**Review**

# Fungal medicine, *Fuscoporia obliqua*, as a traditional herbal medicine: its bioactivities, in vivo testing and medicinal effects

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*Fuscoporia obliqua* is a kind of mushroom growing on silver birch. In northern terrains of Asia, *Fuscoporia* has been used as a traditional herbal medicine for a long period. However, its therapeutic actions were little studied until recently. Scientific research has now begun on the biological function and mechanism of action of *Fuscoporia* extracts. This study reveals *Fuscoporia* active compounds, *in vivo* testing and medicinal effects, referring to recent studies on other mushroom species.

*Keywords:* Anti-cancer, antioxidant, antisepsis, antivirus, beta-glucan.

Many mushrooms, macrofungi with a fruiting body, contain medicinally useful substances, some having been used in medicine over a long period of time. These include *Lentinula edodes*, cultured in China since AD190, and *Piptoporus betulinus* which was found in the basket of an Iceman from the Copper Age [1]. In 1985, the Japanese government approved the use of extracts from cultured *Lentinula edodes* for anticancer therapy. It is widely accepted as a supplemental support during radiation therapy and surgery in Japan [2, 3].

*Fuscoporia obliqua* (abbreviated to *Fuscoporia* hereafter) is one of such fungal medicines. *Fuscoporia* grows on the tops of silver birch trees forming a hard dark- brown fruiting body (**Fig. 1**). Its mycelia grow down inside the trunk digesting the wood. The fruiting body has been taken as herbal tea in Russia since the 16-17th century [1]. The Russian novelist, Solzhenitsy, started his book "Cancer Ward" [4], by describing how there were few cancer patients in rural villages where, for many generations, the

inhabitants had drunk a hot-water extract of *Fuscoporia* instead of tea.

The Ainu people in northern Japan drank hot-water extracts of *Fuscoporia* for treatment of stomach pain and inflammation (see **Fig. 2**). Further, a pipe filled with the powdered fruiting body of *Fuscoporia* was lit when people congregated for religious ceremonies. The leader of the ceremony inhaled the smoke then gave the pipe to his neighbor. Circulation of the pipe continued until all the participants had smoked it. This ritual was described as "eating the smoke" [5]. Although nobody knows the medicinal effects of the smoke, such a tradition shows that *Fuscoporia* was highly regarded and it continues to be a component of dietary supplements.

Despite the long history of *Fuscoporia* usage, its therapeutic actions were little studied until recently. Scientific research has now begun on the biological function and mechanism of action of *Fuscoporia* extracts. This research reveals various active compounds of *Fuscoporia*, *in vivo* testing and medicinal effects, with references to recent studies on other mushroom species.

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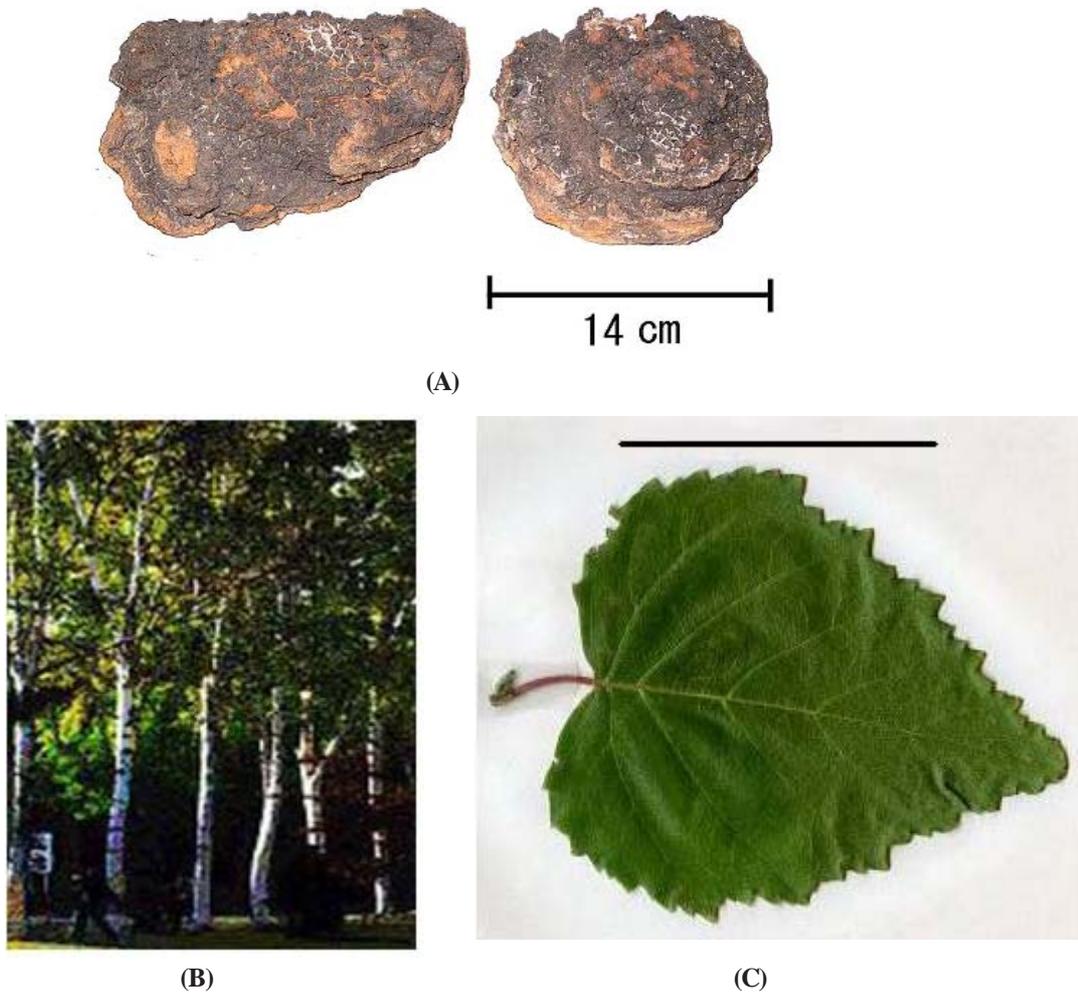
**Fig. 1** **(A)** Two fruiting bodies of *Fuscoporia* (left: 320 grams, 110x170x80 mm, right: 250 grams, 80x140x90 mm). (Courtesy of Dr. Watanabe O, Hokkaido Research Institute of Food Science, Japan). The biggest one ever seen was 1440 grams and 180x230x200 mm. When they grow big, they lose the usual shape of the mushroom. **(B)** Silver birches growing in line. **(C)** A leaf of the silver birch. The horizontal line shows 5 cm.



