Poster Session 3 – Pharmacognosy

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The effect of liquorice extract on serum testosterone level in healthy male volunteers

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Glycyrrhiza glabra (liquorice) is widely used in the Iranian folk medicine. This herb is grown mostly in the central part of Iran. Liquorice root extract has been used traditionally for the treatment of gastro-intestinal disorders which is supposed to be related to its flavonoids. Glycyrrhizin, a triterpenoid saponin, which is a major constituent of the root extract, causes pseudocorticosteroid effects by inhibition of 11-β-hydroxy steroid dehydrogenase. Also recent reports show that this component can influence testosterone blood level by inhibiting 17-β-hydroxyl steroid dehydrogenase (Sakamoto & Wakabayashi 1988; Armanini & Palermo 1999). In order to assess this effect, a clinical trial was carried out during the year 2002 in Tehran. The study was approved by Ethics Committee of the University. Liquorice root was purchased from the local market and extracted by boiling in water. After dispersion in water, 1.3 g of the dried extract was orally administered to 20 healthy male subjects 3 h after breakfast for 10 days. Blood samples from the subjects were collected just before the trial and on days 4, 7, 10, 13 and 20. Testosterone level in the blood samples was measured using a radio immuno assay method (Brenner 1995). The average concentration of testosterone for each day was calculated and the results were analysed using repeated measures analysis of variance (table 1). The decrease in serum testosterone level were found to be significant after 10 days daily consumption of liquorice extract ($P < 0.05$).

Table 1 Average testosterone serum level in twenty subjects (nmol L^{-1})

Day	0	4	7	10	13	20
Mean	19.58	18.18	17.85	13.43	13.46	15.27
s.d.	5.69	3.93	5.99	3.84	3.31	4.92

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Microbial 11β-hydroxylation of cortexolone by *Aspergillus ochraceus*

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Microbial bioconversion has replaced complex synthetic chemical routes for the stereochemical introduction of oxygen into substrates in the large-scale commercial production of steroids because of the high cost and low yield of chemical synthesis of oxygenated steroids and high regio- and stereospecificity of microbial enzyme systems (Vitas *et al* 1994). Among microbial transformation of steroids, 11-oxygenation of Reichstein's Substance S (cortexolone) belongs to the most important ones. It's a way of producing hydrocortisone, which, apart from being a finished medicinal steroid, is also the starting material for the manufacture of several other potent steroids of prednisolone structure (Wilmanska *et al* 1992). It is a structure that causes no drug-induced salt retention at therapeutic dose levels (Ghanem & Yusef 1992). The only difference between cortexolone and hydrocortisone is the absence of 11β-hydroxy group in cortexolone. 11β-hydroxylating ability of cortexolone by *Aspergillus ochraceus* PTCC 5060 is reported for the first time.

A. ochraceus was incubated in 500 mL flasks, containing 100 mL of preculture medium at 30°C and 130 rev min^{-1} for 48 h. Ten millilitres of this preculture was used to inoculate 100 mL of the production medium. After getting to desired biomass which takes 48 h, 0.08% cortexolone was added as an ethanolic solution (final concentration of ethanol less than 1% v/v). Ninety-six hours after adding the substrate, microbial broth was extracted three times with chloroform. The solvent was then evaporated at 50°C. The concentrate was applied on TLC plates coated with silica gel GF 254 (thickness 0.5 mm). A fresh mixture of chloroform–benzene–methanol–water (90:5:4.5:0.5) was used as the mobile phase. The physicochemical properties of the major metabolite, isolated in this manner, are described below:

Melting point: 211–215°C.

Mass spectrometry (EI): MS m/z 362 (M+, $C_{21}H_{30}O_5$), 303 (M-$COCH_2OH$), 285 (M-$COCH_2OH$-H_2O), 227,163,123.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.69 (s, 1H, H_4), δ 4.49(d, 1H, $OH_{11\beta}$), 4.29 (d, 1H, H_{21}), 4.64 (d, 1H, H_{21}).

^{13}C NMR (125 MHz, $CDCl_3$) 211.6(C_{20}) , 198.9(C_3), 171.2(C_5), 122(C_4), 88.2, 67.8, 67 ($C21$,C_{17},C_{11}).

Infrared spectroscopy (IR, CCl_3): λmax 3400 (s, O-H), 1730 (s, C=O), 1650 (s,C=O).

Spectral and chromatographic data along with the melting point of this compound were identical with the authentic sample of hydrocortisone.

It has been shown in this study that *A. ochraceus* has the ability to hydroxylate cortexolone at C-11β position.

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