

## Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*

Kim Kyoung Soon<sup>○</sup>, Lee Sanghyun, Lee Yeon Sil, Jung Sang Hoon, Park Youmie, Shin Kuk Hyun, Kim Bak-Kwang

a College of Pharmacy, Seoul National University, Seoul 151-742, Korea

The anti-oxidant activities of the various fractions from the herbs of *Artemisia apiacea* were investigated. The n-hexane and n-butanol fractions were found to cause significant free radical scavenging effects on DPPH, their scavenging potencies as indicated in IC<sub>50</sub> values, being 230.1 and 183.7 g/ml, respectively. The n-butanol fraction exhibited a significant decrease in serum transaminase activities elevated by hepatic damage induced by CCl<sub>4</sub>-intoxication in rats. All fractions tested exhibited a lipid peroxidation causing a significant decrease in MDA production in TBA-reactant assay. The n-butanol fraction was the strongest in the increase in the anti-oxidant enzymes such as hepatic cytosolic superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-px) activities in CCl<sub>4</sub>-intoxicated rats. These results suggest that the herbs of *A. apiacea* possess not only the anti-oxidant, but also the activities in CCl<sub>4</sub>-intoxicated rats. Especially, the n-butanol extract was found to cause significant increases in the rat liver cytosolic SOD, catalase, GSH-px activities as well as a significant decrease in the MDA production

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### Assay of Human Chorionic Gonadotropin in Urine of Athletes and Evaluation of Assay Kit Performance

Lee Jung Ran<sup>○</sup>, Kim Myungsoo, Choi Myung Ja

Bioanalysis & Biotransformation Research Center,  
Korea Institute of Science and Technology, Seoul, Korea

Special attention has been paid to human chorionic gonadotropin (hCG) for athlete doping control because it stimulates the endogenous production of testosterone and epitestosterone without increasing the T/E ratio which is a doping indicator for the exogenous administration of testosterone. Even though the IOC banned the use of hCG, a detection method has not been decided upon since there are a variety of immunoassay kits available on the market. We evaluated three kits in terms of their performance characteristics. The assay value of the control sample varied depending on the kit, resulting in 198 mIU/ml for the MAIA kit, 172 mIU/ml for the IRMA kit, and 143 mIU/ml for the IMEIA kit. Considering the IOC inter-lab distribution of results (55 ~ 312 mIU/ml) using 27 different kits and the mean value (178±56 mIU/ml), all three kits are within the range of -15.8% ~ +5.6% of the mean value, which proves them useful for the hCG assay. The MEIA kit resulted in lower hCG values because it detects only intact hCG molecules, in contrast to the other two kits which detect intact hCG and β-hCG together. However, it is suitable for screening purposes because its advantage of being an automated system. When 123 urine samples of athletes were analyzed in 22 batches using this system, the variation of control values fell within ±10% of the mean values, and all specimens tested negative with hCG values less than the detection limit of 2 mIU/ml.

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### Analysis of Branched PEG-Conjugated Interferon Alpha by Capillary Electrophoresis and MALDI-TOF Mass Spectrometry

Na DongHee<sup>○</sup>, Park EunJi, Lee SangDeuk, Jo YoungWoo, Lee SungHee, Kim WonBae, Lee KangChoon

Drug Targeting Laboratory, College of Pharmacy, SungKyunKwan University:Research Laboratories, Dong-A Pharm. Co, Ltd

Analysis of interferon alpha (IFN) modified with high molecular weight branched PEG was performed by capillary electrophoresis (CE) and MALDI-TOF mass spectrometry (MALDI-TOF MS). IFN was modified by the reaction of amine residues with an active ester of monomethoxy polyethylene glycol at various molar ratios. As a CE method, capillary electrophoresis sodium dodecyl sulfate nongel sieving (CE-SDS-NGS) was performed using an uncoated capillary filled with a hydrophilic replaceable polymer network matrix. CE-SDS-NGS showed good

resolution between each branched PEG (MW 20 or 40 kDa)-conjugated IFN species as well as the native IFN. The total amount and distribution of PEG-IFN species were directly measured and the relative standard deviation (RSD) was below 5%. The mono-PEG-IFN conjugates were isolated by ion-exchange chromatography and also characterized by MALDI-TOF MS. CE-SDS-NGS provides a novel approach for the analysis of PEGylated IFN and shows the advantages of speed, high resolution, automation, and quantitation over SDS-PAGE.