**Fig. 2** Ainu people, an ethnic group indigenous to Hokkaido, Kuri islands and Sahkhalin, with their traditional and formal costume. (From the Wikimedia Commons).

## Components *of Fuscoporia* and bioactive substances

The *Fuscoporia* fruiting body is composed of various substances. The weight ratio (percentage) of each component is as follows [6]:

Water 13.2%, proteins 2.40%, lipids 2.40%, ash 10.1%, carbohydrates 71.9 % (lignin 32.6% betaglucans 12.0%), ergosterol 35.30mg %, K 2.98%, Na 0.02%, Ca 0.06%, rich in Mn*. The total energy is 159.4 kcal 100 g.

*Mn content is not measured in *Fuscoporia*. It is reported to be 105 and 118 PPM in closely resembling polyporous mushrooms, *Ganoderma lucidum* and *G. applanatum,* respectively.

Usually, when *Fuscoporia* extracts are prepared at home, three grams of the powdered fruiting body are boiled over a low heat in two liters water for two hours. The volume is then brought up to two liters again. This aqueous extract can be stored in the refrigerator and approximately a total of 100 mL are drunk 30 minutes before each meal. It is dark brown, and has no taste.

Various bioactive compounds were found in *Fuscoporia*. *Fuscoporia* includes agaricic, syringic and vanillic acids that were found in other mushrooms and plants. Agaricic acid (from *Meshimacob* and *Eburicoic* mushrooms) has been used as an antihydrotic. Syringic acid, that is the main bioactive compound of *Matricria* camomila, has anti-inflammatory, analgesic, spasmolytic, antihydrotic and tonic actions. Vanillic acid contributes to the flavor of olives and the fruits of the *ginkgo*. Glycyrrhizin (p-hydroxybenzoicacid), one of the major components of Chinese herbal medicines, is the active principal in the root of *Licorice* (known as "Kanzou" in Asia). It is spasmolytic, analgesic and antipyretic and protective against diabetes.

## *Ongoing Research on the bioactivity of major constituents*

### *Sterol compounds*

Ergosterol is present in fungal, but not animal, cell membranes. This makes it a useful target for antifungal drugs. Its presence in the cell membranes of some protozoa such as trypanosomes is the basis for the use of antifungals against West African sleeping sickness [1].

Lanosterol is the basic substrate for the synthesis of triterpenoids in *Fuscoporia*. Lanosterol itself and also trametanolic acid have an anti-viral effect [7]. Zheng et al. [9] compared the sterol composition of field-grown and cultured mycelia of *Fuscoporia*. They found a big difference in sterol composition depending on the environment in which they grew. Field-grown mycelia contained lanosterol, inotodiol and another ten sterols at a ratio of 45.5, 25.4 and 30.2%, respectively, of the total sterols. Cultured mycelia also contained lanosterol, but this represented only 3.7% of the total sterols; the percentages of ergosterol and other sterols were 82.2 and 14.1%, respectively. The addition of $AgNO_3$ to the culture medium increased the content of lanosterol to 56.8% and decreased the content of ergosterol to 18.5%. The sterol composition in mycelia of the field mushroom clearly depends on environmental conditions such as temperature, UV irradiation and so on. The techniques used for artificial culture must be improved to obtain the level of sterols found in the wild mycelia.

### *Triterpenes*

The triterpenes are a large group of steroidal compounds that are widely distributed in plants. They are composed of four or five rings of 30 carbons with several oxygen-containing branches. They form a C30 arrangement from the C5 isoprene unit through the mevalonate pathway [1]. Triterpenes are classified under several groups. These include cholesterol and phytosterols. Ursolic acid and oleanolic acid are further examples of typically bioactive triterpenes such as *ginsenoside* and *glycorrhizin* present in *Panax ginseng* and *Licorice*, respectively. Most of them contain saponin glycosides, various sugars being attached to the basic triterpene unit. These sugars are readily cleaved off in the gut, allowing triterpenes to be absorbed. They enter the lipid bilayers, modifying membrane fluidity and affecting signal transmissions. Saponin glycosides reduce the surface tension of water causing foaming and may break down lipid membranes. Ursolic acid, a naturally occurring triterpene from the mint *Perilla*, reduces cell viability and induces apoptosis of human prostate cancer cells due to or together with a down regulation of the anti-apoptotic factor, bcl-2 [10]. A synthetic triterpenoid was found to induce apoptosis in lung carcinoma cells [11]. Anti-inflammatory and anti-allergic actions, increases in the killer T-cells, and anti-transgenicity have also been reported.

Inotodiol, the most abundant triterpene in *Fuscoporia*, was investigated for an inhibitory effect in a two-stage carcinogenesis test on mouse skin. Skin cancer was induced by the use of dimethylbenzanthracene (DMBA) as an initiator and tetradecanoyl-phorbol-13-acetate (TPA), as a promotor. Inotodiol exhibited a potent anti-tumor promoting activity in the *in vivo* carcinogenesis test [8].

