Recent Research on «Harpagophytum procumbens» DC. (***)

The industrialized countries' return to the use of natural drugs over the last ten years has been accompanied by a critical re-examination of the drugs employed in traditional medicine. For many drugs, empirically used for centuries, pharmacological researches have confirmed the action for which they were employed, or in addition to this one, other important effects, useful in therapy, have been observed. Besides, for a lot of them, the substances really responsible for their biological activity and the interactions between them have been identified.

A lot of examples could be quoted in this regard, and you will hear someone relate about many of them in the course of this Symposium. I will dwell upon a drug used in the traditional medicine of a limited region of Africa, in the treatment of many diseases and, among other things, in rheumatic affections; for its anti-inflammatory effect the employment of this drug was diffused in Europe. It consists of Harpagophytum procumbens DC. roots, a drug that has been the subject of morphological, analytical and biological researches also in the laboratories of our Department [1-4].

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Harpagophytum procumbens DC. (Pedaliaceae) is a perennial herbaceous plant spread in South Africa and in tropical Africa, where it grows particularly in the savannas of the Kalahari, in Namibia, from the capital of which the root takes one of its names — Windhoek’s root —, in Transvaal and in the Orange State.

After the first rains, from the roots there develop creeping shoots bearing...
alternate, petiolate, lobate leaves, at the axil of which there is a solitary, tubular, red violet flower.

The flat, ligneous fruit bears four appendixes, each subdivided into several claws ending by hooks turned towards the inside. The fruits get entangled in animals’ manes and tails, or stick into their hoofs, so that the animals perform a sort of frenzied dance to get rid of them; or, if they remain stuck in the animals’ fauces, these may die if they are not promptly assisted.

And that is the reason why the drug is called “Devil’s claw” and is commercially known with this denomination; the name of the genus “*Harpagophytum*” derives from the Greek ἀρπαγεῖον - ἀρπαγαῖος = hook.

The first information about this drug goes back to 1904, when a farmer, G.A. Menhert, of the Mariental District of Nabas, South Africa, during the revolt of the Hottentots, had witnessed the recovery (thanks to a witch-doctor) of a Hottentot who had been given up by the doctors. As the identity of the drug was kept secret, Menhert followed the witch-doctor and, as people say, with the help of a dog discovered the secret of the plant employed; thanks to him the use of this drug was spread in Europe.

The drug is largely employed in South Africa, both among the natives — Bushmen, Hottentots, Bantu — and among the Europeans, not only in the treatment of rheumatism, but also for its purgative action, which is also one of the side-effects of the use of the drug, and, besides, in febrile illnesses, in allergic reactions and as a bitter-tinge.

For external use, in the form of ointment, it is used in ulcers, wounds and in many cutaneous lesions; moreover, native women apply it to their abdomen to speed up the birth, while the pulverized root is ingested during and after labor to relieve pain.

The drug consists of the secondary roots, which have a characteristic bitter taste, which is useful in picking, to differentiate them from the almost tasteless primary roots.

As soon as the roots, rich in water, are picked, they are cut into slices and put to dry in a well aired room.

Commercially, the drug is sold in the form of “rounds”, which are sometimes cut in half, and then they look like “fans”. The lateral surface is rugous, with a partially cracked, reddish-yellow cork. The cross surfaces are of a reddish-yellow color, irregularly wrinkled, and one can distinguish in them a thin slice of yellowish-white cork, which is easily detachable; a brief cortical zone (about 1/5 of the radius); a raised ring that delimits the wood. In the vascular, spongy cylinder, one can observe raised, concentric rings, corresponding to the annual rings of the wood, and medullary rays.

The whole cut-surface, particularly in the area of the vascular cylinder, is covered by translucent, reddish-brown granules, and with a fine pale yellow granulation.

The granules are partially soluble in methanol, and the solution presents a spectrum with a maximum absorbance at 280 nm. In the chromatograms of
the methanolic solution (HPTLC plates in silica gel 60 Merck; solvent system: ethyl acetate: methanol: water 75: 15: 18) one can observe, amongst others one spot corresponding to the harpagoside and another, smaller, corresponding to the harpagide.

The cross section of the drug, observed by the scanning electronic microscope, is characterized by a few rows of rectangular cells, with thin walls, which, seen frontwise, appear square. The cortical parenchyma, little developed, is made up of cells, with thin walls too, the section of which is rectangular towards the outside, quadrangular towards the inside; among the cells one can find some rare stone cells with lignified walls. The endodermis is not differentiated and in the phloem one can note small portions of deformed and compressed sieve.

The vascular cylinder, which occupies the greatest part of the section [4-5], is delimited by 3-4 undulated rows of cambium, and is made up of abundant reserve parenchyma, which is particularly rich in granulation. The vessels, towards the outside, are rare, isolated or united in groups of 2-3 elements, neatly placed in radial rows of concentric rings; in the central part, they are very close.

