Extracto acuoso de la corteza de Rhizophora mangle (RMABE) en un modelo de ectima contagioso en carneros infectados experimentalmente

de Armas Sanabria, E.\textsuperscript{a} | Oliva Hernández, Y.\textsuperscript{a} | Pérez Hernández, Z.\textsuperscript{a} | Ayala Galindo, J.\textsuperscript{a} | Martínez Marrero, N.\textsuperscript{b} | Fernández Limia, O.\textsuperscript{a} | Faure García, R.\textsuperscript{a} | Barreras, M.\textsuperscript{b} | Alfonso Zamora, P.\textsuperscript{a} | Marrero Faz, E.\textsuperscript{a}

\textsuperscript{a}Direction of Animal Health and Production, National Centre for Animal and Plant Health (CENSA)
\textsuperscript{b}Direction of Microbiology, National Centre for Animal and Plant Health (CENSA)

Email: Elizabeth de Armas: edearmas@censa.edu.cu, Yuleivys Oliva: yuleivys@censa.edu.cu, Zulema Pérez: zulema@censa.edu.cu, Joel Ayala: ayala@censa.edu.cu, Nadia Martínez: nadia@censa.edu.cu, Octavio Fernández: octavio@censa.edu.cu, Roberto Faure: faure@censa.edu.cu, Pastor Alfonso: alfonso@censa.edu.cu, Eva Marrero: evadidem@censa.edu.cu

Corresponding author:
Lic. Elizabeth de Armas Sanabria, MSc. Department of Pharmacology, Direction of Animal Health and Production, National Centre for Animal and Plant Health (CENSA). Carretera de Tapaste y Autopista Nacional, Mayabeque, Cuba. Tel.: (53) 47 863014. Fax: (53) 47 860411. e-mail address: edearmas@censa.edu.cu

Resumen

El ectima contagioso es una de las enfermedades virales que a pesar de su baja mortalidad (<10%), su morbilidad es generalmente alta. El virus Orf causante de esta afeción, ataca principalmente a ovinos y caprinos pudiendo infectar al hombre y causar importantes pérdidas económicas. En la actualidad no se cuenta con tratamientos específicos y la búsqueda de nuevas drogas se ha encaminado al uso de compuestos obtenidos de productos naturales con actividad antiviral. Fue objetivo del trabajo evaluar el efecto antiviral del extracto acuoso de la corteza de Rhizophora mangle (RMABE) (CIKRON*) en un modelo de ectima contagioso en carneros. Se realizaron escoriaciones lineales de 3 cm de longitud en la cara interna de las patas traseras (n = 3). El extracto se aplicó de forma tópica 4 días después de la infección durante 4 días. Se tomaron fotos y se procesaron digitalmente para cálculo de longitud de las lesiones. Además, se determinó presencia de eritema, vesículas y/o pústulas y costras asignando valores (0-2) según su magnitud. Diariamente se
monitorearon las lesiones y se midieron parámetros clínicos. Resultados: el grupo tratado con RMABE logró una disminución en las diferencias de longitud de las lesiones respecto al día 0 comparado al grupo infectado pero no tratado (p<0.05), así como de los signos clínicos. Conclusiones: El uso tópico del extracto natural RMABE resulta prometedor en el tratamiento del ectima contagioso en ovinos y caprinos.

**Palabras claves:** virus ORF, ectima contagioso, polifenoles, *Rhizophora mangle*, tratamiento tópico, CIKRON

---

**Abstract**

Orf is a highly contagious viral disease that causes important economic losses in sheep and goat farms. There are an increasing number of natural compounds with antiviral action that are being studied. This study showed results of *Rhizophora mangle* aqueous bark extract (RMABE) (CIKRON*) in the treatment of lambs experimentally infected with ORF virus. The extract was applied topically once daily 4 days after infection for 4 days. Lesions were scored for the presence of erythema, vesicles and/or pustules and the presence of firmly attached scab. The progression of the lesions was evaluated daily by clinical observations. Our results showed that lesion lengths and clinical sings were higher in Placebo than in RMABE treatment group (p<0.05). The topical application of RMABE can result in milder lesions that resolve faster than untreated lesions confirming that this natural product is useful in the treatment of contagious ecthyma in goat and sheeps.

