VSL 280

Evaluation of the ability of *Perna canaliculus* to reduce superoxide production in a free radical cell model.

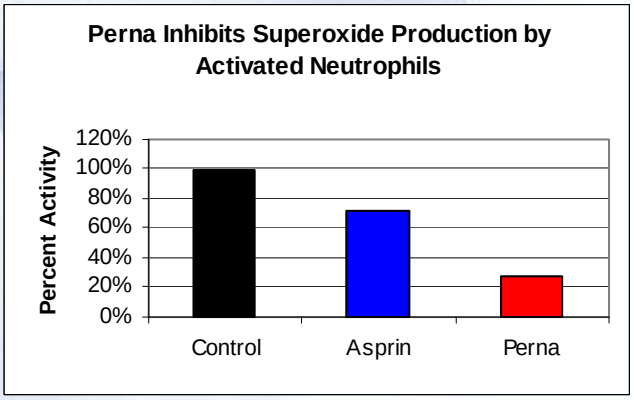
Objective: The purpose of this study was to evaluate the ability of *Perna canaliculus* to reduce superoxide (a free radical) production in cell culture.

Summary: The study was designed to demonstrate that Perna can effectively reduce superoxide production from activated neutrophils in cell culture and compare its inhibitory effect with the positive control Aspirin.

Background: Superoxide is a potent free radical that may be part of the cascade leading to proteolytic breakdown of joint cartilage and a mediator of inflammation. PMA activated neutrophils produce superoxide and the respiratory burst activity can be measured using a simple colorimetric assay. Method can be used to screen and determine the anti-inflammatory activity of ingredients like Perna using Aspirin as a positive control. The experimental procedure is based on the methods described in Tan and Benidge (2000).

Methods: *Perna canaliculus* freeze-dried powder was extracted using 30% phenol, precipitated with ethanol and resuspended in water. The aqueous extract was dialyzed, frozen and then freeze-dried to produce a fine powder of Perna extract for testing in cell culture. Rat neutrophils were isolated from peripheral blood and placed in equal numbers in a 96 welled plate in appropriate culture media. Perna extract at 0, 100, 200 and 400 ug/ml was added to the cultures and the neutrophils activated with 50 ng/ml of PMA (phorbol 12-myristate 13-acetate). Percent inhibition of the superoxide generation was calculated and compared to the response of 0, 100, 200 and 400 ug/ml of Aspirin.

Results: The *Perna canaliculus* extract and the Aspirin showed a direct dose response (i.e. increasing inhibition with increasing concentration) in a statistical manner. Perna shows a dose-dependant inhibition of superoxide production showing less than 25% of the activity of the activated control and twice the inhibition of Aspirin at the same concentration.



Conclusion: In this model, Perna demonstrated more than twice the activity of Aspirin in controlling superoxide production. Perna was shown to be an effective inhibitor of superoxide production from activated neutrophils in a dose-dependant manner. This activity demonstrates that Perna is an effective antioxidant and anti-inflammatory agent, which may help explain its ability to reduce inflammatory mediators in arthritis.

Clinical Significance: This study, along with other data, was submitted for publication in 2007 to substantiate the use of *Perna canaliculus* to reduce inflammation and support overall joint function.

Davis P, et al. Evaluation of the ability of Perna canaliculus to reduce superoxide production in a free radical cell model. Wellington School of Medicine, NZ, 2006. Published in BMC Complimentary and Alternative Medicine, 2007, 7:20