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Over-the-counter melatonin – quality or quackery?

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Melatonin has been proclaimed as the modern panacea with alleged rejuvenating, immune-enhancing, cardiovascular and sexual function potentiating properties [1] as well as the scientifically confirmed benefits in chronobiological disorders (e.g. jet-lag) [2]. Sales in the USA now exceed those of vitamin C and because melatonin is a naturally occuring substance, thus unpatentable, it is sold over-the-counter as a "dietary supplement", without premarket approval by the Food and Drug Administration (FDA). In many other countries, such as Switzerland, it is distributed illegally.

There is limited information available regarding the quality of commercial melatonin products, a cause for concern considering the quantities consumed world wide for various indications. A prior review article [3] showed that four of six melatonin products purchased from healthfood stores contained non-identifiable impurities. Williamson et al. [4] have characterised contaminants found in melatonin preparations which are analogues of the impurities found in contaminated L-tryptophan preparations which were associated with eosinophilia-myalgia syndrome. We report the results of the analysis of 19 commercially available products (tablets, capsules, sublinguals, lozenges, or chewable tablets) from 14 manufacturers which we imported directly from the US or procured from the illegal European market. Samples were checked for quantity, quality and uniformity of weight based on the specifications of the monograph for solid oral dosage forms in the Pharmacopoea Helvetica Octava. Labelling criteria were examined for substance declaration, expiry date and batch number. Melatonin was identified and quantified using HPLC with UV detection [5] and GC/MSD. As there is concern that some melatonin products may contain hypnotics, spectra of our samples were compared with the MS and GC Data Library of Pfleger/Maurer/Weber [6] which contains 4370 spectra of drugs, poisons, pesticides and metabolites. In addition the preparations were screened to determine whether they contained impurities associated with melatonin or its synthesis.

The results are shown in the Table. All products contained melatonin and no hypnotic additives nor any of the above mentioned screened impurities were detected. However, only 9 of the 19 products analysed, conformed to the tested quality criteria. Six products had inadequate labelling; no expiry date or batch number. Two products did not conform to "uniformity of weight" and ffive samples did not contain the declared amount of melatonin (±10%). Our findings revealed no specific impurities but point to a deficit in the pharmaceutical quality of over-the-counter melatonin products. Tighter controls are needed.

Experimental

1. Gas Chromatography (GC) with Mass Selective Detection (MSD)

1.1. Apparatus

GC analysis of the melatonin solutions were performed on a Hewlett Packard Gas Chromatograph 5890 Series II with a mass selective detector 5971 A. The pre-column was a HP-Retentiongap (4.5 m × 0.25 mm, inactivated), and the main column was a GC HP-5 MS Analysis (25 m × 0.2 mm × 0.3 μm).

1.2. Identification of melatonin

The presence of melatonin was determined by comparison of the retention times and the MS with a melatonin solution of known concentration (0.6 mg/ml).

The matching factor of the spectra had to be over 90. Each sample was injected once. Samples for injection were prepared as follows: The average weight of a single tablet was determined by weighing each of 20 tablets, which were then crushed (mortar and pistil). The amount corresponding to 3 mg of melatonin was taken up into 5 ml of ethyl alcohol (volumetric

Table: Qualitative and quantitative analysis of 19 over-the-counter melatonin products

Sample	Melatonin sample (Dosage form)	Contains melatonin	Assay GC[1] (mg/tablet)	Assay HPLC[1] (mg/tablet)	Comments
A	1 mg (tablet)	yes	0.98 ± 0.12	0.94 ± 0.086	
B	3 mg (tablet)	yes	2.80 ± 0.07	2.84 ± 0.088	
C	3 mg (sublingual)	yes	2.32 ± 0.15	1.82 ± 0.181	Dosage too low
D	3 mg (tablet)	yes	2.89 ± 0.07	3.02 ± 0.090	
E	3 mg (tablet)	yes	2.97 ± 0.18	2.88 ± 0.079	
F	3 mg (tablet)	yes	3.00 ± 0.09	3.18[2]	
G1*	2 mg (tablet)	yes	1.95 ± 0.15	1.90 ± 0.047	No batch number
G2*	3 mg (tablet)	yes	3.21 ± 0.18	3.30 ± 0.328	No expiry date Two batch numbers
G3*	2.5 mg (sublingual)	yes	2.50 ± 0.53	2.23 ± 0.016	No expiry date
H	3 mg (capsule)	yes	3.52 ± 0.16	3.74 ± 0.154	No expiry date Dosage too high
I1	3 mg (chewable)	yes	2.04 ± 0.25	2.45 ± 0.177	Dosage too low Does not conform with "uniformity of weight"
I2	5 mg (chewable)	yes	5.12 ± 0.16	5.08 ± 0.279	No expiry date, no batch number Does not conform with "uniformity of weight"
K1*	3 mg (tablet)	yes	2.39 ± 0.17	2.17 ± 0.992	Dosage too low
K2*	3 mg (tablet)	yes	2.42 ± 0.16	3.11 ± 0.293	
K3*	3 mg (tablet)	yes	2.83 ± 0.20	2.84 ± 0.064	
L	3 mg (tablet)	yes	0.23 ± 0.16	0.32 ± 0.016	Dosage too low
M	3 mg (tablet)	yes	2.84 ± 0.13	2.98 ± 0.175	No expiry date
N	3 mg (chewable)	yes	2.88 ± 0.46	2.97 ± 0.241	
O	0.5 mg (tablet)	yes	0.54 ± 0.02	0.49 ± 0.010	

