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Molecules of Interest

Harpagoside: from Kalahari Desert to pharmacy shelf

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ABSTRACT

Harpagoside is an iridoid glycoside that was first isolated from *Harpagophytum procumbens* (devil's claw, Pedaliaceae), a medicinal plant in which it is the major constituent of the iridoid pool. Both the pure compound and devil's claw extracts have potent anti-rheumatic, anti-inflammatory and analgesic effects. According to the European Pharmacopoeia commercial devil's claw products should contain at least 1.2% harpagoside. However, the compound has also been isolated from several other plant species and *in vitro* plant culture systems. Recent advances in knowledge of harpagoside distribution, biosynthesis/accumulation and pharmacology are summarized in this review. We also discuss the possible synergism and/or antagonism between major constituents in harpagoside-containing phytopharmaceutical products. Finally, future perspectives for its potential application are highlighted.

1. Power from the Kalahari Desert: the discovery of harpagoside and related iridoid glycosides

Deep in the Kalahari Desert grows a herbaceous plant with high medicinal value called devil's claw, *Harpagophytum procumbens* subsp. *procumbens* (Burch.) de Candolle ex Meissner, Pedaliaceae (Fig. 1A and B). *Harpagophytum* plants have been used for centuries by the Khoisan people of southern Africa to treat diverse health disorders, including fever, gastrointestinal problems diabetes, hypertension and blood diseases (Stewart and Cole, 2005). In the middle of the last century several systematic studies revealed that extracts of the plant's tubers are effective in the treatment of degenerative rheumatoid arthritis, osteoarthritis, tendonitis, kidney inflammation and heart disease (Stewart and Cole, 2005). *H. procumbens* is currently listed in the European Pharmacopoeia for the treatment of rheumatism and arthritic ailments (Mncwangi et al., 2012; Stewart and Cole, 2005).

In 1962 scientists from Würzburg reported the isolation of a bitter iridoid glycoside from dried devil's claw roots, which they called harpagoside (Fig. 1; Tunmann and Lux, 1962). They found that acids hydrolyze the glycoside to D-glucose and some dark decomposition products, indicating that it behaves similarly to aucubin (another iridoid glycoside known at that time). Under basic conditions harpagoside saponifies to trans-cinnamic acid and harpagide. Several years later the structure of harpagoside was elucidated by the analysis of degradation reaction products and nuclear magnetic resonance (NMR) spectroscopy (Lichti and von Wartburg, 1966). Harpagoside (**1**) was found to be the major compound of the iridoid pool in *H. procumbens*, together with other iridoid glycosides including harpagide (**2**), 8-*O*-*p*-coumaroyl harpagide (**3**), 8-feruloylharpagide (**4**), procumbide (**5**), and phenylethanoid glycoside verbascoside (Baghdikian et al., 1997; Boje et al., 2003; Burger et al., 1987; Qi et al., 2006). As this review focuses on harpagoside, the list of compounds presented is not comprehensive. Moreover, the phytochemistry and ethnobotany of devil's claw plants, along with the biological properties of iridoid glycosides, have been addressed in two excellent recent reviews (Mncwangi et al., 2012; Viljoen et al., 2012). Thus, these aspects are not considered in detail here.

2. The occurrence and distribution of harpagoside

A search of the SCOPUS database (accessed February 2013) identified 240 articles and 14 patents in which the word "harpagoside" appeared, mostly published after 2000 and mainly concerned with harpagoside isolated from devil's claw. Knowledge of its occurrence and distribution in other plants is still limited. It has been detected mainly in the Lamiaceae, Pedaliaceae, Plantaginaceae and Scrophulariaceae families of the Lamiales order of dicot-