The medullary rays are generally formed by two rows of cells, which come up to the phloem.

The tracheae, mostly dotted, sometimes reticulate, are short and formed by superimposed, slightly elongated cells. In the area where the dividing membrane has disappeared, the strata of thickening around the perforation form a raised pad; they are surrounded by dotted trachids, often intercommunicating by means of a wide hole.

The parenchyma, both in the cortical and phloem zone, and in the ligneous one, is cellulosic. In the centre, on the contrary, the cells are smaller with thickened, lignified, rigid walls and, especially in these, numerous granulations are observed.

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The first investigations regarding the active constituents of the drug go back to 1962 [7], when a group of researchers of the Faculty of Pharmacy, Wurzburg University, in Germany (Tunmann, Lux, Stierdorfer et al.), isolated a bitter substance: harpagoside.

The drug is characterized by the presence of three monoterpenic beta-glucosides, iridoids of the kind aucubin: - harpagoside, bitter, to which most authors attribute the action; harpagide (decinnamoyl-8-harpagoside), with a slightly sweet taste, which probably forms from harpagoside, together with a molecule of cinnamic acid, by enzymatic or chemical degradation respectively during the picking and conservation or during the preparation of the extracts; and procumbide, with epoxide cycle in position 7-8, and which is a 6-diastereoisomer of antirrhinoside.

Iridoids are molecules characterized by the presence of a cyclopentapyran ring system; their name derives from iridoidal, isolated in 1956. For each iridoid molecule there are two possible isomers, one with an open chain with two free
aldehydical functions, and the other with a cyclic closed chain, with the formation of an alcohol-ether bond; it is under the latter shape that in *Harpagophytum* iridoids are stabilized by glucosylation. Their concentration in the drug ranges between 0.5 and 3%. 

Czygan and Kruger [8], examining the different parts of *H. procumbens* and *H. zeyheri* Decne plants, have observed that the flowers, the stalks and the ripe fruits of the two species are free of iridoid glucosides. In the leaves there are only traces of harpagoside together with some other at present non-identified iridoid compounds. In the primary roots the harpagoside content, with traces of non-identified iridoids, ranges between 0.4 and 0.8%, whereas in the secondary roots the harpagoside content is not very different in the two species and ranges between 0.9 and 1.8%.

In the samples we examined [2], the glucorhoidoids, determined by colorimetric method (Haag-Berrurier and coll. [9]), have proved to be 2.09%, and harpagoside, by TLC-colorimetric method (Czygan and coll. [10]), 1.70%.

In the drug, moreover, there are pentacyclic terpenic acids (ursolic, oleanolic, etc.) and their esters; flavonoids (kaempferol, physetin, luteolin); phenylcarboxilic acids (cinnamic, caffeic, clorogenic); a quinone (harpagochinin); water soluble carbohydrates such as glucose, fructose, sucrose, raffinose and considerable amounts of stachyose (a tetrasaccharide made up of fructose, glucose and two molecules of galactose); the stachyose, owing to the considerable quantities in which it is present (46%), represents the main reserve carbohydrate of the roots, that are devoid of starch and of other high molecular weight polysaccharides.

In addition, there are phytosterols, fatty acids, waxes, and small amounts of an essential oil and of a gum-resin.

The methods employed by the different authors for the determination of the substances considered responsible for the drug’s activity, whether they are colorimetric, TLC - UV, gas chromatographic or by HPLC [7, 9, 12-15, etc.], have usually taken into consideration only iridoids.

In biological researches we had observed that other constituents such as flavonoids, terpenic acids or phenylcarboxylic acids, had a great importance as regards the cardiovascular activity of the drug. We have therefore applied a separation, identification and determination method, by liquid high-resolution chromatography, of harpagoside, luteolin, clorogenic, caffeic and cinnamic acids, in some extractive solutions obtained from the drug [16].

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Despite the diffused employment of the Devil’s claw in the cure of rheumatic affections in Germany, Switzerland, France and also in Italy, biological researches about this drug or its active principles are not numerous.

The researches so far carried out, have mainly concerned the anti-inflammatory activity. Zorn in 1958. [17] reported about the antiphlogistic and
antiarthritic effect of a tea, prepared from a south-African plant (H. procumbens), in formalin-induced arthritis test in the rat.