Trametenolic acid, a minor constituent of *Fuscoporia*, has an anti-viral effect [8]. It was shown to inhibit Epstein-Barr virus antigen activation induced by TPA, when tested as a potential cancer hemopreventive reagent.

*Antioxidant effect of Lignin*

Lignin (from the Latin word "wood") [1] is a complex chemical compound that forms an integral part of the cell wall in plants and trees. It is covalently linked to cellulose fibers, forms cross-links between different fibers and gives mechanical strength to structural cell walls. Lignin is highly hydrophobic and is not easily digested by animals. However, some fungi, including *Fuscoporia,* can biodegrade lignin by manganese peroxidase. This digestion of host wood is essential for the nutrition of the fungus. A hot-water extract of *Fuscoporia* causes autologous digestion of lignin by manganese peroxidase. The molecular weights of the lignin fragments obtained show a wide range, since lignin is randomly digested during hot water extraction. It is worth mentioning that the molecular weights of fragments obtained by two hours of boiling generally averaged 8000. Lignin contains the polyphenols, ferulaic acid and p-cumaric acid. Both of these strongly contribute to the powerful oxygen-radical scavenging effects of hot water extracts of *Fuscoporia*.

The addition of lignin, or a dry sample of *Fuscoporia* powder, to a solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) results in the scavenging of the radicals. Purified lignin and *Fusciporia* powder were almost equally effective as scavengers. Since the efficacy of the lignin was unaffected by further heating, its activity cannot be due to a radical-scavenging protein enzyme such as superoxide dismutase (SOD) [6]. It has been shown [12] that an aqueous extract of *Fuscoporia* inhibits the oxidative effects of hydrogen peroxide on human keratinocytes [12]. The temperature stability of the antioxidative effects of *Fuscoporia* was examined in experiments

conducted at 80, 100 or 120°C for 8 hours. The oxygen radical scavenging effect in DPPH and ABRS solutions was highest at 120°C. The inhibitory effect on the production of TBRS due to auto-oxidation from linoleic acid in the presence of $Fe^{2+}$ or $Cu^{2+}$ was again highest in 120°C extract [13]. The antioxidant effects of *Fuscoporia* extract were also assessed against $H_2O_2$-induced oxidative stress in human keratinocytes. Isoflavin, anthocyanine and steroids showed no anti-oxidative effects [12].

*Anti-viral effect of the lignin from Fuscoporia*

Prototype proteins of viruses are synthesized by RNA and spliced to functional proteins. These must be enzymatically modified by proteinases to form the virus body. Hot water extracts of lignin prevent this step, not because the enzymatic activity of the proteinases is inhibited, but because it is reduced by the adsorption of the proteinases onto the lignin fragments. Watanabe et al. [6] made two series of *in vitro* experiments to confirm the inhibitory effects of lignin. First, Madin-Darby canine kidney cells were infected with influenza virus and MT-4 cells of human lymphocytes with HIV-1. Microscopic shape changes of infected cells were reduced by the addition of lignin from *Fuscoporia*. The I50 value was 1.4 µg/mL. To confirm this effect of natural lignin, artificial lignin was synthesized by polymerization of ferulaic acid with a peroxidase. The artificial lignin caused a similar inhibitory effect on the morphological changes of cells affected by the virus.

*Beta-glucan*

Glucans, one of the hydrophylic fibrous constituents of plants, are composed of glucose monomers. Beta 1,3-D glucans are chains of D-glucose molecules connected at the 1 and 3 positions. Glucans with short side chains (either $-CH_2OH$ or -OH) at the 1 and 6 positions are referred to as beta-1,3/1,6 glucans. These appear to be the therapeutically most active forms, stimulating but not overactivating the immune system. They are thought to modify biological responses. Beta-1,3/1,6 glucans have been shown to enhance the activity of phagocytes, lower elevated levels of LDL cholesterol, aid in wound healing, prevent infections, increase natural killer cell function and protect against the development of cancer. Glucans combine with the dual occupancy complement receptor, CR3, on macrophages and activate immunological functions followed by increases

in TNF±, IL-1, $H_2O_2$, and $O_2$ production. The activated monocytes induce apoptosis in mutated cells [14, 15].

The basic mechanism of the anticancer effects of beta-glucans has been studied mainly in beta-glucans in bakers yeast. The mechanism for the anticancer effect of beta-glucan is thought to involve the CR3 receptor on the surface of innate immune cells, which usually cannot recognize tumors that are self-originating [16]. One receptor of CR3 combines with a complement molecule, a carbohydrate present on the non-self invader, and the other combines with an exposed antigen molecule on the surface of the invader. Both these receptors must be occupied to produce cytotoxic actions on the invader [17, 18]. If the invader has complement and antigen, it will be readily recognized by immune cells. Since cancer cells have no complement on the cell surface, they are not attacked by immune cells. If processed fragments of glucan combine with the complement receptor of CR3 on neutrophils, their ability to locate the antigen is intensified. When neutrophils supplied with artificial complement come in contact with tumor cells, they can attack the tumors. Thus, this auto-defense system involving beta-glucans is worth further investigation. In Japan, intravenous injection of extracts of *Lentenula edodes*, which are rich in beta-glucans, has been approved for use as an adjunct to chemotherapy [2, 3, 19, 20]. Systematic studies on the water-soluble betaglucan purified from *Fuscoporia* extracts are lacking. However, it is known that betaglucans are abundant in *Fuscoporia* [2]. Hence, it seems likely that the beneficial effects recognized in folk medicine for many generations may be due in part to glucans.