**Keywords:** ORF virus, contagious ecthyma, polyphenols, *Rhizophora mangle*, topical treatment, CIKRON

---

**1. Introduction:**

ORF is one of the most widespread viral diseases worldwide, affecting small ruminants and other species including wild animals (Hosamani et al., 2009). Contagious ecthyma results from infection by ORF virus, a prototype member of the *Parapoxvirus* genus in the family *Poxviridae* (Georgiades et al., 2005). ORF virus is responsible for a highly contagious pustular dermatitis in sheep and goats, which occasionally can spread to humans (Mc Elroy and Bassett, 2006). Therefore, ORF is considered an occupational hazard to farmers, shepherds, veterinarians, animal handlers, among other.
Extracto acuoso de la corteza de *Rhizophora mangle* (RMABE) en un modelo de ec tima contagioso en carneros infectados experimentalmente

http://www.veterinaria.org/revistas/redvet/n060612/061208.pdf

La enfermedad tiene una alta morbimidad y aunque la mortalidad es rara y usualmente no supera el 10 por ciento, la enfermedad es frecuentemente severa lo suficiente para crear problemas de bienestar en los troncos (Nettleton *et al*., 1996; Grumbrell *et al*., 1997). El virus infecta a través de daños cutáneos, escarificando, o de otra manera dañando la piel y replicándose en células epiteliales a través de contacto directo con animales infectados o con fomites contaminados (Lederman *et al*., 2007). Las pérdidas financieras pueden no solo ser debido a la muerte de animales, sino también por varios factores como el crecimiento reducido de peso corporal, costos adicionales de mantenimiento, costo laboral, intervención veterinaria y demora en la terminación para el mercado (Hosamani *et al*., 2009).

Actualmente, no hay fármacos específicos para el tratamiento de la enfermedad en animales infectados. El tratamiento es generalmente de apoyo y consiste en vendajes húmedos, antisépticos locales, inmovilización de dedos y/o antibióticos para tratar las infecciones bacterianas secundarias (Ünal *et al*., 2002). Los esteroides topicos y antihistamínicos orales se usan con eritema (Georgiades *et al*., 2005).

La vacunación de ovejas y cabras puede limitar la gravedad de la enfermedad, pero no preven la infección, y en algunos casos las cepas de los virus han sido el origen de brotes de ec tima contagioso (Gilray *et al*., 1998; Centers for Disease Control and Prevention, 2006).

El tratamiento de las infecciones virales con los fármacos antivirales disponibles a menudo resulta insatisfactorio debido al problema de resistencia viral y el problema de latencia viral (Pillay y Zambon, 1998). La búsqueda de sustancias antivirales con alta eficacia, baja toxicidad y efectos secundarios menores debe continuar.

La etnofarmacología ofrece una alternativa para el descubrimiento de agentes antivirales, es decir, el estudio de plantas medicinales con una historia tradicional como fuente potencial de sustancias con actividades farmacológicas y biológicas significativas (Vlietinck *et al*., 1991).

Tannins son sustancias caracterizadas por su naturaleza polifenólica y se encuentran en muchas plantas. Sus efectos farmacológicos (promotor de la cicatrización, antiinflamatorios, antioxidantes, antimicrobianos, antivirales, etc.) se relacionan con su capacidad de formar complejos con macromoléculas tales como proteínas y polisacáridos. Sus actividades antioxidantes y de scavenging son a menudo ligados a la interacción con iones metálicos, formando complejos quelados (Haslam, 1996).

La actividad antiviral de los compuestos fenólicos de plantas contra varios virus DNA y/o ARN está bien documentada en la literatura (Chattopadhay *et al*., 2006), así como los efectos antisepticos y de cicatrización (Nayak y Pinto, 2007; Azees *et al*., 2007).

*Rhizophora mangle* L, la manglar rojo, es conocido como una medicina tradicional en los diferentes países del Caribe. Su corteza ha sido utilizada como astringente, antiséptico, antioxidante, hemostático, con antifúngico,
antiulcerogenic and wound healing promoter properties (Kollar and Hotolova, 2003). Biologically active complexes include tannins as major active components (54%), free and bound carbohydrates, saturated and unsaturated long chain fatty acids, essential oils and fitosterols (Sánchez et al., 1998).

The antiseptic and antibacterial effects of aqueous extract of *R. mangle* have been demonstrated in open and sutured injuries in rabbits (Figueroa et al., 1995), open wounds in calves (Figueroedo et al., 1995), open surgical wounds in patients with pilonidal cyst and fistula (Fernández et al., 2002) and in patients with aphthous ulcers (de Armas et al., 2005). In all instances the extract showed antiseptic properties and accelerated the healing process compared to placebo or other antimicrobial agent treatments.

In this study, we report a model of contagious ecthyma to evaluate the effect of *Rhizophora mangle* aqueous bark extract (RMABE) in experimentally infected lambs.

2. Materials and Methods:

2.1. Clinical samples for experimental inoculation:

Crusted scabs lesions were obtained from a Pelibuey, sheep with typical ORF lesions in the region of the mouth, eyes and ears. Scabs were homogenised and identified as positive to the presence of ORF virus by semi-nested PCR (Inoshima et al., 2000). The collected scabs from the infected sheep were maintained at -80 ºC and previous to the experimental infection an inoculum was prepared at 10% w/v PBS.

