

## Scientific Studies on Chaga, Betulinic Acid and Beta Glucans

Tom Mower Snr on Facebook on 8 January 2014 provides the following information:

### **LEARN WHY CHAGA AND PANAMA BOQUETE COFFEE TOGETHER MAKE THE WORLDS BEST TASTING HEALTH COFFEE! SISEL SCIENTIST PROUDLY AND FIRMLY BELIEVE.**

Sisel Coffee contains extremely high amounts of Chaga extract arguably providing the most incredible health support ingredients in the world and in any health coffee. This combination or the worlds #1 best tasting coffee flavor found in only the Panama Boquete Coffee Bean links the #1 health supporting ingredient in the world for a health coffee like no other.

Chaga Mushroom extracted actives are used in Sisel KAFFE and concentrated 10X's. Chaga has the highest known antioxidant levels (ORAC) of any plant, mushroom or herb found on earth.

### **CHAGA MUSHROOM (*Inonotus obliquus*) ALCOHOL (ethanol) EXTRACT SHOWED THE STRONGEST SUPEROXIDE DISMUTASE (SOD) ACTIVITY AND ANTIPROLIFERATIVE EFFECT COMPARED WITH WATER EXTRACT**

Polysaccharide, protein and phenolic content, and the 1H-NMR spectra: Hot water (50 degrees C, 70 degrees C, and 80 degrees C) and ethanol crude extracts of *I. obliquus* were investigated for their antioxidant activity with superoxide dismutase (SOD) and (1,1-diphenyl-2-picrylhydrazyl) (DPPH) radical-scavenging activity assays. Also investigated the antiproliferative effects and ability of the extracts to induce apoptosis in human colon cancer DLD-1 cells. Among the four extracts, the ethanol extract (EE) exhibited the strongest SOD-like activity and antiproliferative effect on DLD-1 cells. Reference: *Clinical Study from Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan. Hu H, Zhang Z, Lei Z, Yang Y, Sugiura N. 2009 Jan; 107(1):42-8.*

### **SOLVENT - ETHANOL (Alcohol) ALLOWED EXTRACTION OF TRITERPENOID WITH A HIGHEST CONTENT**

A simple procedure is described for the simultaneous extraction and determination of betulin and betulinic acid in white birch. The extraction was checked using different solvents: dichloromethane, ethyl acetate, acetone, chloroform, methanol and ethanol. It was found that ethanol was a good extraction solvent that allowed extraction of triterpenoid with a highest content. Reference: *Clinical Study from Department of Chemistry, Zhejiang University, Hangzhou 310027, PR China. Zhao G, Yan W, Cao D. PMID: 17084057, PubMed - as supplied by publisher.*

### **ANTI-AGING**

Positive effect on localisation and bringing out of 90Sr from organism were gained in the series of experiments on the rats Wistar after using per orum alcohol extract, water suspension and water extract of *Inonotus obliquus* (Chaga). Everyday per orum infusion of *Inonotus* water extract into the BALB-line mice under conditions of a prolonged (during two months) external total gamma-irradiation with power dose 0.025 sGr/min has a positive effect on increase an average life duration, are slow down the development of leycopenia, hold lipid peroxide oxidation in the blood and in critic tissues and the R-proteins in blood serum on the level, close to the intact control; appearance, activity and behaviour of the animals were the same. Reference: *Clinical Study from Institute of Animals and Plants Ecology, Ural Branch Russian Academy of Sciences, Ekaterinburg, 620144 Russia. Rasina LN. 2002 Jul-Aug;42(4):399-403.*

### **INDUCES APOPTOSIS (PROGRAMMED CELL DEATH) IN THE TUMORS**

Betulinic acid: In 1998 there was a study in Poland that demonstrated Chaga's inhibiting effects on tumor growth. Noda and colleagues found that betulin seems to work highly selectively on tumor cells because the interior pH of tumor tissues is generally lower than that of normal tissues, and betulinic acid is only active at those lower levels. They found that once inside the cells, betulinic acid induces apoptosis (programmed cell death) in the tumors. Reference: *Tillotsen, Alan. Chaga Mushrooms (*Inonotus obliquus*) and Wikipedia.*

### **ANTI-CANCER AND IMMUNO-STIMULATING**

Endo-polysaccharide: *Inonotus obliquus* BELYU1102 was selected from 12 different strains of *Inonotus* as a producer of immuno-stimulating polysaccharide. However, indirect anti-cancer effects via immuno-stimulation were observed. The mycelial endo-polysaccharide of *I. obliquus* is a candidate for use as an immune response modifier. Submerged mycelial cultures are advantageous for industrial production of polysaccharides. Reference: *Clinical Study from Department of Biotechnology, College of Engineering, Yonsei University, Shinchon-dong, Seodaemoon-gu, Seoul 120-749, South Korea. Kim YO, Han SB, Lee HW, Ahn HJ, Yoon YD, Jung JK, Kim HM, Shin CS. 2005 Sep 23;77(19):2438-56.*

**ANTI-TUMOR**

Polysaccharides - Identification of *Inonotus obliquus* and analysis of antioxidation and antitumor activities of polysaccharides. The results indicated that *I. obliquus* polysaccharides exhibit high antitumor and antioxidation effects. Reference: *Clinical Study from School of Life Science, Liaoning University, Chongshan Middle Road 66, Shenyang, 110036, People's Republic of China. Song Y, Hui J, Kou W, Xin R, Jia F, Wang N, Hu F, Zhang H, Liu H. 2008 Nov; 57(5):454-62. Epub 2008 Sep 16.*