**The effects of *Fuscoporia* extracts: basic studies**
***Establishment of an acceptable dosag*e**

*Fuscoporia* was given to mice by mouth at doses of 1000, 3000 and 5000 mg/kg every two days for one month. With a dose of 5000 mg/mL, the survival rate was 80%. A single 5000mg/kg dose caused no deaths according to the Japanese Food Analysis Center, 1993. In human subjects, oral administration of dried *Fuscoporia* at one gram/day for two to three weeks caused no problems [21].

***In vitro tests using isolated cells and tissues***
*Antioxidant*

Among mushrooms examined so far, *Fuscoporia* has the strongest antioxidant activity in terms of superoxide and hydroxyl radical scavenging. Seven small phenolic antioxidant components have been isolated: 4-hydroxy-3.5-dimethoxy benzoic acid, 2-hydroxy-1-hydroxymethyl ethyl ester (BAEE), protocatetic acid (PCA), caffeic acid (CA), 3,4-dihybenzaldehyde (DB), 2,5-dihydroxyterephtalic acid (DTA), and syringic acid (SA), 3,4-dihydroxy-benzalacetone (DBL) [22].

*Immunological effects*

Batch fermentation of *Fuscopria*, yielded endo- and exo-polysaccharides. The endo-polysaccharide was the more effective antioxidant. Enhanced proliferation and increased polyclonal IgM antibody production were observed in B cells treated with purified water-soluble endo-polysaccharide. Nitrite production and expression of IL-1β, IL-6, TNF-α and iNOS in macrophages were also enhanced. However, the proliferation of T cells, IL-2 expression in Th1 cells, and IL-4 expression in Th2 cells were unaffected. These effects are similar to those of gram-negative bacterial lipopolysaccharide (LPS) on B cells and macrophages. A large difference was found, however, between endo-lipopolysaccharides from *Fuscoporia* and bacterial LPS. Cellular activation induced by *Fuscoporia* endo-lipo-polysaccharide was not affected by polymyxin B, a specific inhibitor of LPS [23].

A stimulatory effect of *Fuscoporia* extracts on lymphocyte proliferation was seen in the blood of irradiated mice given *Fuscoporia* extracts. Twenty-two days after irradiation at 6 Gy lymphocyte numbers were significantly reduced. Administration of *Fuscoporia* extracts during the recovery period might maintain lymphocyte numbers at a level higher than in non-irradiated mice.

*Anti-cancer effects*

The cytotoxic effect of two aqueous extracts of *Fuscoporia* on human cervical cancer cells (Hela S3) *in vitro* has been evaluated. It was concluded that *Fuscoporia* extracts at a concentration range of 10 to 2000 μg/mL inhibited cancer cell growth. In Hela S3 cells cultured with extracts of *Fuscoporia* a decrease of the cell proteins and mitotic index was observed. Moreover, the extracts affected mitoses, elevating the number of mitotic cells in metaphase; there were also effects on the 8/G phase of the cell cycle [24].

In human hepatoma cells, the anti-proliferative and apoptotic actions of aqueous *Fuscoporia* extracts caused inhibition of cell growth and arrested the G0/G1-phase of the cell cycle; this was associated with down-regulation of p53, p27, cyclin D1and D2 [25]. The growth rate of Ehrlich carcinoma cells, implanted in the hind leg muscle of mice, might be strongly reduced by 35 days of oral administration of *Fuscoporia* extracts at 500mg/kg/day.

### Protection of gap junctions as a part of the anti-cancer effect of Fuscoporia

Cell-to-cell communication (GJIC) through gap junctions is a critical factor in the life of cells. GJIC has an important function in maintaining tissue homeostasis through the regulation of cell growth, differentiation, and apoptosis. The cancer promoter TPA disrupts the communication and stimulates carcinogenesis. This effect can be inhibited in human hepatic epithelial cells by the application of *Fuscoporia* extract. *Fuscoporia* may act as a natural anticancer agent, preventing the inhibition of GJIC by inactivating ERK1/2 and p38 MAP kinase [26].

### Cell cycle and related mechanisms

Most genes involved in inflammation, anti-apoptosis and cell proliferation are regulated by the transcription factor, nuclear factor-kappaB (NF-kappaB). NF-kappaB-activity is regulated via modulation of 3,4-dihydroxy-benzalacetone (DBL), a polyphenol derived from *Fuscoporia* (see above). DBL does not interfere with the binding of NF-kappaB to DNA but inhibits I-kappaB alpha kinase activity. DBL also suppresses TNF-induced and NF-kappaB-regulated proliferation and anti-apoptosis and the expression of metastatic gene products. In this way DBL indirectly enhances apoptosis and inhibits invasion [27].

The anti-mitotic activity of aqueous extracts of *Fuscoporia* was studied from the viewpoint of the cytotoxicity effect of two aqueous extracts of *Fuscoporia* on human cervical uteri cancer cells (Hela S3) *in vitro*. It was concluded that *Fuscoporia* extracts at a concentration of 10 to 2000 µg/mL inhibited cancer cell growth. In cultures with extracts of the fungus a decrease of the cell proteins and mitotic index was observed. Moreover, the extracts disturbed mitoses by elevating the number of mitotic cells in metaphase. Aqueous extracts of *Fuscoporia* affected not only mitoses but also the 8/G phase of the cell cycle [24].

### Antiviral effects

In a search for a potential hemopreventive reagent for cancer, trametenolic acid was tested for its inhibitory effects on the activation of Epstein-Barr virus antigen induced by tetradecanoyl-phorbol-13-acetate (TPA) [28].

