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Research Paper

Inhibition of the DHT-induced PSA secretion by *Verbascum xanthophoeniceum* and *Serenoa repens* extracts in human LNCaP prostate epithelial cells

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Verbascoside (PubChem CID: 5281800)

ABSTRACT

Ethnopharmacological relevance: *Verbascum xanthophoeniceum* is a mullein plant, typical of Balkan region and some parts of Turkey, traditionally used as phytotherapeutic agent due to its anti-inflammatory properties. It is rich in phenylethanoid and iridoid metabolites whose anti-inflammatory properties are under characterization.
Materials and methods: The role of *Verbascum xanthophoeniceum* crude methanolic extract and its isolated phenylethanoid glycoside verbascoside have been evaluated, in comparison to a saw palmetto extract, on a human *in vitro* model of androgen-regulated prostate epithelium, the LNCaP cell line. Cytotoxicity and DHT-induced free and total PSA secretion have been thoroughly studied.
Results: We have found that similar to saw palmetto, *Verbascum xanthophoeniceum* extract and its isolated phenylethanoid glycoside verbascoside have no cytotoxicity in human LNCaP prostate epithelial cells, whereas an inhibitory effect on the DHT-induced free and total PSA secretion, a recognized anti-androgen like activity, has been shown in case of both *Verbascum xanthophoeniceum* extract and pure verbascoside. Furthermore, in the absence of the endogenous androgen DHT, an androgen-like activity in *Verbascum xanthophoeniceum* is detectable as it is for saw palmetto, suggesting that a mixed androgen-antiandrogen activity is present.
Conclusions: For the first time, *Serenoa repens* and *Verbascum xanthophoeniceum* extracts have shown an absence of cytotoxicity and an inhibitory effect on DHT-induced PSA secretion in an *in vitro* model of human prostate epithelium, whereas the phenylethanoid glycoside verbascoside appeared to explain only part of the *Verbascum xanthophoeniceum* inhibitory activity on PSA secretion.

More information is available at: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5281800#x332

1. Introduction

Verbascum xanthophoeniceum Griseb. is an endemic mullein plant species (genus *Verbascum*, Scrophulariaceae) of the Balkans and some parts of Turkey, particularly enriched in iridoids- and phenylethanoids. In Bulgarian and worldwide folk-medicine, *Verbascum* species have a traditional use in the treatment of inflammatory disorders of skin and epithelial tissues of the respiratory, gastro-intestinal and uro-genital tracts (Ivanov et al., 1971; Turker and Gurel, 2005; EMEA, 2009; Suntar et al., 2010). *Verbascum* species contain several bioactive compounds, including flavonoids and phenylethanoid glycosides among others, whose anti-inflammatory, anti-oxidative, anti-tumor and immune-stimulatory activities (Talib and Mahasneh, 2010; Georgiev et al., 2011a, 2011b) have been reported so far. Recently, a large body of evidence, based on *in vivo* and *in vitro* experimental models, supported the anti-inflammatory properties of *Verbascum xanthophoeniceum* total methanolic extract and its bioactive constituents from the iridoids—and phenylethanoids—containing fractions (Georgiev et al., 2011b, 2012b). Indeed, *Verbascum xanthophoeniceum* crude methanolic extract and its constituents have been shown: i) to inhibit TNF-α and IL-10 production and to decrease NO release in *in vitro* cultured peritoneal macrophages (Dimitrova et al., 2012); ii) to negatively regulate mediators of skin inflammation in normal human epidermal keratinocytes (NHK; Potapovich et al., 2011; Georgiev et al., 2012b); iii) to reduce inducible nitric oxide synthase (iNOS) activity (Speranza