2.2. *Rhizophora mangle* aqueous bark extract (RMABE)

The aqueous extract of the bark of *Rhizophora mangle* used was chemically characterized (Sánchez et al., 1998). It has polyphenols (54%), represented in their majority by polymeric tannins (80%) and hydrolysable tannins (20%); with the presence of epicatechin, catechin, chlorogenic, gallic and elagic acids, as well as gallotannins, elagitannins and condensed tannins. Moreover, this extract also contains non tannin structures as free bound carbohydrates (xilose, ramnose, fucose, glucose, arabinose mannose and galactose), saturated and unsaturated long chain fatty acids from C12:0 to C24:0, essential oils and fitosterols. The batch used was physic and chemically characterized including HPLC analysis and released by the CENSA Department of Quality Assurance. Placebo was composed by water and the excipients presents in the formulation of the RMABE.
2.3. Animals, inoculations and treatment regimens

ORF virus naïve lambs used in this study were either raised in a containment facility at the National Centre for Animal and Plant Health (CENSA), Cuba. Animal studies were conducted in accordance with the International Regulation 86/609/EEC, “Protection of animals used for experimental and scientific purposes” and the National Regulation R.15, “Handle and care of laboratory animals. Ethical principles”. The lambs were fed with solid food and clean water freely until the end of the study. Five animals were infected, by scarification (Nettleton et al., 1996) on the inner aspect of both hind thighs, with the inoculums and thereafter each of the virus scarification sites was subjected to one of three different treatments. Treatment was administered topically once a day from day 4 post-infection (PI) for 4 consecutive days. Treatment I involved no intervention; Treatment II involved the application of Placebo; Treatment III involved the application of RMABE.

2.4. Evaluation of RMABE on viral infection

The progression of the lesions was evaluated daily, until its resolution, by monitoring the clinical score for each lesion (Scaglarini et al., 2007). Briefly, lesions were scored for the presence of erythema, the presence of vesicles and/or pustules and the presence of firmly attached scab. Each criterion was scored from 0 to 3 according to the thickness of the lesion along the scarification line. Respiratory frequency, pulse and temperature were monitored as well.

At the same time, lesions were photographed with Nikon D-200 camera (lens: AF-5 NIKKOR 18-70 mm) and images were digitally processed (UTHSCSA ImageTool Software for Microsoft Windows, version 3.00). Middle length segment between lesion borders (MLS) was calculated as is shown in figure 1 for the evaluation of daily lesion thickness.

![Fig. 1. Schematic representation of the middle length segment between lesion borders (MLS)](http://www.veterinaria.org/revistas/redvet/n060612/061208.pdf)

2.5. Statistical analysis

Data were analysed using SAS® system, SAS-stat software version 8.02 (SAS Institute Inc. Cary, NC, USA). Clinical score data were analysed using ANOVA test. Because the data was not normally distributed...
previous root square transformation was carried out. MLS data were analysed using Kruskal Wallis non-parametric test. Results were considered significant when p was < 0.05.

3. Results:

3.1. Treatment of ORF lesions with RMABE

All the infected lambs developed typical ORF virus lesions in the first four days following infection with an intense erythema and oedema along the lines of scarification and the appearance of vesicles and pustules. Also, temperature, respiratory and cardiac frequency were elevated.

The progression of the lesion over the 30 day observation period is shown in figure 2. Mean clinical scores for each of the 3 different treatment regimens showed that lesions treated with RMABE succeeded much more than those in the placebo and no intervention groups. Scabs from RMABE treatment group were easily removed by day 11 PI (p<0.0001) whereas scabs from placebo and no intervention treated animals last about 27 days to be fully removed. The RMABE-treated lesions were completely resolved by day 21 PI, whereas placebo and no intervention groups needed 9 extra days for complete recovery.

![Graph showing mean daily clinical scores over 30 days.](http://www.veterinaria.org/revistas/redvet/060612/061208.pdf)

**Fig. 2.** Effect of RMABE treatment on the development of ORF virus lesions in lambs. A graph is shown of the group mean daily clinical scores, over 30 day observation period. (* p<0.01).
By day 8 PI, after 4 days of RMABE treatment, differences (p=0.009) in the thickness of the lesions (MLS PI/MLS day 0) were found between the untreated and treated lesions groups (Figure 3). The RMABE-treated lesions were almost completely resolved by day 18 PI.