**ANTI-TUMOR**

Lanostane-type triterpenoids from the sclerotia of *Inonotus obliquus*: Their structures were determined to be lanosta-8,23E-diene-3beta,22R,25-triol (1) and lanosta-7:9(11),23E-triene-3beta,22R,25-triol (2) by spectral data. Was found to exhibit the potent anti-tumor promoting activity in the in vivo carcinogenesis test. Reference: *Clinical Study from Department of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan. Taji S, Yamada T, Wada S, Tokuda H, Sakuma K, Tanaka R. 2008 Nov; 43(11):2373-9. Epub 2008 Feb 8.*

**ANTI-TUMOR**

Polysaccharides such as hetero-beta-glucans and their protein complexes (e.g., xyloglucans and acidic beta-glucan-containing uronic acid), as well as dietary fibers, lectins, and terpenoids have been isolated from medicinal mushrooms. In Japan, Russia, China, and the U.S.A. several different polysaccharide antitumor agents have been developed from the fruiting body, mycelia, and culture medium of various medicinal mushrooms (*Inonotus obliquus*, *Lentinus edodes*, *Ganoderma lucidum*, *Schizophyllum commune*, *Trametes versicolor*, and *Flammulina velutipes*). Both cellular components and secondary metabolites of a large number of mushrooms have been shown to effect the immune system of the host and therefore could be used to treat a variety of disease states. Reference: *Clinical Study from International Centre for Cryptogamic Plants and Fungi, Institute of Evolution, University of Haifa, Israel. Wasser SP, Weis AL. 1999;19(1):65-96.*

**MALIGNANT TUMORS, DIABETES, CARDIOVASKULAR DISEASE AND AIDS**

Abstract *Inonotus obliquus* has high nutritional and medicinal value, especially in treating malignant tumors, diabetes, cardiovascular disease and AIDS, attracting significant attention from scholars in recent years. In this paper, the biological characteristics, chemical composition and pharmacologic effects of *Inonotus obliquus* were summarized. And the applications in medicine and food were introduced. Future research on *Inonotus obliquus* was also discussed in order to make *Inonotus obliquus* obtain effective exploitation and satisfy people's demands.

**Key Words** *Inonotus obliquus* - biological characteristics - chemical composition - pharmacologic effect  
*Journal Chinese Journal of Integrative Medicine. Issue Volume 15, Number 2 / April, 2009.*

*Publisher Chinese Association of Traditional and Western Medicine, China Academy of Chinese Medical Sciences. Studies of*

(1) *Deptment of Pathology Jilin Medical College, Jilin, 132013, China*

(2) *Deptment of Pharmacy, Jilin Medical College, Jilin, 132013, Jilin, China*

(3) *Deptment of Biochemistry, Jilin Medical College, Jilin, 132013, Jilin, China*

(4) *Deptment of Pathology and Forensic Medicine, Medical College of Yanbian University, Yanji, 133000, Jilin, China.*

**INHIBIT CANCER**

Isolation and characterization of a novel platelet aggregation inhibitory peptide (tripeptide) from the medicinal mushroom, *Inonotus obliquus*. This study describes the extraction and characterization of a platelet aggregation inhibitory peptide from *Inonotus obliquus*. Ethanol extract from *I. obliquus* ASI 74006 mycelia showed the highest platelet aggregation inhibitory activity (81.2%). The maximum platelet aggregation inhibitory activity was found when the mycelia of *I. obliquus* ASI 74006 was extracted with ethanol at 80 degrees C for 12 h. The platelet aggregation inhibitor was purified by systematic solvent fractionation, ultrafiltration, Sephadex G-10 column chromatography, and reverse-phase HPLC. The purified platelet aggregation inhibitor is a novel tripeptide with a molecular mass of 365 Da, having a sequence of Trp-Gly-Cys. The purified platelet aggregation inhibitor also showed high platelet aggregation inhibitory activity in Institute of Cancer Research (ICR) mice. Reference: *Clinical Study from Department of Genetic Engineering and Bio-Medicinal Resources Research Center, Paichai University, Daejeon 302-735, Republic of Korea. Hyun KW, Jeong SC, Lee DH, Park JS, Lee JS. 2006 Jun; 27(6):1173-8. Epub 2005 Nov 11.*

**SIGNIFICANTLY INHIBITED THE GROWTH OF TUMOR MASS**

This study showed that the extract of *Inonotus obliquus* mushroom exhibited a potential anticancer activity against B16-F10 melanoma cells in vitro and in vivo through the inhibition of proliferation and induction of differentiation and apoptosis of cancer cells. Furthermore, the anti-tumor effect of *Inonotus obliquus* extract was assessed in vivo in Balb/c mice. Intraperitoneal administration of *Inonotus obliquus* extract significantly inhibited the growth of tumor mass in B16-F10 cells implanted mice, resulting in a 3-fold (relative to the positive control,

(\*) $p < 0.05$ ) inhibit at dose of 20mg/kg/day for 10 days. Reference: *Clinical Study from VestibuloCochlear Research Center and Department of Microbiology, Wonkwang University, #344-2, Shinyoung-dong, Iksan, Jeonbuk 570-749, South Korea. Youn MJ, Kim JK, Park SY, Kim Y, Park C, Kim ES, Park KI, So HS, Park R. 2009 Jan 21;121(2):221-8. Epub 2008 Oct 25.*