Aqueous extracts of *Fuscoporia* have been evaluated *in vitro* for anti-HIV activity [28]. The extract inhibited HIV-1-induced cytopathology in MT-4 cells at concentrations less than 15.6 µg/ml. Pre-treatment of MT-4 cells with the extract before their exposure to the virus reduced HIV-1 infection only slightly. In contrast pre-incubation of HIV-1 particles with the extract before infection dramatically decreased viral infectivity. Flow cytometric analysis revealed that the extract selectively blocked the binding of the monoclonal antibody specific for CD26, thought to be a co-factor for entry of HIV into CD4+ cells [28].

### Inflammation, infection and septic shock

In rats, a methanol extract of *Fuscoporia* (at an oral dose of 100 or 200 mg/kg/day) reduced acute paw edema induced by carageenin. It also showed analgesic activity. A study of the effects of a methanol extract on lipopolysaccharide-induced responses in a murine macrophage cell line revealed inhibition of the production of nitric oxide (NO), prostaglandin E2 and tumor necrosis factor (TNF-alpha) and inhibition of mRNA expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and LPS-induced DNA binding activity of nuclear factor-kappaB (NF-kappaB) [30].

Experimental septic shock mediated by LPS from gram negative bacteria was shown to be alleviated by a hot water extract of *Fuscoporia*. Another species of birch mushroom, *Piptoporus betulinus*, inedible because of the bitter taste, blocks the LPS receptor, CD14, on the lymphocyte surface and provides the antiseptic activity by inhibiting the release of a cascade of inflammatory mediators and reactive oxygen species [31].

### *In vivo tests using experimental animals*
### Anti-cancer

*Fuscoporia* mycelia were cultivated in a 300 incubator. The effective endopolysaccharide was extracted with hot water and precipitated with ethanol. It was purified by DEAE-cellulose ion-exchange

chromatography and gel-permeation chromatography, yielding an alpha-linked fucoglucomannnan (MW=1,000kDa). Its intraperitoneal administration significantly prolonged the survival rate of mice implanted with the melanoma B16F10. Sixty days after treatment, 67% of the mice had no tumors [29].

*Protection of lymphocytes against radiation*

Radiation often causes destructive damage to the immune system. As already mentioned in the above, the concomitant administration of *Fuscoporia* extracts reduced the damage in mice. Flow cytometric measurements revealed that both CD4 and CD8 T-cells decreased significantly 7 days after a single whole body irradiation of 2 Gy. CD4 cells recovered to the control level in 10 days without *Fuscoporia* but reached a level almost twice that of the control when supplied with *Fuscoporia*. Without treatment, the recovery of CD8 cells was much delayed, being only 25% of the control 10 days after irradiation. A daily supply of *Fuscoporia* accelerated recovery, but the effect was up to only 50% of the control level. The biological mechanism responsible for the different effects on CD4 and CD8 is noted.

In addition, a significant increase in SOD-like antioxidant activity is confirmed by the luminescence reader method.

*Effects on blood glucose*

The antidiabetic activity of *Fuscoporia* was investigated in KK-Ay mice, an animal model of the genetic type 2 diabetes with hyperinsulinemia. An aqueous extract of *Fuscoporia* (100 mg/kg orally) reduced hyperglycemia after oral administration of fructose or maltose. *Fuscoporia* reduced the blood glucose of the mice 6 weeks after repeated administration. It also significantly decreased the plasma insulin under similar conditions. In contrast, *Fuscoporia* did not affect the blood glucose of normal mice. It appears that the anti-diabetic action of *Fuscoporia* is derived at least in part from a decrease in plasma insulin due to a decrease in insulin resistance [32]. When combined with exercise, a single dose of *Fuscoporia* reduced the blood glucose. Three weeks of daily administration reduced the blood triglyceride [33].

The effect of *Fuscoporia* extract was studied on postprandial blood glucose in type 2 diabetic mice.

The hyperglycemia was significantly reduced by *Fuscoporia* [34]. In alloxan-induced diabetic mice, which provide an animal model for type-1 diabetes, the dry matter from a culture broth of *Fuscoporia* at a dose of 500 or 100 mg/kg reduced the blood glucose level. The percentage reductions on the 7th day were 11.9 and 15.79%, respectively, and on the 21st day, 30.07 and 31.30%, respectively [35].

*Blood lipids*

The anti-hyperglycemic and anti-lipid-peroxidative effects of the dry matter of culture broth of *Fuscoporia* (500-1000 mg/kg for 21 days) were confirmed in normal and alloxan-induced diabetic mice. There was a significant decrease in blood glucose level by 30.04 and 31.30%, respectively. Serum levels of FFA, TC, TG and LDL decreased significantly [35].

*Effects on arterial blood vessels*

A triterpene, eburicoic acid, from another mushroom, *Phellinus gilvis*, is reported to relax rat aortic rings [36]. No equivalent experimental data are available for blood vessels tested with hot water extracts from *Fuscoporia*. However, since *Fuscoporia* contains several kinds of triterpenes, some of them will dilate arterial vessels in a way similar to eburicoic acid. Several *in vivo* studies have reported that *Fuscoporia* causes a significant decrease in blood pressure in rats [37] and in healthy human subjects [38]. These indicate microvascular vasodilatation.

