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Pterocarpans phaseollin and neorautenol isolated from *Erythrina addisoniae* induce apoptotic cell death accompanied by inhibition of ERK phosphorylation

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Abstract

The genus *Erythrina* (*Leguminosae*), consisting of over 100 different species, is distributed in tropical regions. In traditional medicine, *Erythrina* species are used to treat cancer, but little is known about the anticancer mechanisms. From the stem bark of *Erythrina addisoniae* Hutch. & Dalziel, six prenylated pterocarpans were isolated and analysed for pharmacological activity: While calopocarpin, cristacarpin, orientanol c, and isoneorautenol showed only a weak or moderate toxicity in H4IIE hepatoma cells (EC_{50}-value > 25 μM), the toxicity of neorautenol and phaseollin was in the low micromolar range (EC_{50}-value: 1 and 1.5 μM, respectively). We further focused on these two substances showing that both increased caspase 3/7 activity and nuclear fragmentation as markers for apoptotic cell death. Neorautenol (10 μM, 2 h), but not phaseollin induced the formation of DNA strand breaks (comet assay). Both substances showed no effect on NF-κB signalling (SEAP assay: basal activity and stimulation with TNF-α), on the other hand both pterocarpans (10 μM, 2 h) decreased the activation of the ERK kinase (p44/p42), an mitogen activated protein kinase which is associated with cell proliferation. We conclude that the pterocarpans phaseollin and neorautenol may be responsible for the anticarcinogenic actions of the plant extract reported in the literature. Further analysis of these substances may lead to new pharmacons to be used in cancer therapy.

Keywords: Apoptosis; Caspase; Cytotoxicity; ERK; *Erythrina addisoniae*; NF-κB; Prenylated pterocarpans; TNF-α

1. Introduction

The genus *Erythrina* (*Leguminosae*), a group of more than 100 different species, is distributed in all tropical areas of the world (Krukoff and Barneby, 1974). These plant species are widely used in folk medicine to treat diverse diseases, e.g. different kinds of infections as well as inflammation of skin and mucous membranes (Ghosal et al., 1972; Cox, 1993; Saiduh et al., 2000). They are also used due to their analgesic as well as tranquiliz-

Abbreviations: ERK, extracellular regulated protein kinase; FBS, fetal bovine serum; HPLC, high performance liquid chromatography; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium-bromide; PBS, phosphate buffered saline; ROS, reactive oxygen species; SEAP, secreted alkaline phosphatase; TNF-α, tumor necrosis factor α.

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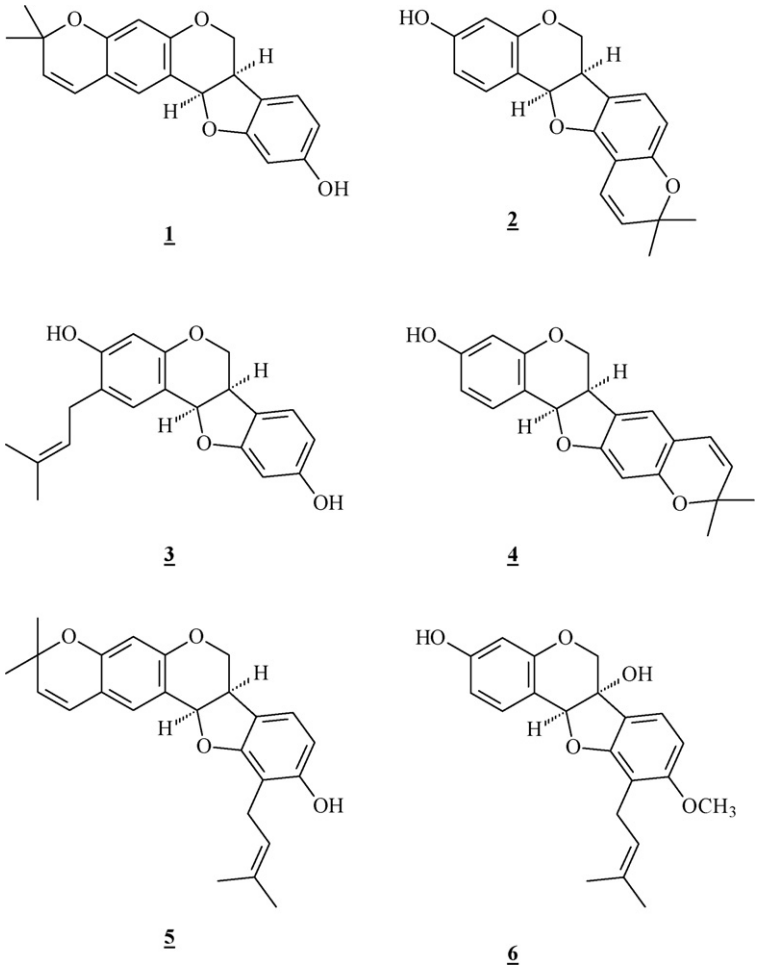

Fig. 1. Structures of isolated compounds: neorautenol (**1**), phaseollin (**2**), calopocarpin (**3**), isoneorautenol (**4**), orientanol c (**5**), cristacarpin (**6**).

ing and sedative activities (Ghosal et al., 1972; Burkill, 1995; Garin-Aguilar et al., 2000). *Erythrina* species are also used against cancer, e.g. stomach cancer, in folk medicine (Hartwell, 1970). In spite of this therapeutic use, little is known about the anticancer mechanisms because only few investigations on cellular level have been conducted.

Erythrina addisoniae Hutch. & Dalziel occurs in tropical areas of Ghana and other West-African countries. Its stem and root bark is mainly used against dysentery, hepatitis, rheumatic disorders, and pain (Burkill, 1995). In some areas of Ghana *E. addisoniae* is also used against swellings and cancer (Hartwell, 1970). We previously isolated six pterocarpans (neorautenol, phaseollin, calopocarpin, isoneorautenol, orientanol c, and cristacarpin, Fig. 1) from the stem bark of *Erythrina addisoniae*. Here we analysed pharmacological effects (cytotoxic, pro-apoptotic effects and effects on signal transduction processes) of the isolated compounds in H4IIE hepatoma cells to elucidate anticancer effects of the substances.

2. Materials and methods

2.1. General

All chemicals were of analytical grade and were purchased from Sigma (Deisenhofen, Germany). All tissue culture reagents were purchased from PAA (Coelbe, Germany), plastic material for cell culture was obtained from Falcon (Heidelberg, Germany). Pterocarpans were isolated form the stem bark of *Erythrina addisoniae*.

2.2. Cell culture

Metabolically active H4IIE rat hepatoma cells were grown in DMEM medium containing 4.5 g/L glucose and 2 mmol/L L-glutamine, supplemented with 10% FBS. The cell culture medium contained 100 units/mL penicillin and 100 μg/mL