#### **INHIBITED GROWTH OF TUMOUR CELLS**

The effect of aqueous extracts from *Inonotus obliquus* on the mitotic index and enzyme activities. The effect of aqueous extract from *Inonotus obliquus* on the mitotic index and some enzyme activities in human cervical uteri tumour cells HeLa S3 in vitro was evaluated. It was concluded that *Inonotus* extract inhibited the growth of tumour cells. The fungal extract caused a decrease of the cell protein amount and mitotic index value. Moreover, this extract disturbed metabolism in cells caused decreased activity of LDH, HBDH, MDH, GGT and increasing the activity of catalase. Reference: **Clinical Study from Department of Human Genetics, Medical Academy, Lublin, Poland. Rzymowska J. 1998 Jan; 137(1):13-5.**

#### **INHIBITED CANCER CELLS GROWTH**

Antimitotic activity of aqueous extracts of *Inonotus obliquus*. The cytotoxic effect of two aqueous extracts of *Inonotus obliquus* on human cervical uteri cancer cells (Hela S3) in vitro was evaluated. It was concluded that *Inonotus* extracts at a concentration of 10 micrograms/ml to 2000 micrograms/ml inhibited cancer cells growth. Reference: **Clinical Study from Department of Pharmacognosy, Silesian Medical Academy, Sosnowiec, Poland. Burczyk J, Gawron A, Slotwinska M, Smietana B, Terminska K. 1996 May; 135(5):306-9.**

#### **INHIBITS OXIDATIVE DNA DAMAGE**

Chaga mushroom extract inhibits oxidative DNA damage in human lymphocytes as assessed by comet assay. We evaluated the effect of aqueous Chaga mushroom extracts for their potential for protecting against oxidative damage to DNA in human lymphocytes. Cells were pretreated with various concentrations (10, 50, 100 and 500 microg/mL) of the extract for 1 h at 37 degrees C. Cells were then treated with 100 microM of H<sub>2</sub>O<sub>2</sub> for 5 min as an oxidative stress. Evaluation of oxidative damage was performed using single-cell gel electrophoresis for DNA fragmentation (Comet assay). Using image analysis, the degree of DNA damage was evaluated as the DNA tail moment. Cells pretreated with Chaga extract showed over 40% reduction in DNA fragmentation compared with the positive control (100 micromol H<sub>2</sub>O<sub>2</sub> treatment). Thus, Chaga mushroom treatment affords cellular protection against endogenous DNA damage produced by H<sub>2</sub>O<sub>2</sub>. Reference: *Clinical Study from Department of Medical Nutrition, Kyunghee University, 1 Hoekidong, Dongdaemoonku, Seoul 130-701, South Korea. Park YK, Lee HB, Jeon EJ, Jung HS, Kang MH. 2004;21(1-4):109-12*

#### **STRONG ANTIOXIDANT AND HIGHEST ANTIMUTAGENIC ACTIVITY**

3beta-hydroxy-lanosta-8, 24-dien-21-al and inotodiol - Antimutagenic effects and antioxidative activities of subfractions of Chaga mushroom had a strong antioxidant activity against DPPH radicals and were identified by MS, (1)H NMR and (13)C NMR analyses as 3beta-hydroxy-lanosta-8, 24-dien-21-al and inotodiol, respectively. The ethyl acetate extract was separated by vacuum chromatography into three fractions, and the fraction bearing the highest antimutagenic activity was subsequently separated into four fractions by reversed phase (ODS-C(18)) column chromatography. Study showed that the 3beta-hydroxy-lanosta-8, 24-dien-21-al and inotodiol components of *Inonotus obliquus* bear antimutagenic and antioxidative activities. Reference: *Clinical Study from Department of Food Science and Biotechnology, School of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Republic of Korea. Ham SS, Kim SH, Moon SY, Chung MJ, Cui CB, Han EK, Chung CK, Choe M. 2009 Jan 10;672(1):55-9. Epub 2008 Oct 17.*

#### **ANTIOXIDANT**

Phenolic compounds produced by sclerotia of *I. obliquus* in the control medium consisted of melanins, flavonoids, polyphenols and small phenolics - Phenolic compounds produced by sclerotia of *Inonotus obliquus* are the active constituents responsible for antioxidant activities. In this study, *I. obliquus* was grown in a continuously stirred tank reactor (CSTR) to explore how it accumulates phenolic Their accumulation was affected by adding H<sub>2</sub>O<sub>2</sub> to the medium, where increased levels of total intracellular phenols (TIP) and melanins, but less total extracellular phenol (TEP) occurred. Simultaneous exposure to H<sub>2</sub>O<sub>2</sub> and arbutin resulted in a further increase in TIP production and reduced accumulation of TEP. Both TIP and TEP obtained at different culture ages and media were active in scavenging Superoxide anion and DPPH radicals. Therefore, production of phenolic compounds by *I. obliquus* is enhanced by imposing oxidative stress, which might allow it to be exploited as a reliable source of pharmaceutically important phenolic compounds. Reference: *Clinical Study from Key Laboratory for Biotechnology on Medicinal Plants of Jiangsu Province, Xuzhou Normal University, Xuzhou, China. [yzyw@xznu.edu.cn](mailto:yzyw@xznu.edu.cn). Zheng W, Zhang M, Zhao Y, Wang Y, Miao K, Wei Z. 2009 Feb;100(3):1327-35. Epub 2008 Sep 27.*

**CHAGA MUSHROOM HAD THE STRONGEST ANTIOXIDANT ACTIVITY** (both Superoxide and Hydroxyl radicals scavenging activities) compared with other medicinal fungi)