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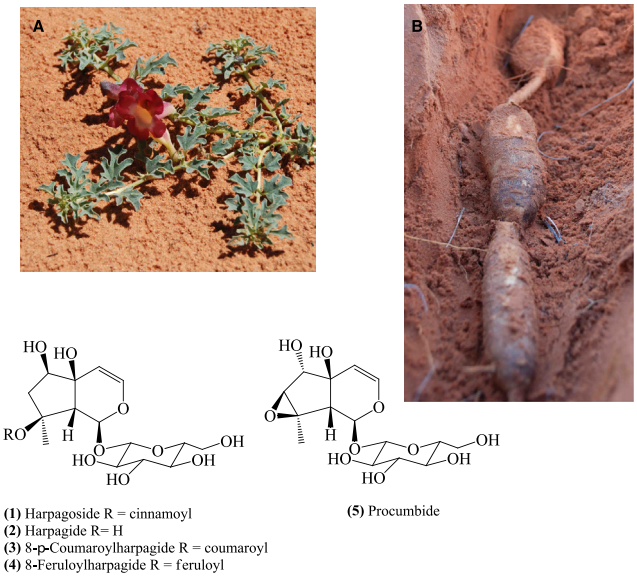

Fig. 1. Pictures of *Harpagophytum procumbens* ((A), above-ground parts with annual creeping stems; (B) underground parts with secondary tubers) and chemical structures of harpagoside (**1**) and some related iridoid glycosides (**2**–**5**). Photos courtesy of Dr. Ilze Vermaak (Tshwane University of Technology, South Africa).

yledonous angiosperms (Table 1). However, Yuan et al. (2011) recently reported its presence in a Gentianales species, *Hedyotis tenelliflora* (Rubiaceae), suggesting that harpagoside, like other iridoids, might have a wider distribution in the plant kingdom.

Harpagoside has been detected in both underground (*e.g.* primary and secondary roots) and above-ground (*e.g.* stems and leaves) parts of plants, but in widely varying levels. For instance, secondary tubers of devil's claw accumulate ten times higher levels of harpagoside than the leaves (Levieille and Wilson, 2002). In a recent NMR-based metabolomics analysis of five *Verbascum* (mullein) species we found that two, *Verbascum xanthophoeniceum* and *Verbascum nigrum*, accumulate potentially commercially attractive levels of harpagoside in their leaves (~0.5% on a dry weight basis). Hierarchical clustering analysis revealed that these *Verbascum* species have a similar leaf metabolome, which is quite different from that of the other analyzed mullein species (Georgiev et al., 2011). This study also showed that NMR spectroscopy can be used for the rapid quantification of harpagoside in plant samples, *e.g.* for quality control of pharmaceutical products and/or botanical supplements.

3. The (bio)production and biosynthesis of harpagoside

It is important to develop sustainable biotechnological methods to produce devil's claw products (particularly the valuable harpagoside) for pharmaceutical applications due to the restricted availability of plant material. In early studies, callus cultures were induced from fresh root tubers of *H. procumbens*, but they failed to produce harpagoside (Abou-Mandour, 1977; Franz et al., 1982). This is not surprising, since callus cultures consist of undifferentiated cells, in which gene expression patterns markedly differ from those of whole plants, so genes involved in the production of desirable secondary metabolites may be repressed (Sesterhenn et al., 2007). Thus, organ cultures might be more appropriate for its production (Ludwig-Müller et al., 2008). Attractive options are hairy root cultures induced via *Agrobacterium rhizogenes*-mediated genetic transformation, which have received increasing attention in recent years, *inter alia* because they have relatively fast growth rates in hormone-free media, are genetically and biochemically stable, and can have similar secondary metabolite profiles to the plants from which they are generated (Georgiev et al., 2012a). By transforming shoot tip explants of devil's claw with *A. rhizogenes* strain A4, Grabkowska et al. (2010) obtained a hairy root clone capable of growing under submerged conditions and accumulating 0.32 mg harpagoside/g dry root mass. This is lower than harpagoside contents in intact plants. However, the study demonstrated the feasibility of biotechnological production, although further selection of strains and knowledge of the pathways involved are required for viable commercial exploitation of

Table 1
Taxa in which harpagoside has been detected.