Subsequently Eichler and Koch [18], on the basis of Zorn's researches, tested an aqueous extract, harpagoside and a product of enzymatic hydrolysis (emulsin) of this one, for the analgesic activity (on the rabbit ear), for the anti-inflammatory one (on the croton-oil-induced granuloma in the rat), for the antiarthritic one (formalin-induced arthritis in the rat), and for the spasmolytic one (isolated guinea-pig ileum). In the experimental patterns they tested, they could observe that while the aqueous extract had an activity in arthritis comparable to that of phenylbutazone, the active principle isolated exercised an analgesic and anti-inflammatory action and the degradation product (harpagide) an anti-inflammatory and anti-arthritis action; no spasmolytic action was noted.

Erdos and coll. [13] showed that the antiphlogistic activity of a basic methanolic extract of the drug was more evident in semichronic animal patterns and such effect was comparable to that of pure harpagoside.

The results obtained by the above-mentioned workers and by others, however, are not univocal because the tested extracts were differently obtained and not standardized.

In 1971 the first studies were carried out by Schmidt [19, 20] regarding the employment, in the therapy of polyarthritis, of a tea prepared from the drug and of other unspecified "Harpagophytum-Ampullen", which allowed him to find, clinically, a clear improvement in 60% of the patients treated, disappearance of the pain and greater mobility in 20%; whereas in the remaining 20% of the patients that presented an acute inflammatory process the effect was less evident.

As the "Harpagophytum" preparations were administered as a support to the usual antiphlogistic therapy, it was noticed that their use had allowed a halving of the corticoid doses.

The positive clinical results have been confirmed by a study on double caecum against placebo [21].

It would seem that the harpagogenins, which would form by hydrolysis of the harpagosides, are responsible for the anti-inflammatory activity; but, as they also are degradable in an acid environment, the administration of a pharmaceutical preparation that may protect the iridosides from the gastric acids, such as the enteric capsules of a nebulized preparation from an aqueous extract, (which extracts 30-40%) would allow us, according to the researches carried out by Van Haelen [22], to reduce its dosage.

Still from Van Haelen's researches it appears that the amount of harpagoside linked to plasma proteins is 35% and this fraction represents a drug reserve, as, the molecule not being ionized, the bonds are weak.

In the toxicity tests carried out by some authors [23, 24], both the drug extracts and the harpagoside, through oral administration, have proved to be little toxic. Through the parenthetical route, even though a little higher, toxicity is always low and that is valid both for extracts (nebulized preparation 1 g/Kg/i.p.
in rat) and harpagoside (DL50 in the mouse 1 g/Kg/i.p.) and harpagide (DL50 in the mouse 3.2 g/Kg/i.p.). The side-effects are infrequent too.

Rare are the pharmacological investigations concerning other activities of the drug and of its active principles.

Iridoids have proved inactive against P388 leukemia in the mouse [22] and against other experimental tumors [25].

In the researches we carried out [4] on the guinea-pig ileum and on the rabbit jejunum, we observed how the methanolic extract and the harpagoside cause a reduction of tone only at the highest doses employed (80 μg/ml and 6.9 μg/ml medium, respectively); while with pure harpagide, at concentrations of 0.4 μg/ml, we could note an increase in tone and at concentrations of 1.6 and 3.2 μg/ml an increase of the contractions amplitude and a diminution of tone.

The extract causes a light increase of the acetylcholine (Ach) induced contractions on the isolated guinea-pig ileum and on the rabbit jejunum, and such effect is inversely proportional to concentration.

Harpagoside, on the contrary, at concentrations corresponding to those contained in the doses of the drug's methanolic extract above tested, causes a dose-dependent reduction of the Ach-induced contractions; while harpagide at the lowest doses (0.4 and 0.8 μg/ml) increases the response to Ach and at the highest doses (1.6 and 3.2 μg/ml) the response to agonist is lowered.

Both the extract and the harpagoside produce a partial inhibition of the stimulant effect of barium chloride on the guinea-pig ileum. The cumulative dose-response curves show a noncompetitive antagonism mechanism, while, also in this case, the harpagide increases at the lower doses, and reduces the Ach stimulant effect at the higher.

These results show a complex interaction of the different active principles of the drug with cholinergic receptors.

The partial inhibition of the barium chloride induced contractions suggests an interference with the mechanisms that regulate the influx of calcium in the cells.

Also according to Van HaeLEN [22], harpagoside and harpagide have little specific inhibiting effect on the isolated guinea-pig ileum.

Harpagogenin, which, as we said, shows the greatest anti-inflammatory activity in the experiments on the isolated guinea-pig ileum carried out by Van HaeLEN [22], exercises an anti-histaminic action and inhibits the responses to partially cholinergic antagonists such as 5-hydroxytryptamine and PGE2, which indicates an inhibitory action on the cholinergic neuronal structures of the intestine; while the harpagide acts as a sensitizer of cholinergic response. Harpagoside, owing to its inhibitory effects on the responses of nicotine, seems to exercise a weak ganglioplegic action.