Small phenolics as follows- 4-hydroxy-3,5-dimethoxy benzoic acid 2-hydroxy-1-hydroxymethyl ethyl ester (BAEE), protocatechic acid (PCA), caffeic acid (CA), 3,4-dihydroxybenzaldehyde (DB), 2,5-dihydroxyterephthalic acid (DTA), syringic acid (SA) and 3,4-dihydroxybenzalacetone (DBL): In the present study, the antioxidant activity of Chaga was precisely compared with those of other medicinal fungi (*Agaricus blazei* Mycelia, *Ganoderma lucidum* and *Phellinus linteus*) showing Chaga had the strongest antioxidant activity among fungi examined in terms of both superoxide and hydroxyl radicals scavenging activities. Reference: *Clinical Study from Niigata University of Pharmacy and Applied Life Science, Niigata, Japan. Nakajima Y, Sato Y, Konishi T. 2007 Aug;55(8):1222-6.*

## **STRONG ANTIOXIDANT EFFECT**

### **CHAGA MUSHROOM ALCOHOL (Methanolic) EXTRACT SHOWED SIGNIFICANT SCAVENGING ACTIVITY AGAINST THE ABTS RADICAL CATION AND DPPH RADICAL**

New antioxidant polyphenols from the medicinal mushroom *Inonotus obliquus*. The fruiting body of *Inonotus obliquus*, a medicinal mushroom called Chaga, has been used as a traditional medicine for cancer treatment. Although this mushroom has been known to exhibit potent antioxidant activity, the mechanisms responsible for this activity remain unknown. In our investigation for free radical scavengers from the methanolic extract of this mushroom, inonoblins A (1), B (2), and C (3) were isolated along with the known compounds, phelligridins D (4), E (5), and G (6). Their structures were established by extensive spectroscopic analyses. These compounds exhibited significant scavenging activity against the ABTS radical cation and DPPH radical. Reference: *Clinical Study from Functional Metabolites Research Center, KRIBB, 111 Gwahangno, Yuseong-gu, Daejeon 305-806, Republic of Korea. Lee IK, Kim YS, Jang YW, Jung JY, Yun BS. 2007 Dec 15;17(24):6678-81. Epub 2007 Oct 25.*

### **STRONG ANTIOXIDANT AND GENOPROTECTIVE EFFECTS**

Melanin complex of the fungus *Inonotus obliquus*: The fungus *Inonotus obliquus* (Pers.) Pil. synthesised high-molecular-weight phenolic pigments that were assigned to melanins according to their physicochemical properties. It was showed that copper ions (0.008%), pyrocatechol (1.0 mM), and tyrosine (20.0 mM) stimulated the melanogenesis. The production of melanin correlated with the synthesis of o- and p-diphenoloxidases. The fungal melanin had strong antioxidant and genoprotective effects. Reference: *Clinical Study from Institute of Microbiology, National Academy of Sciences of Belarus, Minsk, Belarus. Babitskaia VG, Shcherba VV, Ikonnikova NV. 2000 Jul-Aug; 36(4):439-44.*

## **STRONG ANTIOXIDANT EFFECT**

Triterpenoids and steroids, Antioxidant effect of *Inonotus obliquus*. The purpose of this study was to elucidate the antioxidant capacities of *Inonotus obliquus*. Four extracts from the fungus were evaluated for antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH), Superoxide, and Peroxyl radicals. The polyphenolic extract had a strong antioxidant activity, and the extract containing triterpenoids and steroids presented a relatively strong antioxidant effect. The polysaccharide extract, however, was inactive. The protective effects of these four extracts were assessed against hydrogen peroxide-induced oxidative stress using a human keratinocyte cell line, HaCaT. Our results show that the polyphenolic extract protected these cells against hydrogen peroxide-induced oxidative stress, while the polysaccharide, triterpenoid and steroid extracts were ineffective. Additionally, the remnant polyphenolic and low molecular weight polysaccharide extracts showed a weakly protective effect at a concentration of 50 microg/ml. Our results indicate that *Inonotus obliquus* has the capacity to scavenge free radicals at concentrations higher than 5 microg/ml and that the polyphenolic extract can protect cells against oxidative stress. Reference: *Clinical Study from Department of Dermatology, Seoul National University, Bundang Hospital, 300 Gumi-Dong, Bundang-Gu, Seongnam-Si, Kyungki-Do 463-707, Republic of Korea. Cui Y, Kim DS, Park KC. 2005 Jan 4;96(1-2):79-85.*

## **ANALGESIC, ANTI-INFLAMMATORY**

Lipopolysaccharide (LPS): This study was designed to investigate the anti-inflammatory and anti-nociceptive effects of the methanol extract from *Inonotus obliquus* (MEIO) in vivo and in vitro. MEIO (100 or 200 mg/(kg day), p.o.) reduced acute paw edema induced by carrageenin in rats, and showed analgesic activity, as determined by an acetic acid-induced abdominal constriction test and a hot plate test in mice. To reveal the mechanism of the anti-inflammatory effect of MEIO, we examined its effect on lipopolysaccharide (LPS)-induced responses in a murine macrophage cell line RAW 264.7. MEIO was found to significantly inhibit the productions of nitric oxide (NO), prostaglandin E2 (PGE2) and tumor necrosis factor-alpha (TNF-alpha) in LPS-stimulated RAW 264.7 macrophages. Consistent with these observations, MEIO potently inhibited the protein and mRNA expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Reference: *Clinical Study from Department of Biochemistry, College of Pharmacy, Kyung-Hee University, Dongdaemun-Ku, Hoegi-Dong, Seoul 130-701, South Korea. Park YM, Won JH, Kim YH, Choi JW, Park HJ, Lee KT. 2005 Oct 3;101(1-3):120-8.*