Family	Plant	Tissue	References
Lamiaceae	*Stachys officinalis*, *S. alpina*, *S. germanica*, *S. sylvatica*, *S. grandiflora*, *S. macrantha*, *S. palustris*, *S. byzantina*, *S. recta*	Aerial parts	Haznagy-Radnai et al. (2006)
Pedaliaceae	*Harpagophytum procumbens*	Tubers, primary roots, leaves	Czygan and Krüger (1977)
	H. zeyheri	Tubers	Czygan and Krüger (1977)
	Rogeria adenophylla	Aerial parts	Potterat et al. (1991)
Plantaginaceae	*Plantago lagopus*	Aerial parts	Velazquez-Fiz et al. (2000)
Scrophulariaceae	*Oreosolen wattii*	Aerial	Jensen et al. (2008)
	Scrophularia ningpoensis	Roots	Kajimoto et al. (1989)
	S. scorodonia	Roots	Fernandez et al. (1992)
	S. korainensis	Roots	Pachaly et al. (1994)
	S. koelzii	Aerial parts	Garg et al. (1994)
	S. frutescens	n.s.[a]	Garcia et al. (1996)
	S. yoshimurae	Stems, leaves, roots	Lin et al. (1998)
	S. buergeriana	Roots	Kim et al. (2002)
	S. nodosa	Whole plant	Sesterhenn et al. (2007)
	Verbascum nigrum, *V. aphentulium*, *V. undulatum*, *V. pulverulentum*, *V. thapsus* ssp. *crassifolium*, *V. wiedemannianum*, *V. densiflorum*	Roots	Seifert et al. (1985)
	V. lychnitis	Shoot	Seifert et al. (1985)
	V. laxum	Roots	Agababyan et al. (1987)
	V. lasianthum	Roots	Akdemir et al. (2004)
	V. letourneuxii	Whole plant	Emam (2010)
	V. xanthophoeniceum, *V. nigrum*	Leaves	Georgiev et al. (2011, 2012b)
Rubiaceae	*Hedyotis tenelliflora*	Whole plant	Yuan et al. (2011)

[a] Not specified.

this *in vitro* plant organ system. Levieille and Wilson (2002) reported the development of a two-step protocol for *in vitro* propagation of devil's claw, and another *Harpagophytum* species (*Harpagophytum zeyheri*), involving the regeneration of new plantlets from nodal cuttings and their acclimatization to *ex vivo* conditions. Tuber tissues of their micropropagated plants had comparable harpagoside and harpagide levels to those of the wild plant material (Levieille and Wilson, 2002), demonstrating the potential for further optimization of hairy root systems of devil's claw and related plants.

To the best of our knowledge there are no published methods for artificially synthesizing harpagoside, therefore plants (and *in vitro* plant systems eventually) remain the only sources of the compound. Fortunately, efficient, convenient methods for separating and purifying harpagoside from plant extracts have been developed (Tian et al., 2012; Tong et al., 2006) involving the use of high-speed countercurrent chromatography (HSCCC). This technique eliminates irreversible adsorption, a common problem in column chromatography, and can provide highly purified harpagoside (98%) from roots of *Scrophularia ningpoensis* Hemsley. The significance of harpagoside-containing plant sources and pharmaceutical products has also prompted the development of several analytical methods using high-performance liquid chromatography (HPLC) coupled with either diode array detection or electrospray ionization-mass spectrometry (Lee et al., 2007; Sesterhenn et al., 2007). Both of these approaches are suitable for determination of their main active constituents and quality control. HPLC analysis of commercial tinctures of *H. procumbens* has also shown that harpagoside and concomitant iridoids are stable; their concentrations reportedly fell by less than 10% during 6 months storage at 40 °C at 75% relative humidity (Karioti et al., 2011).

The harpagoside biosynthetic pathway has not yet been fully elucidated. The early steps are known, but several intermediates, key enzymes and the corresponding genes remain to be discovered. Current knowledge of the pathway, including several hypothetical intermediates based on related pathways (*e.g.* iridoid biosynthesis in *Catharanthus roseus*) is summarized in Fig. 2. Iridoids comprise a large group of monoterpenoids with a cyclopentane-[C]-pyran skeleton, and an early biosynthetic step in plants is generally condensation of dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), forming geranyl diphosphate (GPP) (Sampaio-Santos and Kaplan, 2001). Two independent routes are known to supply IPP: the mevalonate and 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathways, which occur in the cytosol and