*Cardiovascular functions*

Effects on cardiovascular functions were studied in spontaneously hypertensive rats (SHR). When stroke-prone SHR received either a hot-water extract of *Fuscoporia* for 50 days, the arterial pressure decreased significantly as shown in **Fig. 3**. The number of leukocyte increased mildly with a trend towards a decrease in s-hemoglobin A1c (s-HbA1c) (**Fig. 4**). The total capillary density increased in the subendocardium (**Fig. 5**), while the ratio of capillary to myocyte showed no clear change. This was accompanied by a decrease in the arteriolar capillary portions. In conclusion, *Fuscoporia* might be beneficial for cardiovascular function, hematological status and, probably, immunological function [37].
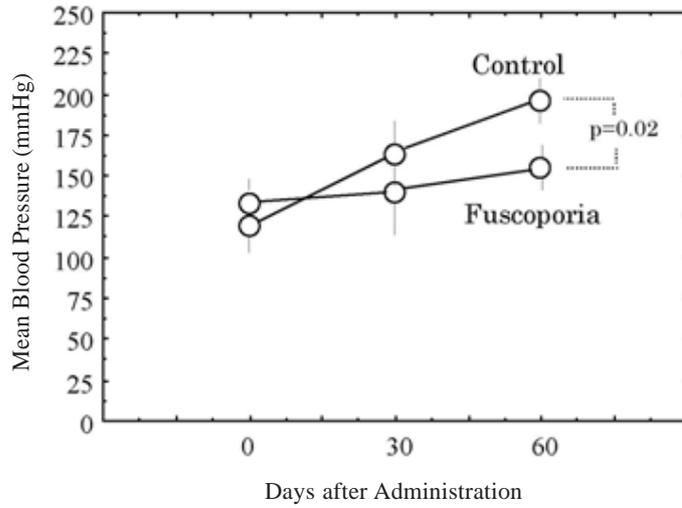
**Fig. 3** Effects of *Fuscoporia* on the mean blood pressure (MBP) in stroke-prone spontaneously hypertensive rats (SHRSP) 0, 30 and 60 days after the administration (modified from [37]). Note that MBP is reduced significantly by *Fuscoporia* 60 days after administration, compared with the control level without *Fuscoporia* administration) (p=0.02).
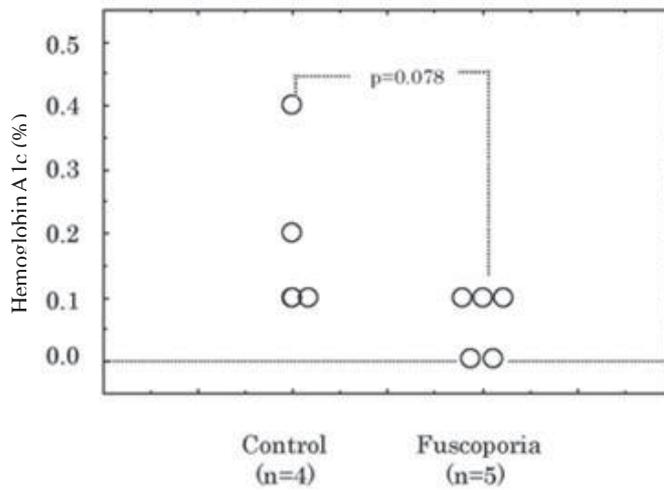


**Fig. 4** Effects of *Fuscoporia* on s-hemoglobin A1c (s-HbA1c) in SHRSP 60 days after administration (modified from [37]).
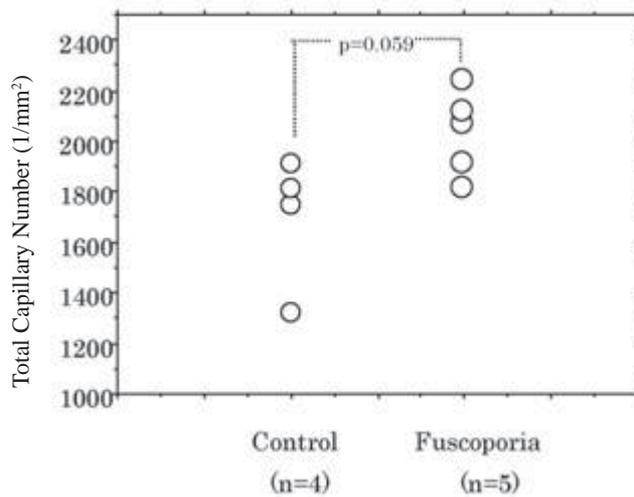


**Fig. 5** Effects of *Fuscoporia* on total capillary number density in the subendocardial muscle of SHRSP 60 days after administration (modified from [37]).

### Administration to human volunteers
#### Healthy subjects

A double blind test on *Fuscoporia* extract was made on 60 healthy human volunteers [38] divided into three groups of 20 subjects. The first group received 15 mL of diluted vinegar, the second, 5 mL of diluted *Fuscoporia* extract and the third, 15 ml of *Fuscoporia* extract prepared from cultured mycelia (Fujiwara Technoart, Japan). Aortic blood pressure, body weight and blood glucose, all decreased slightly but significantly, with a bigger decrease in superoxidized blood lipids. Most subjects in the two *Fuscoporia* groups experienced subjective improvements in cold-sensation, insomnia and volition. On the other hand, they reported diuresis and increased sweating. Another objective negative result was a slight but significant increase in blood triglycerides. Thus, the researchers were hesitant to make a general recommendation for the daily use of *Fuscoporia* extracts.

#### Type-2 diabetic patients

The effects of *Fuscoporia* on postprandial blood glucose and arterial endothelial cells function assessed by reactive hyperemia, were compared in 14 patients with type-2 diabetes, and 12 healthy controls [39]. Reactive hyperemia was produced in the forearm by transient occlusion followed by reperfusion. In normal subjects, the peak forearm blood flow response and total reactive hyperemic flow were unaffected by the meal. A remarkable difference was seen in the diabetic patients, both indices being significantly decreased at 120 and 240 minutes after the test meal. Prior administration of *Fuscoporia* decreased the postprandial glucose peak accompanied by the recovery of the peak forearm blood flow and hyperemia. Thus, *Fuscoporia* may offer protection to arterial endothelial cells against ischemic damage; this action could be valuable in preventing vascular complications in diabetes.