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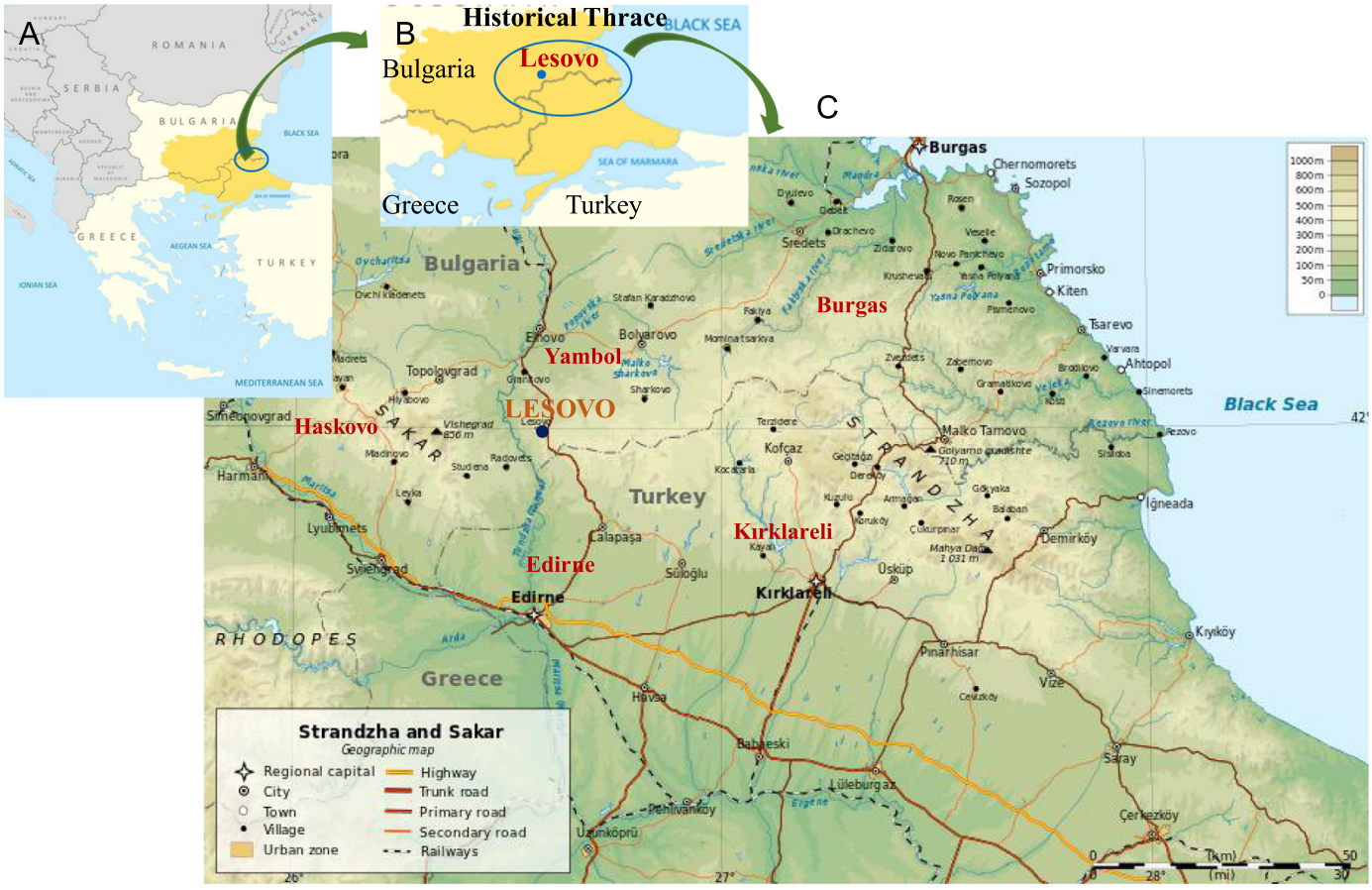

Fig. 1. Geographical location of Lesovo Village (Yambol province) within the historical region of Thrace along with nowadays state borders (A and B) and map of the Strandzha or Stranja (Yildiz in Turkish) and Sadar mountains (C).