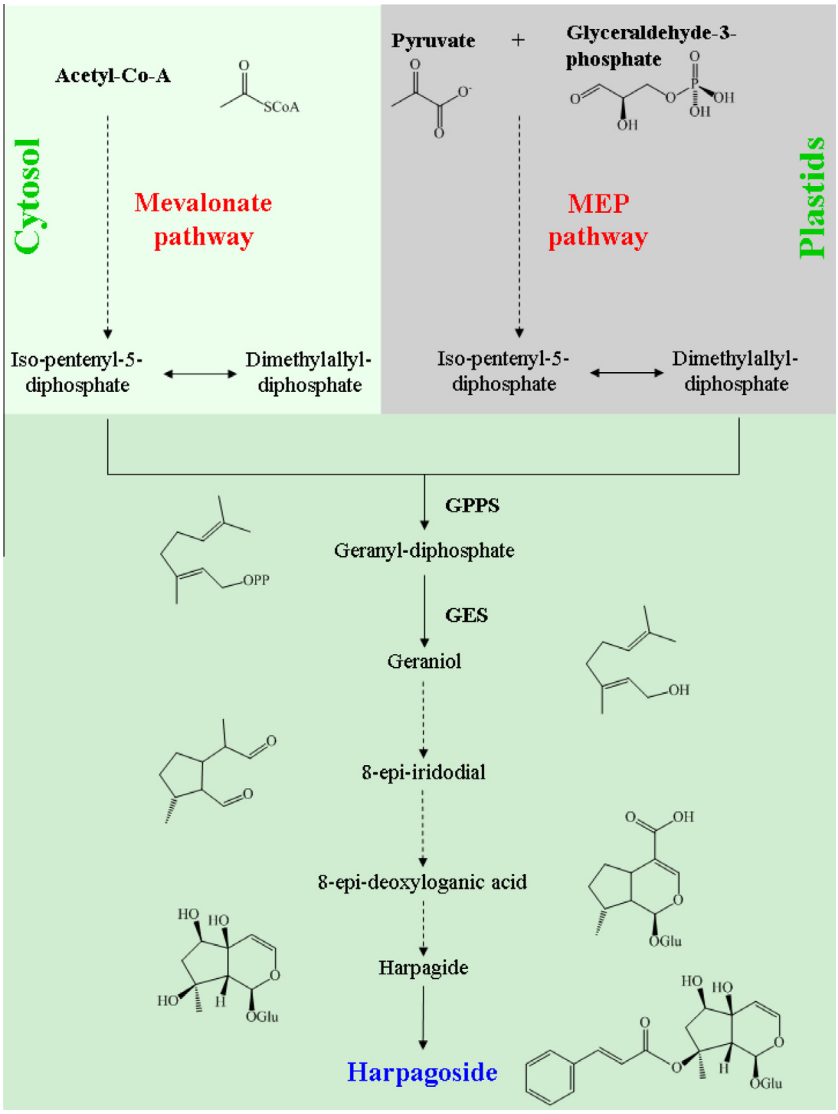

Fig. 2. Tentative pathway of harpagoside biosynthesis. MEP, 2-*C*-methyl-D-erythritol-4-phosphate; GPPS; geranyl diphosphate synthase; GES, geraniol synthase.

plastids, respectively (reviewed in Oudin et al., 2007). The condensation of DMAPP and IPP is catalyzed by geranyl diphosphate synthases (GPPS). These enzymes, and transcripts encoding them, have been detected in several plant species and characterized. In some species GPPS are homodimeric enzymes, while in others they are heterodimeric (Oudin et al., 2007). The next step in iridoid biosynthesis is formation of geraniol from GPP, a dephosphorylation catalyzed by geraniol synthase (GES). cDNAs of *GES* transcripts from several plant species have been cloned, including sweet basil and camphor (Oudin et al., 2007 and literature cited therein). However, we have found that overexpressing *GES* (from *Valeriana officinalis*) in transformed root cultures of *H. procumbens* and *V. nigrum* does not result in increased harpagoside production (Georgiev et al., unpublished results), suggesting that further manipulation would be required to boost levels of downstream intermediates. Damtoft et al. (1993, 1994) found that feeding *Scrophularia umbrosa* with deuterium-labeled 8-*epi*-iridoidial, 8-*epi*-iridotrial, 8-*epi*-iridotrial glucose and 8-*epi*-deoxyloganic acid results in incorporation of labeling into harpagide (and aucubin). Thus, key intermediates in harpagide and harpagoside biosynthesis probably include these molecules, or natural derivatives thereof.

