

Effect of ME-3451-106, an aqueous extract of *Stichaster striatus* with inhibitory activity of voluntary alcohol intake, in genetically drinker rats: Isolation and identification of the active fraction

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Abstract

The aqueous extract obtained from *Stichaster striatus* Müller & Troschel (Asteroidea, Stichasteridae) has been shown to possess activity as an alcohol appetite inhibitor after oral administration in a rat model with a genetically established excessive appetite for alcohol (Wistar rats, lineage UChB). A significant decrease in the consumption of ethanol was observed (unrelated to a possible disulfiram effect) without a change in the normal food or water intake during the experimentation period.

A bio-guided fractionation of the extract was carried out in order to identify the most active fraction, in which the presence of a group of natural endogenous polyamines in undetermined proportions is suspected. Our hypothesis was to relate the activity obtained for the original ME-3451-106 extract with the presence of these polyamines in the extract in question. The activity shown by a series of commercially available polyamines (putrescine (Pu), spermidine (SPD) and spermine (SP)) in inhibiting voluntary ethanol intake lends support to our hypothesis.

The extract was selected on the basis of oral tradition, which claimed that the consumption of a “soup” obtained by boiling starfish, later identified as *Stichaster striatus*, prevented the appearance of alcoholism in laborers on properties entrusted to the Jesuit order during the middle period of the Spanish conquest of America (17–18th century).

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1. Introduction

Historical records show that in the “haciendas” assigned by the Spanish crown to the Jesuits, a religious congregation, at the end of the 16th century until the time of their expulsion at the end of the 18th century, there was no excessive alcohol intake among their workers and absolutely no manifestation of alcoholism. This phenomenon led to higher productivity in comparison to that reported for other similar centers in the area. Traditional stories (no written documentary references have been found) told in Chile, between the 16th and 18th centuries, relate this behavior to the fact that the laborers in question were forced to ingest, each morning prior to initiating their work and again at the end of the day, a “soup” obtained by boiling a starfish later

identified as *Stichaster striatus* Müller & Troschel (Asteroidea, Stichasteridae) (Jenselius Madsen, 1956; Parke, 1982).

Using these stories as a point of reference, an initial hypothesis was established that the intake of aqueous extracts, obtained by boiling certain marine echinoderms, decreases excessive voluntary consumption of alcoholic drinks in man. This hypothesis is based on the assumption that such extracts contain certain substances that affect the appetite for alcohol. Nevertheless, to the best of our knowledge, there is no literature reference that reports the existence of compounds isolated from marine organisms that possess this type of activity.

Alcoholism affects all of the organs due to chronic alcohol consumption, with the central nervous system (CNS) and peripheral nervous system (PNS) being two of the most damaged in the inflammatory or degenerative processes that take place after this type of consumption (Estruch, 1999; Eriksson, 2001). Indeed, alcoholism is now associated with the simultaneous actions on excitatory and inhibitory neuronal routes and

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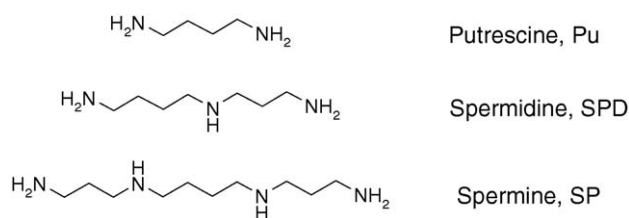


Fig. 1. Structures of natural polyamines.

receptors (Spanagel and Zieglansberger, 1997). Several studies have shown that ethanol is a potent and selective inhibitor of the *N*-methyl-D-aspartate receptors (NMDAr) and that prolonged exposure to ethanol leads to a compensatory “up-regulation” of these receptors, resulting in enhanced NMDA receptor-mediated functions after the removal of ethanol. It is assumed that these alterations contribute to the development of a tolerance of and dependence on ethanol as well as the acute and delayed signs of ethanol withdrawal (Kiefer et al., 2003; Nagy, 2004). NMDAr are large hetero-oligomeric complexes including at least two copies of an NR1 subunit and two copies of an NR2 subunit (Mayer et al., 1989), with a group of modulating sites, among which the polyamine site stands out (Williams et al., 1990; Araneda et al., 1999; Gibson et al., 2002). Thus, it has been demonstrated that the endogenous polyamines spermidine (SPD) and spermine (SP) (Fig. 1) modulate the NMDA receptor complex, with low μM and high μM concentrations potentiating and inhibiting its function, respectively (Berger et al., 1998). The affinity of the polyamines for many types of receptors and ion channels at which ethanol is also believed to act, suggests many potential interactions between these agents (Littleton et al., 1988, 2001). In addition, interactions between ethanol and polyamines are of potential importance with regard to many sequels of ethanol abuse, including different states of ethanol withdrawal.