**Fig. 3.**
Effect of RMABE treatment on the development of ORF virus lesions in lambs. The graph is shown of daily lesion thickness (MLS PI/MLS day 0), over the 18 day observation period. (¤ p=0.06; * p<0.01).

The results also demonstrated that there is a correspondence between mean daily clinical scores and daily lesion thickness in relation to time needed for lesions to be completely resolved.

4. **Discussion:**

Contagious ecthyma is a highly contagious, zoonotic, viral skin disease that is considered one of the top twenty most important diseases of sheep and goats in terms of impact on the poor in undeveloped countries (Perry *et al.*, 2002). Morbidity is high and mortality can reach 10-20%; mortality is often caused by starvation and secondary infections.

Our results showed the effect of RMABE in the treatment of contagious ecthyma in lambs. In the first four days, all infected animals developed typical ORF virus lesions with an intense erythema and oedema along the lines of scarification and the appearance of vesicles and pustules. Also, temperature, respiratory and cardiac frequencies were elevated. Other studies have used Massese lambs for the evaluation of a cream of
Cidofovir and they have confirmed that all infected lesions were fully developed by day 4 PI (Scaglarini et al., 2007).

Treating with RMABE for 4 days immediately following the initial infection certainly slow the development of the lesions. By day 18 PI, thickness of the lesions (MLS PI/MLS day 0) in RMABE treated group were reduced in 75 percent. Also, it was observed that lesions treated with a cream of Cidofovir (1 % and 0.5 %, w/v) were completely resolved by day 15 and 17, respectively (Scaglarini et al., 2007).

Furthermore, the clinic evaluation showed a reduction in time in lesions to be healed concerning RMABE treatment. The antioxidant activity of polyphenols and their ability of forming complexes with proteins, aminoacids and polysaccharides (Scalbert, 1991) and also, the effect on capillary permeability and cellular proliferation (Villegas et al., 1992) could contribute to these results. Polyphenols are known to act as a free radical scavenger acting like superoxide anions and hydroxyl radicals, which have strong oxidant properties (Haslam, 1996) producing tissue damage. In addition, it has been shown that Rhizophora mangle aqueous extract reduced the wound area and modified the redox balance during the tissue reparation process in an aseptic wound healing model in rats (Sánchez et al., 2009).

On the other hand, the antiseptic properties of polyphenols could influence the results due to secondary infections that could appear in lesions. They can form complexes with microbial cell wall proteins and with ion metals such as V, Mn, Al, Ca, Cu and Fe; in E. coli Fe has been identified very important as being an active component in oxygen transport (Haslam, 1996). This mechanism has also been corroborated by electron microscopy for evaluating integrity of microorganism wall cell where a formulation of red mangrove was tested. These observations showed that after a 10min incubation period with S. Agalactiae and S. aureus cell wall disruption was noted. This can be linked to either in cellular death or alteration in cellular metabolism that influence growth and replication (Armenteros and Ginorio, 1999).

*In vitro* studies have shown the antiviral effect of plant polyphenols from leaves against poxviridae family virus. Thus, Acacia arabica showed inhibition of 99.7 % and Eugenia jambolana 99.2 % (Bhanuprakash et al., 2008). Other studies have used E. jambolana for inhibiting buffalopox virus (98.52 % of inhibition) (Bhanuprakash et al., 2007).

An aqueous acetone extract of Guiera senegalensis J. F. Gmel was evaluated *in vitro* against fowlpox virus. The antiviral effect of the extract was partially attributed to phenolic components (flavonoids, tannins and anthocyanins) (Lamien et al., 2005). In this sense, polyphenols in RMABE could be responsible of the antiviral effect against same family virus.
Taken together our results demonstrate that the topical application of RMABE can result in lesions that resolved faster than untreated lesions, confirming that Rhizophora mangle is valuable in the treatment of contagious ecthyma.

The ORF virus does not appear to spread systemically from the site of initial infection, instead it is shed with scab material to seed the environmental pool. ORF virus resists harsh environmental conditions, surviving in the environment for long periods with the possibility of being transmitted to susceptible hosts (Nettleton et al., 1996). Therefore the faster resolution of lesions is a contribution against ORF virus persistence in the environment and disease incidence.

Present study represents what could happen in natural cases of ORF when treatment would be applied to evident ORF lesions at the beginning state, therefore this animal model can be useful to evaluate promissory products against different virus of poxviridae family.

Acknowledgements:

The authors would like to thank Alessandra Scaglierini from the Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale – Alma Mater Studiorum, Bologna for her support and contribution to the work.

References:


Extracto acuoso de la corteza de *Rhizophora mangle* (RMABE) en un modelo de ectima contagioso en carneros infectados experimentalmente

http://www.veterinaria.org/revistas/redvet/n060612/061208.pdf