Clearly, despite recent advances, further information about harpagoside biosynthesis is needed to develop a viable biotechnological production process using metabolically engineered material. Obtaining this information will require robust identification of all the intermediates, followed by thorough characterization of the genes involved in the committed steps.

4. Pharmacology and applications of harpagoside

Arthritis is a common, chronic (long lasting), progressive and disabling autoimmune disease that causes inflammation and pain in the joints (usually in the hands and feet first, but any joint may become affected), tissues around the joints, and other organs in the human body (Firestein, 2003; Luyten et al., 2006). Current drugs have severe limitations for treating this crippling disease: thus there are urgent needs for new leads (Breedveld and Combe, 2011). Devil's claw offers attractive potential as a source of such leads, since various kinds of preparations (powder, water or alcoholic extracts) have been traditionally used to treat musculoskeletal complaints.

Based on the wide recognition of their potency as folk remedies for rheumatic complaints, the effects of *Harpagophytum* extracts have been studied for nearly 60 years using various animal models, including sub-acute formaldehyde-induced and egg albumin-induced paw oedema in rat (Arrigoni-Martelli, 1977; Mahomed and Ojewole, 2004) and croton oil-induced granuloma in guinea pig (Eichler and Koch, 1970). Several investigations have also found that extracts have good anti-inflammatory and analgesic activities in carrageenan-induced acute inflammation (Andersen et al., 2004; Baghdikian et al., 1997; Dimitrova et al., 2013; Haznagy-Radnai et al., 2012; Lanhers et al., 1992; Soulimani et al., 1994). However, others workers have reported non-significant oedema reduction (Grahame and Robinson, 1981; Whitehouse et al., 1983). The divergence between the results could be due to the use of extracts with different concentrations of harpagoside and/or other, uncharacterized compounds. The major active constituents of *H. procumbens* are the iridoid glycosides harpagoside (**1**), harpagide (**2**), 8-*O*-*p*-coumaroyl harpagide (**3**; Fig. 1) and the phenylethanoid glycoside verbascoside, but minor constituents may have significant synergetic or antagonistic effects. Thus, overall therapeutic effects may depend on both absolute and relative amounts of their constituents (Abdelouahab and Heard, 2008). However, the main pharmacological activity of *Harpagophytum* extracts is attributed to harpagoside, and the harpagoside content is used to standardize commercial *H. procumbens* products, which should contain at least 1.2% of the compound according to the European Pharmacopoeia (Mncwangi et al., 2012; Stewart and Cole, 2005). Nevertheless, the divergence in results of pharmacological experiments clearly shows that the extracts have varying activities: thus rigorous standardization is needed. Analysis of a single compound is insufficient; detailed systems biology analysis is required to correlate the multi-omic profiles of the plant materials, and metabolomic profiles of phytotherapeutic preparations obtained from them, with their pharmacological properties in order to realize their full potential.

An assessment of 15 studies on the pharmacology of *Harpagophytum* extracts concluded that daily doses of at least 50 mg of harpagoside are effective for treating arthritis (Chrubasik et al., 2003; Chrubasik, 2004). It reportedly provides pain relief for 60% of patients with an osteoarthritic hip or knee, or nonspecific lower back pain (Chrubasik et al., 2007a,b; Gagnier et al., 2004; Sporer and Chrubasik, 1999). Improvement in knee osteoarthritis has also been observed following oral administration of doloteffin (a standardized Devil's claw extract; Ardeypharm), providing 60 mg harpagoside per day, for up to 54 weeks (Chrubasik et al., 2002, 2005). The clinical studies clearly confirm the positive effects of standardized harpagoside-containing products in the treatment of arthritis.