## **USEFUL FOR CLINICAL APPLICATIONS IN THE MANAGEMENT OF INFLAMMATORY DISEASES AND MEDICAL FOOD**

Tested the ability of the *I. obliquus* extract to inhibit the inflammatory cascades in lipopolysaccharide (LPS)-induced RAW 264.7 macrophage cells. RESULTS: Have useful clinical applications in the management of inflammatory diseases and may also be useful as a medicinal food. Reference: *Clinical Study from Department of Biochemistry and Research Institute of Life Sciences, Kangwon National University, Republic of Korea. Kim HG, Yoon DH, Kim CH, Shrestha B, Chang WC, Lim SY, Lee WH, Han SG, Lee JO, Lim MH, Kim GY, Choi S, Song WO, Sung JM, Hwang KC, Kim TW. 2007 Mar;10(1):80-9*

## **HEART / CARDIAC**

Effects of bioglycans from birch fungus *Inonotus obliquus* on the electrical activity of cells from the cardiac venous sinus of the frog. Abstract Bioglycans isolated from Chaga in a concentration of 0.0001% reduced frequency of action potential in venous sinus cells of frog heart during the first 15–30 min of exposure, then this parameter increased by 10% per hour over 3.5 h, and was  $41 \pm 3 \text{ min}^{-1}$  from the 4th to the 20th hour of incubation. The frequency of action potentials in heart strips in the Chaga extract was 40% higher than in Ringer's solution. The effect of Chaga bioglycan is probably associated with adsorption on myocyte membranes. Binding of  $\text{Ca}^{2+}$  to bioglycans observed during the first 30 min inhibited efflux of intracellular  $\text{Ca}^{2+}$ . Reference: *Laboratory of Physiological Researches, Department of Biotechnology and Molecular Immunology, Institute of Physiology, Komi Research Center, Ural Division of the Russian Academy of Sciences, Syktyvkar Biull Eksp Biol Med. 1999 Sep;128(9):264-6. Golovko VA. PMID: 10560041 [PubMed - indexed for MEDLINE]*

## **LEUKEMIA**

Inotodiol, a lanostane triterpenoid: Antitumor effect of *Inonotus obliquus* Pilat, the antiproliferative effect of lanostane triterpenoids from a chloroform extract of *I. obliquus* sclerotia against mouse leukemia P388 cells was assessed. examined, only inotodiol inhibited P388 cell proliferation. DNA fragmentation and caspase-3/7 activation were observed in the P388 cells treated with inotodiol (30 microM). A caspase-3 inhibitor, DEVD-CHO (N-acetyl-Asp-Glu-Val-Asp-al, 100 microM) partially inhibited the DNA fragmentation and growth-inhibition induced by inotodiol. The intraperitoneal administration of 10 mg/kg inotodiol prolonged the number of survival days of the P388-bearing mice. CONCLUSION: Inotodiol inhibits cell proliferation through apoptosis induction by activating caspase-3. Reference: *Clinical Study from Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Hokuriku University, Nomura M, Takahashi T, Uesugi A, Tanaka R, Kobayashi S. Kanazawa, Ishikawa 920-1181, Japan. 2008 Sep-Oct;28(5A):2691-6.*

## **LEUKEMIA**

Studies on the digestion by the DNA ase and RNA ase of the blood and bone marrow cells were performed by Prof. J. Aleksandrowicz et al. (Blicharski, Perkowska, Spirer) in the IIIrd Internal Diseases Clinic A.M. in Cracow, Poland. The results were published in 1954. In 1957, published the results of study of digestion of blood and bone marrow smears by urine of normal people and of patients with chronic granulocytic leukemia. Substances of vegetable origin (*Inonotus obliquus*) pass specific digestive properties for some cell nuclei. Recently we isolated a substance from fungus *Inonotus obliquus* which selectively digests the nuclei of cells of chronic granulocytic leukemia, but does not change normal cells. The difference can also be observed on some blood and bone marrow smear slides. The mechanism of this is not known. It seems that chemical changes caused by DNA as are related to the presence of leukemic isomer of desoxyribonucleic acid. Reference: *DIGESTION OF CELL NUCLEI BY ENZYMES. Dr. Janina Krauss-Zaki, Illiw CLINIC OF INTERNAL DISEASES, CRACOW, POLAND*

## **SIGNIFICANT ANTI-HYPERGLYCEMIC AND ANTI-LIPIDPEROXIDATIVE EFFECT ON NORMAL AND ALLOXAN-DIABETES**

This study showed that the DMCB of *Inonotus obliquus* possesses significant antihyperglycemic, antilipidperoxidative and antioxidant effects in alloxan-induced diabetic mice. Reference: *Clinical Study from Lab of Pharmaceutical Engineering, School of Medicine and Pharmaceutics, Jiangnan University, Wuxi, PR China. Sun JE, Ao ZH, Lu ZM, Xu HY, Zhang XM, Dou WF, Xu ZH. 2008 Jun 19;118(1):7-13. Epub 2008 Mar 4.*

## **BETULINIC ACID STUDIES**

### **INHIBITS PROSTATE CANCER GROWTH**

Betulinic acid inhibits prostate cancer growth through inhibition of specificity protein transcription factors. Betulinic acid is a pentacyclic triterpene natural product initially identified as a melanoma-specific cytotoxic agent that exhibits low toxicity in animal models. Subsequent studies show that betulinic acid induces apoptosis and antiangiogenic responses in tumors derived from multiple tissues; however, the underlying mechanism of action is unknown. Using LNCaP prostate cancer cells as a model, we now show that betulinic acid decreases expression of vascular endothelial growth (VEGF) and the antiapoptotic protein surviving. The mechanism of these betulinic acid-induced antiangiogenic and proapoptotic responses in both LNCaP cells and in tumors is due

to activation of selective proteasome-dependent degradation of the transcription factors specificity protein 1 (Sp1), Sp3, and Sp4, which regulate VEGF and surviving expression. Thus, betulinic acid acts as a novel anticancer agent through targeted degradation of Sp proteins that are highly overexpressed in tumors.