et al., 2010). Among the isolated iridoids- and phenylethanoids-glycosides, the most effective anti-inflammatory compounds appeared to be verbascoside and forsythoside B (Georgiev et al., 2012b). Indeed, verbascoside, also known as acteoside, is a phenylethanoid glycoside frequently found in different plants, whose use in the traditional medicine has been reported and experimentally supported, such as — besides *Verbascum* species—among others, *Anisomeles indica*, *Cistanche tubulosa*, *Rehmannia glutinosa*, *Buddleja* species in traditional Taiwan, Chinese or Japanese medicine (Liao et al., 1999; Rao et al., 2009; Yamada et al., 2010; Lee et al., 2013), *Penstemon gentianoides*, *Penstemon campanulatus*, and *Castilleja tenuiflora* in Mexican traditional medicine (Domínguez et al., 2011; Carrillo-Ocampo et al., 2013; Sanchez et al., 2013), *Aloysia triphylla*, *Lippia citriodora* (lemon verbena) in South America and Southern Europe (Caturla et al., 2011; Lenoir et al., 2012), *Teucrium chamaedrys* and *Nepeta cataria* in England (Prescott et al., 2011), *Syringa vulgaris* in Balkan regions and overall in Europe (Paola et al., 2011), *Harpagophytum procumbens* in South African traditional medicine (Georgiev et al., 2012a), *Clerodendron* species and *Rumex aquatica* in Egyptian folk medicine (Lee et al., 2011; Wahba et al., 2011).

Most human tissues, including the male reproductive organs, the prostate in particular, are susceptible to inflammation. Chronic prostatitis could conceivably result in altered homeostasis that may, in the longer term, trigger more severe patho-physiological conditions (Delongchamps et al., 2008; Vendramini-Costa and Carvalho, 2012; Nakai and Nonomura, 2012; Bachir and Jarvi, 2014), such as benign prostate hyperplasia (BPH) and even prostate cancer (PCa). In all cases, the inflammatory status may potentially impair the prostate functionality leading to male infertility through altered quality of the secreted prostatic fluid, an essential component of the ejaculate regulating sperm function (Lorenzetti et al., 2012; Bachir and Jarvi, 2014). The prostatic fluid constitutes about one-third of the volume of the male ejaculate and provide it with, among other factors, a subgroup of extracellular serine proteases involved in complex proteolytic cascades called kallicreins (KLKs), that are fundamental in processes such as the liquefaction of the seminal clot (Lorenzetti et al., 2010, 2012 and refs therein). The best known KLK is the Prostate-Specific Antigen (PSA/KLK3), a clinical biomarker known to be prostate specific, but not PCa specific; indeed, PSA is not considered to be a really robust biomarker since it lacks the sensitivity to specifically detect all prostate adenocarcinomas, particularly in the early stage of PCa. Besides PCa, among the many factors known to affect PSA levels are the inflammatory-based prostate diseases, such as prostatitis and BPH (Obort et al., 2013), whose treatment with anti-inflammatory drugs have been recently promoted and supported by a large body of evidence (Mahmud et al., 2010; Rothwell et al., 2011; Kahokehr et al., 2013).

Several plant-derived extracts have been promoted as complementary and alternative medicines targeting prostate diseases although, so far, placebo-controlled clinical trials did not support their use as pharmacological drugs (Kim et al., 2012; Posadzki et al., 2013). Among them, *Serenoa repens* (American saw palmetto or dwarf palm plant) extract has been suggested as a promising phytotherapeutic agent, whose anti-inflammatory properties as well the modulation of all known key pathways leading to prostate diseases have been shown in *in vitro* (Habib et al., 2005; Yang et al., 2007; Silvestri et al., 2013) and animal models (Talpur et al., 2003; Abe et al., 2009; Borovskaya et al., 2012).