The anti-inflammatory action of pure harpagoside has been mainly evaluated in mouse and rat carrageenan-induced oedema models, in which intraperitoneal (i.p.) treatment with 10 mg/kg (Haznagy-Radnai et al., 2012; Lanhers et al., 1992) or 20 mg/kg (Dimitrova et al., 2013) doses significantly abolishes paw swelling. Paw swelling in mouse models is also reportedly inhibited by oral administration of harpagoside in 10 mg/kg (Ahmed et al., 2003) or 100 mg/kg (Recio et al., 1994) doses. However, further studies are required to define optimal doses and administration routes. Furthermore, knowledge of harpagoside's effects on chronic inflammation is very sparse, particularly for long-term treatment, although histopathological data we recently obtained indicate that i.p. application (20 mg/kg daily for 10 days) abrogates joint destruction in a mouse model of zymosan-induced arthritis (Dimitrova et al., 2013).

In attempts to elucidate the reported analgesic and anti-inflammatory activities of *H. procumbens* preparations in animals and man, the effects of various extracts and pure harpagoside have also been thoroughly studied *in vitro* (see summary in Table 2). Their influence on the arachidonic acid pathway has been particularly closely examined, because inhibitors of cyclooxygenases 1 and 2 (COX-1/2, key enzymes of the pathway) have emerged as important targets for treating rheumatoid arthritis. Anauate et al. (2010) found that an *H. procumbens* fraction containing 88.8% harpagoside moderately inhibited COX-1/2 activity and nitric oxide (NO) production in whole human blood. Various extracts and pure harpagoside can reportedly inhibit COX-2 expression in freshly excised porcine skin (Abdelouahab and Heard, 2008; Ouitas and Heard, 2009, 2010), and (at concentrations of 27% or 8.9%, but not 2%) inducible nitric oxide synthase (iNOS) expression, in rat mesangial cells (Kaszkin et al., 2004). Aqueous extracts can suppress NO production and both COX-2 and iNOS expression in fibroblast cell line L929 (Jang et al., 2003). Enriched extracts containing up to 30% harpagoside can reportedly completely inhibit 5-lipoxygenase *in vitro* (Gunther et al., 2006). Pure harpagoside and *H. procumbens* extracts (from cell suspension cultures and hairy roots generated by *A. rhizogenes*-mediated transformation) inhibit COX-1/2 expression and NO production by peritoneal macrophages (Gyurkovska et al., 2011). In addition, harpagoside suppresses lipopolysaccharide (LPS)-induced COX-2 and iNOS expression induced in RAW 264.7 cells via the NF-κB (nuclear factor kappa B) signaling pathway (Huang et al., 2006). All of these findings strongly suggest

Table 2
Activities of harpagoside and *Harpagophytum procumbens* extracts in models.

Assays	Extract or pure harpagoside	Concentration	Target cells	Effect	References
COX-1/2 expression, NO and cytokine production	Aqueous extract	1 mg/ml	Rat renal mesangial cells	Inhibition of NF-kB activation and iNOS expression	Kaszkin et al. (2004)
	Ethanolic extract	30 μg/ml	Whole blood	Inhibition of COX-2 and PGE2 activity	Anauate et al. (2010)
	Aqueous extract	1 mg/ml	L 929 cells	Inhibition of COX-2 and iNOS mRNA expression, PGE2 synthesis and NO production	Jang et al. (2003)
	Harpagoside and different extract formulations	1 mg/ml	Porcine skin cells	Inhibition of COX-2	Abdelouahab and Heard (2008) and Ouitas and Heard (2009, 2010)
	Methanolic extract	500 μg/ml	Mouse peritoneal macrophages	Inhibition of NO, TNF-α and IL-6 production and COX-1/2 expression	Gyurkovska et al. (2011)
	Ethanolic extract	400 μg/mouse	Mouse skin	Inhibition of COX-2 expression, ERK-kinase activity, c-FOS expression, AP-1 and CREB DNA binding	Kundu et al. (2005)
	Hydroalcoholic extract (60% ethanol v/v)	100 μg/ml	Human monocytes	Inhibition of TNF-α, IL-6, IL-1β and PGE2 production, COX-2 expression in RAW 264.7 cells; blocking of the AP-1 pathway	Fiebich et al. (2001, 2012)
	Harpagoside	250 μg/ml	Mouse peritoneal macrophages	Inhibition of NO production and COX-1/2 expression	Gyurkovska et al. (2011)
		10–200 μM	HEPG2 and RAW 264.7 cells	Inhibition of NF-kB activity and expression of iNOS and COX-2	Huang et al. (2006)
		50–100 μM	RAW 264.7 cells	Inhibition of IL-1β, IL-6, and TNF-α production	Inaba et al. (2010)
Leukotriene biosynthesis	Ethanolic extract	IC_{50} 1.45 mg/ml	Human blood	Reduction of ionophore-stimulated Cys-LT levels	Loew et al. (2001)