Reference: *Clinical Study from Institute of Biosciences and Technology, Texas A&M University Health Science Center, Houston, Texas 77843-4466, USA. Chintharlapalli S, Papineni S, Ramaiah SK, Safe S.*

### **AS A CHEMOSENSITIZER MAY BE A NEW STRATEGY TO ENHANCE THE EFFICACY OF CHEMOTHERAPY**

Effect of betulinic acid on anticancer drug-resistant colon cancer cells. Primary or acquired resistance of tumours to established chemotherapeutic regimens is a major concern in oncology. Attempts to improve the survival of cancer patients largely depend on strategies to prevent tumour cell resistance. 5-Fluorouracil (5-FU)-based chemotherapy with a combination of other drugs such as irinotecan (IRT) and oxaliplatin (OXT) has been reported to be effective, even though an optimal regimen has yet to be defined due to the relatively high toxicity of the procedure. The aim of this study was to examine the effect of betulinic acid (BetA) as a chemosensitizer for anticancer drug treatment in chemoresistant colon cancer cell lines. A chemoresistant cell line to 5-fluorouracil (SNU-C5/5FU-R), irinotecan (SNU-C5/IRT-R) and oxaliplatin (SNU-C5/OXT-R) treatment were derived from the wild-type colon adenocarcinoma cell line (SNU-C5/WT). The effect of BetA or a combination of anticancer drugs and BetA on the multidrug resistance-related genes, caspases, Bcl-2, Bad and cell death in the SNU-C5/WT and SNU-C5/R cell lines was analysed. BetA alone was an effective chemotherapeutic drug for the SNU-C5/WT, SNU-C5/5FU-R and SNU-C5/OXT-R cells. The combination of BetA with IRT or OXT was effective against SNU-C5/5FU-R cells, and the combination of BetA with 5-fluorouracil, IRT or OXT was effective against SNU-C5/OXT-R cells. BetA induced cancer cell death by apoptosis through the mitochondrial pathway. These findings indicate that the use of BetA as a chemosensitizer may be a new strategy to enhance the efficacy of chemotherapy.

Reference: *Clinical Study from Department of Pharmacology, College of Medicine, Chosun University, 488 Seosuk-dong, Dong-gu, Gwangju 501-140, Korea. Jung GR, Kim KJ, Choi CH, Lee TB, Han SI, Han HK, Lim SC.*

### **CHEMOTHERAPEUTIC AGENT FOR THE MOST PREVALENT HUMAN CANCER TYPES**

Broad in vitro efficacy of plant-derived betulinic acid against cell lines derived from the most prevalent human cancer types. Betulinic acid (BA) is a widely available plant-derived triterpene with reported activity against cancer cells of neuroectodermal origin and leukaemias. Treatment with BA was shown to protect mice against transplanted human melanoma and led to tumor regression. In contrast, cells from healthy tissues were resistant to BA and toxic side-effects in animals were absent. These findings have raised interest in the chemotherapeutic anti-cancer potential of BA. A comprehensive assessment of the efficacy of BA against the clinically most important cancer types is currently lacking. Therefore, we tested the in vitro sensitivity of broad cell line panels derived from lung, colorectal, breast, prostate and cervical cancer, which are the prevalent cancer types characterized with highest mortalities in woman and men. Multiple assays were used in order to allow a reliable assessment of anti-cancer efficacy of BA. After 48 h of treatment with BA, cell viability as assessed with MTT and cell death as measured with propidium iodide exclusion showed clear differences in sensitivity between cell lines. However, in all cell lines tested colony formation was completely halted at remarkably equal BA concentrations that are likely attainable in vivo. Our results substantiate the possible application of BA as a chemotherapeutic agent for the most prevalent human cancer types. Reference: *Clinical Study from Laboratory for Experimental Oncology and Radiobiology, Center for Experimental and Molecular Medicine, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Kessler JH, Mullauer FB, de Roo GM, Medema JP.*

