S., Guerreiro, E., Pestchanker, M.J., Guzman, J., Pastor, D. and Guardia, T. (1990) The gastric cytoprotective effect of several sesquiterpene lactones. *Journal of Natural Products* **53**(4), 803–809.

Gramajo-Bühler, M.C., Zelarayán, L. and Sánchez-Toranzo, G. (2016) Involvement of protein cAMP-dependent Kinase, Phospholipase A2 and Phospholipase C in sperm acrosome reaction of *Chinchilla lanigera*. *Reproduction in Domestic Animals = Zuchthygiene* **51**(1), 150–157. https://doi.org/10.1111/rda.12659

Guardia, T., Guzman, J.A., Pestchanker, M.J., Guerreiro, E. and Giordano, O.S. (1994) Mucus synthesis and sulfhydryl groups in cytoprotection mediated by dehydroleucodine, a sesquiterpene lactone. *Journal of Natural Products* **57**(4), 507–509.

Hehner, S.P., Heinrich, M., Bork, P.M., Vogt, M., Ratter, F., Lehmann, V., Schulze-Osthoff, K., Dröge, W. and Schmitz, M.L. (1998) Sesquiterpene lactones specifically inhibit activation of NF-kappa B by preventing the degradation of I kappa B-alpha and I kappa B-beta. *The Journal of Biological Chemistry* **273**(3), 1288–1297. https://doi.org/10.1074/jbc.273.3.1288

Heinrich, M., Robles, M., West, J.E., Ortiz de Montellano, B.R. and Rodriguez, E. (1998) Ethnopharmacology of Mexican asteraceae (Compositae). *Annual Review of Pharmacology and Toxicology* **38**, 539–565.

Lee, B.K., Park, S.J., Nam, S.Y., Kang, S., Hwang, J., Lee, S.J. and Im, D.S. (2018) Anti-allergic effects of sesquiterpene lactones from Saussurea costus (Falc.) Lipsch. determined using in vivo and in vitro experiments. *Journal of Ethnopharmacology* **213**, 256–261. https://doi.org/10.1016/j.jep.2017.11.018

Lee, K.H., Huang, E.S., Piantadosi, C., Pagano, J.S., Geissman, T.A. (1971) Cytotoxicity of sesquiterpene lactones. *Cancer Research* **31**(11), 1649–1654.

Li, H., Zhou, C., Zhou, L., Chen, Z., Yang, L., Bai, H., Wu, X., Peng, H. and Zhao, Y. (2005) In vitro antiviral activity of three enantiomeric sesquiterpene lactones from Senecio species against hepatitis B virus. *Antiviral Chemistry & Chemotherapy* **16**(4), 277–282. https://doi.org/10.1177/095632020501600407

Lopez, M.E., Giordano, O.S. and Lopez, L.A. (2002) Sesquiterpene lactone dehydroleucodine selectively induces transient arrest in G2 in Allium cepa root meristematic cells. *Protoplasma* **219**, 82–8.

Medina, M.F., Bühler, M.I. and Sánchez-Toranzo, G. (2014) Chemical activation in Rhinella arenarum oocytes: effect of dehydroleucodine (DhL) and its hydrogenated derivative (2H-DhL). *Zygote*. doi: 10.1017/S0967199414000641

Mohamed, T.A., Hegazy, M.F., Abd El Aty, A.A., Ghabbour, H.A., Alsaid, M.S., Shahat, A.A. and Paré, P.W. (2017) Antimicrobial sesquiterpene lactones from Artemisia sieberi. *Journal of Asian Natural Products Research* **19**(11), 1093–1101. https://doi.org/10.1080/10286020.2017.1302939

Moreno, L.E., Juárez, A.O. and Pelzer, L.E. (2012) Lethal effect of dehydroleucodine (DhL) on amphibian Bufo arenarum embryos. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* **50**(3-4), 672–674. https://doi.org/10.1016/j.fct.2011.12.037

Ozçelik, B., Gürbüz, I., Karaoglu, T. and Yeşilada, E. (2009) Actividades antivirales y antimicrobianas de tres lactonas sesquiterpénicas de Centaurea solstitialis L. ssp. solstitialis. *Investigación Microbiológica* **164**(5), 545–552. https://doi.org/10.1016/j.micres.2007.05.006

Penissi, A.B., Fogal, T.H., Guzman, J.A. and Piezzi, R.S. (1998) Gastroduodenal mucosal protection induced by dehydroleucodine: mucus secretion and role ofmonoamines. *Digestive Diseases and Sciences* **43**, 791–8.