that harpagoside can perturb the arachidonic acid pathway. Intriguingly, a recent study demonstrated that harpagide has COX-2-mediated pro-inflammatory properties, therefore it can apparently antagonize the anti-inflammatory effect of harpagoside (Abdelouahab and Heard, 2008).

In addition to the effects already described, extracts of devil's claw (and several other medicinal plants) influence levels of various cytokines, such as TNF-α, interleukin (IL-1 and IL-6) and gamma-interferon (IFN)-γ, which are characteristically involved in inflammation (Spelman et al., 2006). *Inter alia*, the standardized extract Steiner *H. procumbens* 69 reportedly suppresses LPS-induction of these cytokines in primary human monocytes (Fiebich et al., 2001). The *in vitro* data are supported by findings that at a dose of 50 mg/kg a hydroalcoholic extract (50% ethanol v/v) significantly inhibited inflammation and LPS-induced TNF-α, IL-6 and IL-1β production in a rat adjuvant-induced chronic arthritis model *in vivo*; an effect the authors attributed to harpagoside (Inaba et al., 2010). A recent study also showed that harpagoside isolated from *V. xanthophoeniceum* can reduce the amounts of IL-8, MCP-1 and IP-10 secreted by cultured normal human keratinocytes. However, it was significantly less inhibitory than verbascoside and forsythoside B, indicating that the anti-inflammatory effects of iridoid glycosides are probably tissue- and/or cell-specific (Georgiev et al., 2012b).

A standardized *Harpagophytum* extract, WS1531, also reportedly reduces ionophore-stimulated cysteinyl leukotriene (Cys-LT) levels in whole blood *in vitro*, and in plasma obtained from healthy male volunteers, more strongly than both pure harpagoside and harpagoside-free fractions (Loew et al., 2001). Furthermore, *Harpagophytum* extracts can influence transcriptional level events through inhibiting extra-cellular signal regulated protein kinase (ERK) activity, c-Fos expression and DNA binding of activator protein-1 (AP-1) and cAMP response element-binding protein (CREB) (Kundu et al., 2005). These findings call for further investigations to elucidate whether harpagoside contributes to such activities, and to identify other active agents in the extracts.

Several studies have found that harpagoside and standardized *H. procumbens* extracts have other interesting activities. Notably, in rats they are found to have protective action in aconitine-induced arrhythmia (Circosta et al., 1984) and reperfusion-induced ventricular arrhythmia (Costa De Pasquale et al., 1985). In addition, harpagoside inhibits release of the inflammatory mediator RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) by stimulated human bronchial epithelial (BEAS-2B) cells, indicating that it has potential utility for treating respiratory disorders (Boeckenholt et al., 2012). It also ameliorates dopaminergic neurodegeneration and movement disorder in a mouse model of Parkinson's disease by elevating the glial cell line-derived neurotrophic factor (Sun et al., 2012).

Based on the published data, possible mechanisms of the anti-inflammatory action of harpagoside and standardized *Harpagophytum* extracts are proposed (Fig. 3). The inhibition of COX-1/2 enzymes, which catalyze the generation of prostaglandins and thromboxane, makes harpagoside a useful agent for intervention strategies targeting inflammatory disorders and/or inflammatory pain. The reduction of NO production and iNOS expression adds to the beneficial pharmacological profile of the molecule. The suppression of COX-1/2 and NF-kB activity, observed *in vitro*, by harpagoside might contribute to possible neuroprotective effects by limiting progression of neuronal degeneration. Another promising effect of harpagoside is the inhibition of pro-inflammatory cytokines (including TNF-α, IL-1β and IL-6), which regulate a broad range of inflammatory processes implicated in the pathogenesis of arthritis. Molecules that inhibit both COX and cytokines have multiple advantages because they influence two major inflammatory pathways. However, before the full potential of harpagoside as a new anti-inflammatory drug can be realized more work is required: effects of its long-term administration in chronic models of joint inflammation must be thoroughly elucidated; its efficacy must be elucidated in rigorously controlled, long-term clinical trials; its mechanism of action must be systematically clarified; and its minimum effective doses, safety and