### **INHIBIT HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)**

Betulinic Acid Derivatives That Target gp120 and Inhibit Multiple Genetic Subtypes of Human Immunodeficiency Virus Type 1. Betulinic acid (BA) derivatives can inhibit human immunodeficiency virus type 1 (HIV-1) entry or maturation depending on side chain modifications. While BA derivatives with antimaturation activity have attracted considerable interest, the anti-HIV-1 profile and molecular mechanism of BA derivatives with anti-HIV-1 entry activity (termed BA entry inhibitors) have not been well defined. In this study, we have found that two BA entry inhibitors, IC9564 and A43D, exhibited a broad spectrum of anti-HIV-1 activity. Both compounds inhibited multiple strains of HIV-1 from clades A, B, and C at submicromolar concentrations. Clade C viruses were more sensitive to the compounds than clade A and B viruses. Interestingly, IC9564 at subinhibitory concentrations could alter the antifusion activities of other entry inhibitors. IC9564 was especially potent in increasing the sensitivity of HIV-1(YU2) Env-mediated membrane fusion to the CCR5 inhibitor TAK-779. Results from this study suggest that the V3 loop of gp120 is a critical determinant for the anti-HIV-1 activity of IC9564. IC9564 escape viruses contained mutations near the tip of the V3 loop. Moreover, IC9564 could compete with the binding of V3 monoclonal antibodies 447-52D and 39F. IC9564 also competed with the binding of gp120/CD4 complexes to chemokine receptors. In summary, these results suggest that BA entry inhibitors can potentially inhibit a broad spectrum of primary HIV-1 isolates by targeting the V3 loop of gp120. Reference: *Clinical Study from Duke University Medical Center, Box 2826, Durham, NC 27710-2926. [chc@duke.edu](mailto:chc@duke.edu). Lai W, Huang L, Ho P, Li Z, Montefiori D, Chen CH. <http://aac.asm.org/content/52/1/128.full.pdf>*

### HAVE THE POTENTIAL TO BECOME CLINICALLY USEFUL FOR AIDS THERAPY

Synthesis and anti-HIV activity of bi-functional betulinic acid derivatives. Betulinic acid (BA) derivatives with a side chain at C-3 can inhibit HIV-1 maturation. On the other hand, BA derivatives with a side chain at C-28 can block HIV-1 entry. In order to combine the anti-maturation and anti-entry activities in a single molecule, new bi-functional BA derivatives containing side chains at C-3 and C-28 have been synthesized. The most potent compound ([N-[3beta-O-(3',3'-dimethylsuccinyl)-lup-20(29)-en-28-oyl]-7-aminoheptyl]-carbamoylethyl)methane inhibited HIV-1 at an EC<sub>50</sub> of 0.0026 microM and was at least 20 times more potent than either the anti-maturation lead compound DSB or the anti-entry lead compound IC9564. This bi-functional BA derivative was active against both HIV entry and maturation. These results suggest that bi-functional BA derivatives with dual mechanisms of action have the potential to become clinically useful for AIDS therapy. Reference: *Clinical Study from Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA. Huang L, Ho P, Lee KH, Chen CH.* <http://aac.asm.org/content/52/1/128.full.pdf>

### ANTI-HIV AGENT

The discovery of a class of novel HIV-1 maturation inhibitors and their potential in the therapy of HIV. Although HIV infection is now primarily treated with reverse transcriptase and protease inhibitors, HIV therapy must look toward new drugs with novel mechanism(s) of action to both improve efficacy and address the growing problem of drug resistance. Using natural products as a source of biologically active compounds, our drug discovery program has successfully optimised the natural product betulinic acid to the first-in-class maturation inhibitor 3-O-(3',3'-dimethylsuccinyl)-betulinic acid (DSB). DSB's unique viral target has been identified as a late step in Gag processing. Specifically, it inhibits the cleavage of the capsid precursor, CA-SP1, resulting in a block to the processing of mature capsid protein leading to a defect in viral core condensation. DSB represents a unique class of anti-HIV compounds that inhibit virus maturation and provide additional opportunities for anti-HIV therapy. In this review, the discovery of DSB and its mode of action are summarised. Anti-AIDS Agents part 64. For part 63 in the series, see YU D, LEE KH: Recent progress and prospects on plant-derived anti-HIV agents and analogs. In: Medicinal Chemistry of Bioactive Natural Products. XT Liang, WS Fang (Eds), Wiley, New York, USA (2005) (In Press). Reference: *Clinical Study from Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA. Yu D, Wild CT, Martin DE, Morris-Natschke SL, Chen CH, Allaway GP, Lee KH.* [http://www.academia.edu/5104931/Plant-derived\\_natural\\_product\\_research\\_aimed\\_at\\_new\\_drug\\_discovery](http://www.academia.edu/5104931/Plant-derived_natural_product_research_aimed_at_new_drug_discovery)

### ANTIFUNGAL

Antifungal activity of cysteine, its effect on C-21 oxygenated lanosterol derivatives and other lipids in *Inonotus obliquus*, in vitro. Reference: *Clinical Study from Department of Pharmacy, University of Helsinki, Pharmacognosy Division, Box 15, 00014 Fabianinkatu 35, Finland. K. Kahlos1 and V. H. Tikka1. Received: 27 December 1993 Revised: 28 March 1994 Accepted: 18 April 1994*