#### HIV-1 patients

On the debate page of a patent application (Sakuma K, Publication No. 20040105869), reference is made to the use of *Fuscoporia* in the treatment of two cases of HIV. In one patient, with a T4 lymphocyte count of $1250/mm^3$, the virus disappeared following six months of repeated administration of *Fuscoporia* extract. In the other patient whose T4 lymphocyte count was $822/mm^3$, the treatment was unsuccessful.

The medicinal effects of *Fuscoporia* extract on HIV patients may depend on the severity of the condition, as indicated by the lymphocyte population, when treatment is begun.

### Concluding remarks

Although mushrooms are a simple organism, they have developed their own unique system to survive environmental stresses and to maintain their biological integrity and genetic lines for a very long period. Mushrooms appear to contain various types of substances that are effective in systems much more complex than their own. In particular, *Fuscoporia* contains various kinds of bio-effective materials, which may help protect us from the increasingly carcinogenic environments where many people now live.

Western medicine, which maintains the principle, "one drug for one disease", has made a remarkable contribution to human life. On the other hand, Asian traditional medicine has used different kinds of herbs in mixtures. It is hoped that "one plus one" adds up to three or four (not simply two) [40-42]. Although Asian countries have a long history of using herbs as nutrients and medicines, analytical studies are still scant. There are some reports to indicate side actions by traditional medicine. However, it seems justified to acknowledge the value of the long heritage in traditional mushroom medicine, at least as a supplement to modern pharmacological and medicinal treatments. Further analyses and understanding of herbal medicine, including the side action, will open up new fields and increase its usefulness to current medical practice.

### Acknowledgements

### References

1. Lindequist U, Niedermeyer THJ, Juelich W-D. The pharmacological potential of mushrooms. Evidence-based Complem Alternat Med. 2005;2:285-99.
2. Morikawa K, Takeda R, Yamazaki M, Mizuno D. Induction tumoricidal activity of polymorphonuclear leukocytes by a linear bet-1,3-D-glucan and other

immuno-modulators in murine cells. Cancer Res .1985; 45:1496-501.

3. Matsushita K, Kuramitsu Y, Ohiro Y, Hosokawa M. Combination therapy of active hexose correlated compound plus UFT significantly reduces the metastases of rat mammary adnocarcinoma. Anti-Cancer Drugs. 1998;9:343-50.

4. Solzhenitsy A. Cancer Ward. London:The Brodley Head. Cited from a Japanese edition (translated by Ogasawara T), Tokyo:Shinchou Co, 1969.

5. Sekiba F. An account of Ainu medicine. In: A collection of materials on the Ainu history. Private publication (in Japanese), 1895.

6. Watanabe O, Abe T, Kawakami M, Kakimoto M; Antioxidation by water-soluble lignin-like substance from a northern terrain basidiomycetes, Fuscoporia obliqua. Bull Hokkaido Food Processing Res Center. 2005;6:13-6.

7. Shyadehi AZ, Lamb DC, Kelly SL, Schunck WH, Wright JN, Corina D, Akhtar M. The mechanism of the acyl-carbon bond cleavage reaction catalyzed by recombinant sterol 14 alpha-demethylase of Candida albicans(other names are lanosterol 14 alpha-demethylase, P45014DM, and Cyp51). J Biol Chem. 1996;271:12445-50.

8. Nakata T, Yamada T, Taji S, Ohishi H, Wada S, Tokuda H, et al. Structure determination of inototsuoxide A and B and in vivo anti-tumor promoting activity of inotodiol from the sclerotia of Tinonotus obliquus. Bioorg Med Chem. 2007;15:257-64.

9. Zheng WF, Liu T, Xiang XY, Gu Q. Sterol composition in field-grown and cultured mycelia of Inonotus obliquus. Yao Xue Xue Bao. 2007;42:750-6.

10. Pratsinis Z, Aligiannis N, Manoussakis M, Moutsatsou P. Antioxidants. J Cancer Res Clin Oncol. 2007;133:493-500.

11. Kim KB, Lotan R, Yue P, Sporn MB, Suh N, Gribble GW, et al. Identification of a novel synthetic triterpenoid, methyl-2-cyano-3,12-dioxooleana-1, 0-dien-28-oate, that potently induces caspase-mediated apoptosis in human lung cancer cells. Mol Cancer Ther. 2002;3:177-84.

12. Cui Y, Kim DS, Park KC. Antioxidant effect of Inonotus obliquus. J Ethnopharmacology. 2005;96:79-85.

13. Lee SO, Kim MJ, Kim DG, Choi HJ. Antioxidative activities of temperature-stepwise water-extracts from Inonotus obliquus. J Korean Soc Food Sci Nutrition. 2005;34:139-47.

14. Sakurai K, Shinkai S. Molecular recognition of adenine, cytosine, and uracil in a single-stranded RNA by a natural polysaccharide: Schizophyllan. J Am Chem Soc. 2000;122:4520-1.

15. Miyoshi K, Uezu K, Sakurai S. Polysaccharide-polynucleotide complexes. Part 32. Structural analyses of the curdian/poly (cytidylic acid) complex with semiempirical molecular orbital calculations. Biomacromolecules. 2005;6:1540-6.

16. Vetvicka V, Thornton BP, Ross GD. Soluble beta-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell compartment receptor type 3 b (CD11b/CD18 generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J Clin Invest. 1996; 98:50-61.

17. Hong F, Yan J, Bran JT, Ross GD. Mechanisms by which orally administered beta 1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. J Immunol. 1996; 173:797-806.

18. Hong F, Hanson RD, Yan J, Ross GD. Beta-glucan functions as an adjuvant for monoclonal antibody immunotherapy by recruitiung tumoricidal granulocytes as killer cells. Cancer Res. 2003;63: 9024-31.