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#### Development of Immunostrip for DDT Detection

Hong Ji Youn<sup>0</sup>, Kim Joung-Hyun, Lee Eun Ah, Choi Myung Ja

Bioanalysis & Biotransformation Research Center,  
Korea Institute of Science & Technology, Seoul 136-791, Korea  
Department of Chemistry, Seoul Women's University, Seoul. 139-774, Korea

To develop immunostrip of DDT (4,4'-dichlorodiphenyl-2,2,2-trichloroethane) and its metabolites, DDT derivatives (DDA-, DDHP-, DDCP-, DDHH-, and DDHAP-) were conjugated to carrier proteins (OVA and BSA) and three DDT derivatives (DDA, DDHP, DDCP) were conjugated to KLH for the use of coating ligand and immunogen, respectively. To screen the immunoreactivity of antibody to DDT derivatives, the coating ligand was evaluated by a competitive ELISA and DDHP-OVA was selected. Three polyclonal antibodies (DDA-1, DDHP-2, DDCP-3) were purified using protein A affinity column for the preparation of immunostrip. The immunostrip was assembled using a combination of membranes. 17 Chro cellulose membrane (sample pad), glassfiber, polyester supported nitrocellulose membrane, 17 Chro cellulose membrane (absorbance pad), in the listing order. DDT polyclonal antibodies (DDA-1, DDHP-2, DDCP-3) labeled with colloidal gold was applied on glassfiber membrane as a tracer. DDHP-OVA and second antibody (anti-rabbit IgG) were immobilized on the result line and the control line of NC membrane, respectively. As a result, the membrane strip could detect 30 ppm of DDT derivative mixture using 8.4 ug DDCP-3 antibody labeled with colloidal gold, 2.8 ug of DDHP-OVA on the result line and 150 uL of sample.

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#### Chemiluminogenic imaging for highly sensitive detection of DNA

Kai Masaaki<sup>01</sup>, Hirayama Fumiko<sup>1</sup>, Ohta Kazuko<sup>1</sup>, Kabashima Tsutomu<sup>1</sup>, Lee MyungKoo<sup>2</sup>

<sup>1</sup>School of Pharmaceutical Sciences, Nagasaki University, Bunkyo-Machi 1-14, Nagasaki 852-8521, Japan; <sup>2</sup>College of Pharmacy, Chungbuk National University, Cheongju 361-763, Republic of Korea

We have been studying sensitive non-enzymatic chemiluminescence (CL) imaging methods for the detection of DNA. For one of our methods, a unique chemical derivatization reagent, 3',4',5'-trimethoxyphenylglyoxal (TMPG) was utilized. This reagent reacted specifically with guanine bases in nucleic acids to quickly produce a chemiluminescent derivative under mild reaction conditions. TMPG gave an increasing CL intensity depending on the content of guanine base in the analyte DNA molecule, and thus a linear relationship between the intensity and guanine content at the same molar concentration of DNAs or oligonucleotides was obtained. Then we tried immobilized-hybridization assay of a target DNA sequence, telomere (TTAGGG)<sub>n</sub> binding to its cDNA on a nylon membrane. Telomere gene protects chromosome from fusion and degradation, and it becomes shortened by cell division. Thus telomere length in chromosome has been interested in the correlation with aging and tumorigenesis. The maximum CL intensity was reached around 1.0 min in the presence of DMF after the TMPG reaction, and as low as 0.5 ng of DNA was detected and visualized on the membrane. Overall, this simple and sensitive CL imaging system is expected to be very useful in biomedical analysis.

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#### Optimization of a fiber optic probe for non-invasive blood glucose monitoring

Kang Naroo<sup>0</sup>, Baek JuHyun, Woo YoungAh, Lee HyunChul, Kim HyoJin