* more than one sample from the same manufacturer
[1] mean value of 3 samples, 95% confidence interval
[2] only two values

flask) and sonicated for 15 min. Then 2 ml of this suspension were filtered through a nylon membrane filter (0.45 μm) and 1 μl (0.6 mg/ml) of this solution was injected once on the gas chromatograph (helium, 5.6, 50 kPa; total flow: 40 ml/min; temperature: 60 °C for 3 min, 6 °C/min to 220 °C, 8 °C/min to 280 °C, 280 °C for 15 min; injector temperature 250 °C; detector temperature: 300 °C, range of MS: 40–550 [m/z]).

1.3. Chromatographic purity of melatonin in tablets

The purity of melatonin was analysed by GC. Peaks in the chromatogram were compared with all libraries of spectra. MS of the following compounds were considered as reference spectra: 6-hydroxy-melatonin, 5-methoxytryptamin, N-acetylserotonin, serotonin hydrochloride, L-tryptophan, 5-methoxy-tryptophan. The same chromatographic system as described above has been used. For further details see [7].

1.4. Quantification of melatonin in tablets

The concentration of melatonin in the tablets was determined by peak integration and comparison to a reference melatonin curve, in the presence of phenacetin as the internal standard. Melatonin solutions were prepared with ethyl alcohol, sonicated and filtrated, for further details see [7]. The same chromtographic conditions described in chapter 1.2 have been used, except the range of MS: SIM (selected ion monitoring)-mode; Ions [m/z]: 108.0, 109.0, 179.0, 137.1 for phenacetin; [m/z]: 160.1, 173.1, 145.1, 232.1 for melatonin).

2. High Performance Liquid Chromatography (HPLC)

Melatonin in oral solid state formulations was identified and quantified by HPLC using a modified version [7] of a validated method described in a proposed monograph of melatonin for the United States Pharmacopeia (USP) [5].

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Immunomodulatory activity of the saponin-rich fraction from roots of *Silene vulgaris* Garcke: initial study

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Triterpenoid saponins isolated from many medicinal plants as well as mixtures of triterpenoid saponins exhibit a broad spectrum of biological and pharmacological activities, as discussed in a number of extensive reviews [e.g. 1, 2]. Various saponin-containing plants are used in traditional medicine as biomodulators of the central nervous system facilitating both physical and mental activities (*Panax ginseng*), antiphlogistics (*Aesculus hippocastanum*), diuretics (*Solidago* sp., *Herniaria* sp.), expectorants (e.g. *Hedera helix, Primula* and *Glycyrrhiza* sp.) and extracts containing standardised mixture of saponins are registered as drugs in many countries. Moreover, several new activities of saponins have been described during the last few years, such as: antitumor, cytotoxic action [1–4], antiinflammatory and immunomodulatory activities [1, 2, 5–9].

In this paper we report initial results of studies of the *in vitro* immunomodulatory activity of saponin mixtures (the S_w fraction) obtained from roots of *Silene vulgaris* Garcke (Caryophyllaceae), a perennial herb commonly found in meadows, grassy slopes and at waysides. Structures of saponins from the S_w fraction have been described in a previous paper as bidesmosides of gypsogenin and quillaic acid [15].

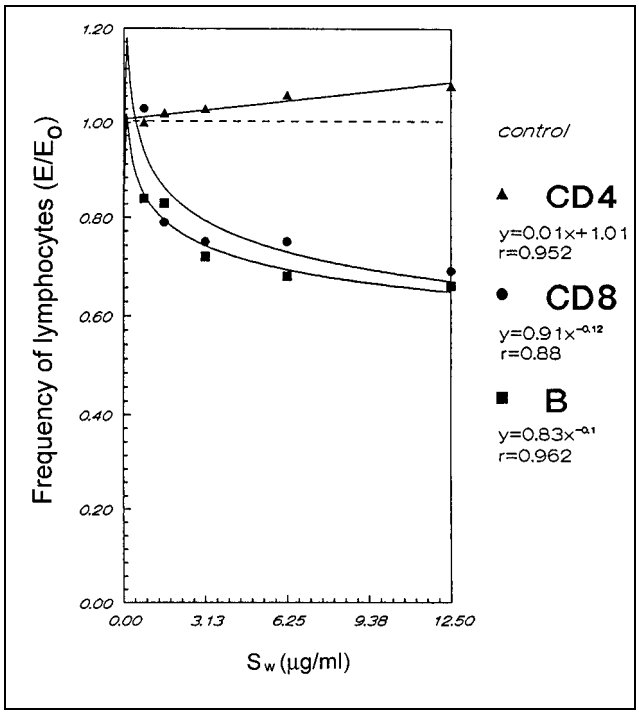

Fig.: Frequency of the main subpopulations of human lymphocytes after 72 h of culture with lectin (PHA; 10 μg/ml) and the S_w fraction. Results were compared to the relative control (without the S_w) and expressed as E/E_0 ratio. Regression equations estimate the dose-response relations