BelaiChe [26] reported the case of a patient suffering from chemosis (oedematous infiltration of the conjunctiva, which produces a voluminous pad around the cornea), which, by a prolonged pretreatment with 3g aqueous nebu-
lized preparation of the drug 3 times a day, after 4 months presented a considerable improvement of sight function and of eye mobility, reabsorption of chemosis, disappearance of the retro-orbital oedema and of the conjunctiva anti-inflammatory phenomena.

Tuunan and Lux report [7] that, in pharmacological researches carried out by Dr. Karl Thomae GmbH, up to a dose of 1 mg/Kg harpagoside, it had not been possible to find any toxic or stimulant action on a guinea-pig heart-lung preparation and doses of up to 50 mg/kg did not produce any diuretic or salutary action in the rat.

In researches carried out [2] in our Pharmaco-Biological Department with dried methanolic extract of the drug, whose content of harpagoside (8.5%) and of total iridoids (10.5%) had been determined, we observed how in normotensive, non anesthetized rats, at the dose of 400 mg/kg by gavage, and still more i.p., a significant reduction of the arterial blood pressure and a concomitant decrease of heart rate occurred. Pure harpagoside, under the same experimental conditions, exhibits an activity lower than that of doses of drug extracts containing corresponding amounts of harpagoside.

On the rabbit heart, isolated and perfused by Landerdoff's method, the methanolic extract caused a light decrease of heart rate with a concomitant slight positive inotropic effect at lower doses and a negative one at higher doses. Only at highest doses a diminution of coronary flow was observed.

The negative chronotropic and positive inotropic effects of harpagoside are comparatively higher with respect to those of the extract; whereas harpagide has only a slight negative chronotropic effect and a considerable negative inotropic one, which may explain the opposite dose-dependent variations of inotropism caused by the extract.

Both in vivo in the rat and on the isolated heart of rabbit, the extract demonstrates a protective action towards aconitine-induced arrhythmias, and even more towards those induced by calcium chloride and by epinephrine-chloroform. The effect of the extract is always stronger than that of corresponding doses of pure harpagoside.

Subsequently [3] we studied the effects of pretreatment by the same crude methanolic extract of the drug, or by pure harpagoside, in an experimental pattern determined by hyperkinetic ventricular arrhythmias (HVA) induced by reperfusion, which reproduces a clinical situation similar to that of human infarct after anti-thrombotic reperfusion. The problem of statistically high incidence and of the serious clinical consequences of HVA is of considerable clinical and practical importance.

The ischaemic perfusion causes conduction trouble with considerable slowing of the rhythm provoked by an atrio-ventricular progressive block. During the first minute of reperfusion with oxygenated medium it is possible to observe polypeptic extrasystoles, and in the 2nd minute ventricular tachycardia occurs. By pretreatment with 2 mg of extract (= 0.170 mg of harpagoside) in the first
minute there is no occurrence of HVA, whereas in the second minute of reperfusion only one case presented one delayed ventricular extrasystole.

Whereas with 0.170 mg of harpagoside, corresponding to that contained in the dose of the extract proved active, during the first minute of reperfusion, we could observe some ventricular extrasystoles.

The considerable protective action towards reperfusion-induced arrhythmias, and the one observed in in vivo experiments and on isolated heart of rabbit towards calcium chloride-induced arrhythmias, suggest an effect on the transmembrane ionic fluxes (slow currents of calcium).

The hypothesis of an inhibition of calcium penetration in the myocardial cells may be confirmed by the fact that both the extract and the harpagoside have a protective effect towards digitalis-induced arrhythmias, which are probably linked to the appearance of a post-potential or automatism. In fact, we observed that the drug extract (2 mg) and the harpagoside (0.170 mg) prevent the insur- gence of hyperkinetic arrhythmias induced by 100 µg of digitoxin, limiting the latter’s toxic effects to troubles of conduction and of the repolarization phase.

Consequently, it would seem that the inhibition of HVA may be due to modifications of cellular metabolism causing transmembrane ionic fluxes, as happens with verapamil.

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Harpagophytum is therefore another example of a drug, largely employed for a specific action (mainly anti-inflammatory in this case), which shows a strong action of potential therapeutic utility (antiarrhythmic action). This drug, moreover, is a good example of the assumption that sometimes the use of the extract in toto is preferable to that of an isolated principle considered responsible for the activity of the drug.

Our researches are going on in order to isolate, identify and determine other constituents existing in the drug, which undoubtedly interact in its antiarrhythmic activity besides, in order to establish the cause of the protective effect in the presence of structural metabolic alterations. In fact, this action, taking into consideration the low toxicity of the drug, might be useful in the acute phase of an ischaemic attack for the possible calcium-antagonistic effect.
REFERENCES

[23] ALBUS G., Private communication in TUNKMANN & LUX, [7].