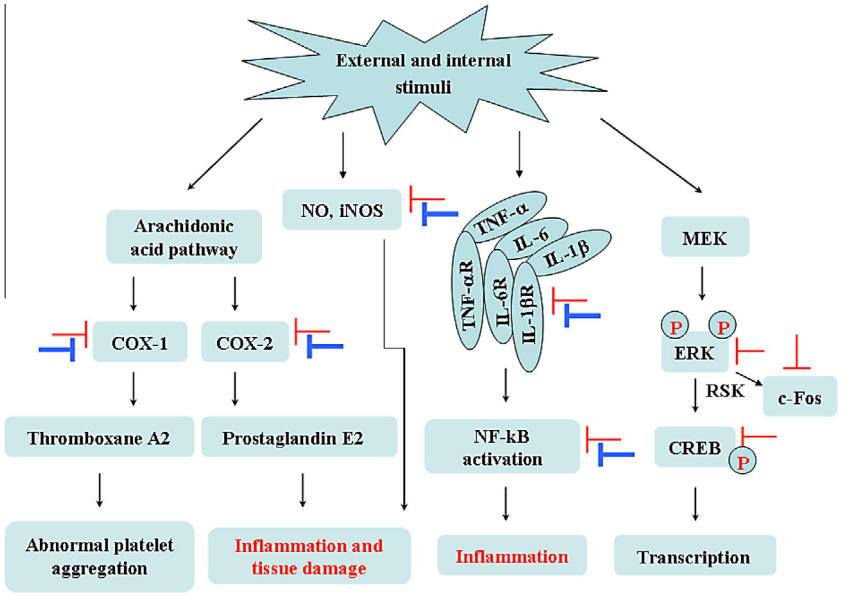

Fig. 3. Proposed molecular action mechanism of harpagoside (⊥) and standardized *Harpagophytum* extracts (⊥). RSK, ribosomal s6 kinase; ERK, extra-cellular signal regulated protein kinase; CREB, cAMP response element-binding protein; MEK, mitogen-activated protein kinases; c-FOS, cellular proto oncogene; NO, nitric oxide; iNOS, inducible nitric oxide synthase.

optimal administration routes must be thoroughly determined. It also appears that different plant extracts contain active ingredients that might act synergistically (and antagonistically) with the known iridoids. Clearly, system biology analyses are required to correlate the pharmacological effects with the active, synergistic agents, characterize the metabolic pathways involved in their synthesis and identify targets for metabolic engineering to boost their production.

5. Future perspectives

Fifty years after the discovery of harpagoside, very little is still known about its biosynthetic pathway. Several key enzymes, and the genes encoding them, remain to be discovered. Thus, better understanding of harpagoside biosynthesis is required to identify means to boost its biosynthesis (and that of putative synergists) by metabolic engineering and subsequently to develop efficient cell factories for its mass-production. Recent advances in deep sequencing and metabolomic technologies are likely to greatly facilitate such efforts.

As a major constituent in preparations of devil's claw and various other plants, the iridoid glycoside harpagoside has highly attractive pharmaceutical properties. It is also of interest for further chemical modification, as its structure offers an interesting scaffold (with various reactive sites) for combinatorial chemistry.

Further intensive studies are required to confirm harpagoside's potential for treating rheumatologic and/or other inflammatory diseases, thereby enabling its acceptance as a therapeutic agent. For this, thorough elucidation of the pathways involved in its anti-inflammatory and/or immunomodulatory activities is required, together with more research using various animal models. Furthermore, clinically-active plant extracts seem to contain much lower levels of the compound than the concentration of the pure compound required for activity in animal models. Thus, the presumed synergists need to be identified, and both their activities and metabolic pathways need to be fully characterized through rigorous systems biology analysis.

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