Polo, L.M., Castro, C.M., Cruzado, M.C., Collino, C.J., Cuello-Carrión, F.D., Ciocca, D.R., Giordano, O.S., Ferrari, M. and López, L.A. (2007) 11,13-dihydro-dehydroleucodine, a derivative of dehydroleucodine with an inactivated alkylating function conserves the anti-proliferative activity in G2 but does not cause cytotoxicity. *European Journal of Pharmacology*, **556** (1-3), 19–26. https://doi.org/10.1016/j.ejphar.2006.10.049

Priestap, H.A., Abboud, K.A., Velandia, A.E., Lopez, L.A. and Barbieri, M.A. (2011) Dehydro-leucodin: a guaiane-type sesquiterpene lactone. *Acta Crystallographica. Section E, Structure Reports Online* **67**(Pt 12), o3470. https://doi.org/10.1107/S1600536811048938

Rodríguez-Expósito, R.L., Nocchi, N., Reyes-Batlle, M., Sifaoui, I., Suárez-Gómez, B., Díaz-Marrero, A.R., Souto, M.L., Piñero, J.E., Fernández, J.J. and Lorenzo-Morales, J. (2021) Antiamoebic effects of sesquiterpene lactones isolated from the zoanthid Palythoa aff. clavata. *Bioorganic Chemistry* **108**, 104682. https://doi.org/10.1016/j.bioorg.2021.104682

Sánchez Toranzo, G., Giordano, O.S., López, L.A. and Bühler, M.I. (2007) Effect of dehydroleucodine on meiosis reinitiation in Bufo arenarum denuded oocytes. *Zygote (Cambridge, England)* **15**(2), 183–187. https://doi.org/10.1017/S0967199407004212

Sanchez Toranzo, G., Lopez, L.A., Zapata Martinez, J., Gramajo Buhler, M.C. and Buhler, M.I. (2009) Involvement of the dehydroleucodine alpha-methylene-gamma lactone function in GVBD inhibition in Bufo arenarum oocytes. *Zygote (Cambridge, England)* **18**(1), 41–49. https://doi.org/10.1017/S0967199409990086

Siriwan, D., Miyawaki, C., Miyamoto, T., Naruse, T., Okazaki, K. and Tamura, H. (2011) Chemopreventive activity of sesquiterpene lactones (SLs) from yacon against TPA-induced Raji cells deformation. *Pakistan Journal of Biological Sciences: PJBS* **14**(10), 605–609. https://doi.org/10.3923/pjbs.2011.605.609

Suhaiman, L., Carlos de-Rosas, J., Sartor, T., Palmada, N., Giordano, O.S. and Lopez, L.A. (2011) Effect of dehydroleucodine on the reproductive tract of male mice. *Andrologia* **43**, 297–302. doi: 10.1111/j.1439-0272.2010.01053.x

Vega, A.E., Wendel, G.H., Maria, A.O.M. and Pelzer, L. (2009) Antimicrobial activity of Artemisia douglasiana and dehydroleucodine against Helicobacter pylori. *Journal of Ethnopharmacology* **124**(3), 653–655.

Vichera, G., Alfonso, J., Duque, C.C., Silvestre, M.A., Pereyra Bonnet, F., Fernández-Martín, R. and Salamone, D. (2010) Chemical activation with a combination of ionomycin and dehydroleucodine for production of parthenogenetic, ICSI and cloned bovine embryos. *Reproduction in Domestic Animals* **45**, e306–12.

Zapata-Martínez, J., Medina, M.F., Gramajo-Bühler, M.C. and Sánchez-Toranzo, G. (2016) Participation of PLA2 and PLC in DhL-induced activation of Rhinella arenarum oocytes. *Zygote (Cambridge, England)* **24**(4), 495–501. https://doi.org/10.1017/S096719941500043X

https://doi.org/10.1017/S096719942400042X Published online by Cambridge University Press