### ANTIVIRAL AGENT

Highlights in the development of new antiviral agents. The potential of a large variety of new compounds and new strategies for the treatment of virtually all major virus infections has been addressed. This includes, for the treatment of HIV infections, virus adsorption inhibitors (cosalane derivatives, cyanovirin-N), co-receptor antagonists (TAK-779, AMD3100), viral fusion inhibitors (pentafuside T-20, betulinic acid derivatives), viral uncoating inhibitors (azodicarbonamide), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs: emtricitabine, amdoxovir, dOTC, d4TMP prodrugs, tenofovir disoproxil fumarate), non-nucleoside reverse transcriptase inhibitors (NNRTIs: thiocarboxanilide UC-781, capravirine, SJ-3366, DPC 083, TMC 125/R165335), integrase inhibitors (diketo acids), transcription inhibitors (temacrazine, flavopiridol), protease inhibitors (atazanavir, mozenavir, tipranavir); for the treatment of RSV and paramyxovirus infections, viral fusion inhibitors (R170591, VP-14637, NMS03); for the treatment of picornavirus infections, viral uncoating inhibitors (pleconaril); for the treatment of pesti- (hepaci-, flavi-) virus infections, RNA replicase inhibitors (VP-32947); for the treatment of herpesvirus (HSV, VZV, CMV) infections, DNA polymerase inhibitors (A-5021, L- and D-cyclohexenylguanidine); for the treatment of VZV infections, bicyclic furopyrimidine analogues; for the treatment of CMV infections, fomivirsen; for the treatment of DNA virus infections at large (papilloma-, polyoma-, herpes-, adeno- and poxvirus infections), cidofovir; for the treatment of influenza, neuraminidase inhibitors (zanamivir, oseltamivir, RWJ-270201); for the treatment of HBV infections, adefovir dipivoxil; for the treatment of HBV and HCV infections, N-glycosylation inhibitors (N-nonyl-deoxynojirimycin); and, finally, IMP dehydrogenase inhibitors and S-adenosylhomocysteine hydrolase inhibitors, for the treatment of various virus infections, including hemorrhagic fever virus infections. Reference: *Clinical Study from Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, B-3000, Belgium. De Clercq E.*

### ANTIVIRAL ACTIVITY AGAINST SOME ENVELOPED AND NON-ENVELOPED VIRUSES, HERPES SIMPLEX VIRUS

Antiviral activity of betulin, betulinic and betulonic acids against some enveloped and non-enveloped viruses

Antiviral properties of betulin, betulinic and betulonic acids were investigated in cell cultures infected with herpes simplex type 1, influenza FPV/Rostock and ECHO 6 viruses. All studied triterpenes were active against herpes simplex virus. Betulin and especially betulinic acid also suppressed ECHO 6 virus reproduction. Reference: *Clinical Study from Belarussian Research Institute for Epidemiology and Microbiology, 4 K Zetkin str, Minsk 220050, Belarus. Pavlova NI, Savinova OV, Nikolaeva SN, Boreko EI, Flekhter OB.*

## **BETA-GLUCANS**

$\beta$ -Glucans (or beta-glucans) are polysaccharides occurring in the bran of cereal grains, the cell wall of baker's yeast, certain types of fungi, and many kinds of mushrooms and bacteria. The cereal based beta-glucans occur most abundantly in barley and oats and to a much lesser degree in rye and wheat. They are useful in human nutrition as texturing agents and as soluble fiber supplements, but problematic in brewing as excessive levels make the wort too viscous. An insoluble (1,3/1,6) beta glucan derived from baker's yeast has a different molecular structure than that of its soluble (1,3/1,4) counterparts and has a greater biological activity due to its structural "branching". Yeast derived beta glucans are notable for their immunomodulatory function. The differences between soluble and insoluble beta glucans are significant in regards to application, mode of action, and overall biological activity. Clinical applications: Cancer, Prevention of infection, Radiation exposure, Septic shock, Surgery, Wound healing, Allergic rhinitis, Arthritis. Additional applications: lowering of atherosclerosis and cardiovascular disease hazards, can be used in amelioration of intestinal problems and also able to modulate mucosal immunity of the intestinal tract. In the central nervous system,  $\beta$ -glucans activate microglial cells. These cells act as scavengers of the brain cell debris and play a positive role in Alzheimer's disease, AIDS, ischemia injury and multiple sclerosis. Beta-1,3 glucans improve the body's immune system defense against foreign invaders by enhancing the ability of macrophages, neutrophils and natural killer cells to respond to and fight a wide range of challenges such as bacteria, viruses, fungi, and parasites.

Recent studies with several hundred women, given a cosmetic regimen of which a topical cream containing Beta Glucan was a major part. The effects on the signs of aging in the skin were evaluated. Compared with the control group, A 27 percent improvement in skin hydration was observed after eight weeks of using the regimen twice a day. An improvement in lines and wrinkles of 47% was measured, firmness and elasticity increased by 60%, and skin color improved by 26%. The rate of skin renewal increased by 34%. These dramatic results were achieved because Beta Glucan rejuvenates the skins cells by invigorating the macrophages.

Beta Glycan Study: The study was done in part by a University of Alberta spin-off company and dispels the hard-held belief about the natural ingredient, Beta Glucan. The finding is significant, not only in the treatment of skin disorders and removing fine lines and wrinkles but in the promotion of wound healing and reduction in scarring following surgical procedures, says Dr. Mark Redmond, president and CEO of Ceapro Inc, a spin-off company formed in the late 1980s to commercialize technology from the University of Alberta's faculties of pharmacy and medicine for the treatment of cold sores. "As a result of our study, we now know that Glucan works through the inter-cellular lipid matrix, or the cells' cement, to enter the lower levels of the skin. Of medical significance is the fact that Beta Glucan creams promote wound healing and reduction in scarring following surgical procedures." The research team, made up of Redmond, Ravi Pillai and Joachim Roding both from Symrise, then measured the depth of the skin that the Glucan penetrated. Photographs show the actual reduction of wrinkles and consumers should expect to see similar results on themselves in as little as ten days, says Redmond. "The proof that we provide in this paper and other research that we have conducted is that Glucan can have a specific and measurable effect on skin beyond making you look good and feeling great," says Redmond. "We also have indications that a number of applications in cosmetics are in the works to use Glucan as the non-invasive alternative to Botox for those who are afraid of needles." Ceapro has also discovered that beta Glucan can be used as a transdermal delivery system to feed drugs and other compounds into the skin. This development may lead to new and better ways of delivering such medicines as antihistamines and pain relievers.