The literature concerning the biochemical characteristics of *Stichaster striatus* and of other species belonging to the same Asteroidea class (Guillen, 1959; Brusca and Brusca, 1990; Larrin, 1995; Dando and Burchett, 1996) emphasizes the high content of polyamine-type compounds present in echinoderms. This is particularly true for starfish, such as *Asterias vulgaris*, which has a high concentration of SP in the digestive glands (Watts et al., 1987), or *Pycnopodia helianthoides* (Asotra et al., 1988), in which the largest concentration of SPD and SP is found. The specific mission of these compounds is still unclear but various hypotheses exist. Given the characteristics of these molecules and the habitat of these invertebrates, the polyamines could be acting as a “protection” system and aid in their adaptation to the environment, as has been demonstrated for other marine invertebrates (Lovett and Watts, 1995; Stuck et al., 1996; Watts et al., 1996). On the other hand, considering the special physiological characteristics of these invertebrates, the presence of these endogenous polyamines could be related to the necessity of a complex control mechanism of cell division and cellular growth (Audit, 1996). In mammals it has been demonstrated that SPD and SP and their diamine precursor, putrescine (Pu) (Fig. 1), which are intracellular cationic molecules that are

biosynthetically related are essential for the cell growth, division and differentiation (Pegg et al., 1981; Pegg, 1988; Wang et al., 1992; Marton and Pegg, 1995).

Our hypothesis suggests the possibility that the endogenous polyamines, which appear in a different proportion in the animal species under study, could be responsible for the ethanol appetite inhibitory activity attributed for the original starfish “soup”. It is expected that these molecules would be extracted by the aqueous medium during the boiling process.

The previous references led us to aim initially at verifying the alcohol appetite inhibitory activity of an aqueous extract of *Stichaster striatus*. This investigation was carried out by performing a free-choice paradigm on genetically high ethanol consuming Wistar UChB rats. This strain of rats is characterized by its innate tolerance to ethanol and high voluntary ethanol consumption (Mardones and Segovia-Riquelme, 1983)—a characteristic that is a consequence of genetic modifications, as recently established (Valle et al., 2002). These modifications include changes in acetaldehyde metabolism (Tampier et al., 1984, 1994; Sapag et al., 2003), in mitochondrial function (Quintanilla and Tampier, 1989), as well as modifications in calcium channel function (Tampier et al., 1997).

Having verified the activity of the original extract, in which the presence of alkaloids was ruled out by means of the Mayer reaction, we considered another objective: the isolation of the active fraction by application of the bio-guided fractionation methodology, a recognized efficient method in the chemistry of natural products (Rahman et al., 2001).

The first separation of the different fractions was achieved by applying a molecular fractionation method involving the use of cut-off membranes with different molecular weight (MW) ranges. At the same time, the polar and non-polar compounds present in the original ME-3451-106 extract were separated by treatment with ammonium hydroxide and subsequent extraction with CH_2Cl_2 , boiling water, or with CH_2Cl_2 (room temperature and/or reflux). The protein content in each of the fractions was determined using the test described by Bradford and the inhibitory activity was investigated by assessing the appetite for ethanol in UChB rats.

Finally, as a way to verify indirectly our hypothesis, compounds related to those assumed to be responsible for the biological activity of the original extract, i.e., endogenous polyamines, were evaluated in terms of activity as ethanol-intake inhibitors. The compounds tested were Pu, SPD and SP, which are commercially available.

It was also possible that the inhibitory effect on ethanol appetite shown by ME-3451-106 might be related to a possible inhibitory activity on acetaldehyde dehydrogenase (ALDH). This is the enzyme following alcohol dehydrogenase in the major pathway of alcohol metabolism and is the target of disulfiram, the ALDH blocker taken as a drug causing aversion to alcohol (Hughes and Cook, 1997). This possibility was ruled out by performing a biological assay on extract ME-3451-106 using a method developed for Wistar UChB rats. The rats were later injected with a standard challenge dose of ethanol (Quintanilla et al., 1993; Tampier et al., 1994) and the blood acetaldehyde levels were determined by chromatographic techniques.

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