19. Spierings EL, Fujii H, Sun B, Walshe T. A phase 1 study of the safety of the nutritional supplement, active hexose correlated compound, AHCC, in healthy volunteers. J Nutr Sci Vitaminol. 2007;53:536-9.

20. Ritz BW, Nogusa S, Ackerman EA, Gardner EM. Supplement with active hexose correlative compound increases the innate immune response of young mice to primary influenza infection. J Nutr. Nutrition Immunol. 2006;136:2868-73.

21. Kupin VI, Dictionary for Herbal Medicine, Moskva Medical and Engineering University, 1990; p.187-189, p.255. Cited from Mizuno T. Chem Times. 1997;1:1-15.

22. Nakajima Y, Sato Y, Konishi T. Antioxidant small phenolic ingredients in Inonotus obliquus (person) Pilat (Cahaga). Chem Pharma Bull (Tokyo). 2007;55: 12222-6.

23. Kim YO, Han SB, Lee HW, Ahn HJ, Yoon YD, Jung JK, et al. Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of Inonotus obliquus. Life Sci. 2005;77:2438-56.

24. Antimitotic activity of aqueous extracts of Inonotus obliquus. Burczyk J, Gawron A, Slotwinska M, Smietana B, Terminska K. Boll Chim Farm. 1996;135:306-9.

25. Youn MJ, Kim JK, Park SY, Kim Y, Kim SJ, Lee JS, et al. Chaga mushroom (Inonotus obliquus) induces G0/G1 arrest and apoptosis in human hepatoma HepG2 cells. World J Gastro-Enterol. 2008;14:511-7.

26. Park JR, Park JS, Jo EH. Reversal of the TPA-induced inhibition of gap junctional intercellular communication by Chaga mushroom (Inonotus obliquus) extracts: effects on MAP kinases. Biofactors .2006;27:145-55.

27. Sung B, Pandey MK, Nakajima Y, Nishida H, Konishi T, Chaturvedi M, et al. Identification of a novel blocker of IkappaBalpha kinase activation that enhances apoptosis and inhibits proliferation and invasion by suppressing nuclear factor-kappaB. Mol Cancer Ther. 2008;7:191-201.

28. Mori H, Otake T, Yamasaki K, Nishio M, Morimoto M, Ueba N. Anti-HIV-1 activity of the extract of Fuscoporia obliqua. Abstract Int Conf AIDS. 1994; 1PA0318.

29. Kim YO, Park HW, Kim JH, Lee JY, Moon SH, Shin CS. Anti-cancer effect and structural characterization of endo-polysaccharide from cultivated mycelia of Inonotus obliquus. Life Sci. 2006;79:72-80.

30. Park YM, Won JH, Kim YH, Choi J, Park H, Lee K. *In vivo* and *in vitro* anti-inflammatory and anti-nociceptive effects of the methanol extract of Inonotus obliquus. J Ethnopharmacol. 2005;101:120-8.

31. Koch J, Witt S, Lindequist U. The influence of selected basidiomycetes on the binding of lipopolysaccharide to its receptor. Int J Med Mushrooms. 2002;4:229-35. Cited from [1].

32. Miura T, Tsuchiya M, Itoh Y, Okamoto H, Noji M, Suzuki I. Anti-diabetic activity of Fuscoporia obliqua in KK-Ay mice. J Traditional Med. 2004;21:39-41.

33. Miura T, Kurata M, Takagi S, Ishibashi C. Effect of Fuscoporia obliqua with exercise on blood glucose and lipid in type 2 diabetic mice. J Traditional Med. 2007;24:137-9.

34. Miura T, Kurata M, Takagi S, Ishibashi C. Postprandial hyperglycemia, Fuscoporia. J Trad. Chin Med. 2008; 28:49-57.

35. Sun JE, Ao ZH, Lu ZM, Xu HY, Zhang XM, Dou WF, et al. Antihyperglycemic and antilipidperoxidative effects of dry matter of culture broth of Inonotus obliquus in submerged culture on normal and alloxian-diabetes mice. J Ethnopharmacol. 2008;118:7-13.

36. Hosoe T, Iizuka T, Chiba Y, Itabashi T, Morita H. Eburicoic acid: relaxing effects of phellinus gilvus extract and purified eburicoic acid on rat aortic rings. J Natural Med. 2006;60:130-4.

37. Koyama T, Taka A, Togashi H. Cardiovascular effects produced by a traditionl fungal medicine, Fuscoporia obliqua extract, and microvessels in the left ventricular wall of stroke-prone spontaneously hypertensive rat (SHRSP). Clin Hemorheol Microcirc. 2006;35:491-8.

38. Yonei Y, Takahashi Y, Matsushita K, Watanabe M, Yoshioka T. Double blind study of health claims for food containing extract of kabanoanatake (Chaga, Fuscoporia obliqua) CRCT: randomized controlled study. Anti-aging Med. 2007;4:1-10.

39. Maenaka T, Oshima M, Itokawa Y. Effects of Fuscoporia obliqua on postprandial glucose excursion and endothelial dysfunction in type 2 diabetic patients. J Tr Ch Med. 2008; 28:49-57.

40. Ryan TJ. Traditional health knowledge; integration of Western and Asian medicine. In: Niimi H, Xiu RJ, Sawada T, et al. editors, Microcirculatory Approach to Asian Traditional Medicine: Strategy for the Scientific Evaluation. Amsterdam:Elsevier. 1996; p. 11-16.

41. Koyama T. Expectations of traditional Chinese medicine. In: Niim H, Xiu RJ, Sawada T, et al. editors, Microcirculatory Approach to Asian Traditional Medicine: Strategy for the Scientific Evaluation. Amsterdam:Elsevier. 1996; p. 17-22.

42. Ryan TJ. Report from Asia: what is new in the field of microcirculation. J Alternat Complem Med. 2002;8: 91-92.