ChapterVIIBioactive substances and medicinal effects of the REISHI, and polyporacea fungi

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1.General chemical components in REISHI

Takashi MIZUNO

Examples of the analysis of general components and free amino acid composition in Mannentake fruiting body (REISHI) are shown in **Table 1 and Table 2**.

As a matter of course, differences in the contents of the components were found between naturally grown and artificially cultivated samples of REISHI.Differences were also found in the chemical composition both quantitatively and qualitatively depending on the lines, places of the production, cultivation conditions, etc. of REISHI.

2.Medicinal effects of REISHI extract with hot water

Takashi MIZUNO

Table 3 shows the medicinal effects of decocted solution and its extract (yield, ca. 10% per dry REISHI) obtained from REISHI, which has been told from generation to generation as an oriental drug (Sanyaku).Little has been studied on the isolation of pure substances having these medicinal effects. Studies on the pharmacologically active substances have grown prosperous recently as the culture and cultivation of Mannentake became possible.These active substances are described below in order. Among them, antitumor substances whose properties have recently studied well will especially be described later in the Chapter VIII in detail.

3.Bitter Terpenoides

Tsuyoshi NISHITOBA

The fruiting body of Mannentake (REISHI) has a remarkably strong bitterness that cannot be found in any other mushroom and the bitterness varies in quality depending on the place of production, cultivation conditions, its strains, etc.On the contrary, such bitterness cannot be found in cultured mycelia or substances produced in the culture medium, and Kokushi (black REISHI, see Chapter V-4.1) does not contain the bitter substance. Though relationship between the bitterness and the pharmacological effect has not fully been

revealed yet, the bitterness attracts attention as a marker substance for pharmacological evaluation and chemical quality judgment of REISHI and chemical classification of *Ganoderma* sp.

As the bitter components and related compounds, 45 lanostane triterpenoids (35 of them being novel compounds) have been isolated from ethanol extracts of REISHI by various chromatographic means **(6)**.

Some other triterpenoids isolated from Mannentake have also been studied on their antiallergy action **(10)**, antiandrogen action **(11)**, antigypertensive action, **(12)** Etc.

A lot of highly oxidized lanostane triterpenoids, some of bitter principles, were isolated from the fruiting body of the fungus *Ganoderma lucidum* (REISHI) **(13, 14)**.This fungus produces bitterness during the course of fruiting, and was classified in at least two types, one is C_{30} ganoderic acid type and the other is C_{27} lucidenic acid type **(15)**.**Fig. 1** and **Table 4**show the structures of some triterpenoid components and theirbitterness.Whilethe relationshipbetweenthe chemicalstructure and bitterness has not so far been clarified, it was pointed out that theoxygenfunctional groups **(16)** and the hydrophobic moieties **(17, 18)** played significant roles in generating bitterness.In thecase of *G. lucidum*bitterness, it was revealed that they have a similarity in their chemical structures and the spatial distances amongthree oxygen atoms and thehydrophobicmethyl groupsplayedan important role.**Fig. 2** represents the structural similarity between lucidenic acid A and lucidone A, both of whichshowedalmostthe same bitterness.Among the bitter terpenoids of *G. lucidum,* ganoderic acids A, B, C_1 and C_2 have inhibitory activity on histamine release from rat mast cells **(19)**, and ganoderic acids B and C_2 have ACE-inhibitory activity. **(20)**

The bitter triterpenoids were also obtained from the fruiting body of *Ganoderma applanatum,* whose structures resembled those of *G. lucidum* **(Fig. 3)** **(21)**.Taste threshold values for ganoderenic acids A, G and furanoganoderic acid were obtained to be $1x10^{-5}$ (M), $1x10^{-6}$ (M) and $1x10^{-6}$ (M), respectively**.**

Crytoporus volvatus, belonging to polyporaceae, shows a bitter taste and drimane sesquiterpenoid ethers, cryptoporic acids A-G **(Fig. 4)**, were obtained from the fruiting body of the fungus**(22-24)**.Dimeric cryptoporic acids C, E, F and G showed the inhibitoryactivity on the release of$0_2^-$from guinea-pig perituneal macrophage.

4.Steroids **(25)**

Takashi MIZUNO

0.3-0.4% of ergosterol (provitamin D_2) to be contained in REISHI has been reported.However, further analysis has confirmed that main component of the steroid fraction was 24-methyl-cholesta-7, 22-dien-3beta-ol and ergosterol and 24-methylchol-est-7-en-3-beta-ol were subcomponents.Recently, ganodesterone hasbeen isolated as a steroid.

5.Nucleotides **(26)**

Takashi MIZUNO

Like other mushrooms, REISHI contains adenosine, 5'-GMP, 5'-XMP,RNA, etc. as basic components, all ofthem being related to taste (deliciousness, Umami).It has been found recently that nucleotides, such as adenosine and guanosina in a water/alcohol extract of REISHI were possessed of a platelet aggregation inhibition action (antithrombotic activity) **(95)**.

6. Hypoglycemic proteoglycans

Masashi TOMODA

The hypoglycemic activity of the crude drug "REISHI", the dried fruiting body of *Ganoderma lucidum*,hasbeenclinically recognized.However, nohypoglycemic principle has been reported until 1985.This article deals with the isolation, characterization and glycan structure of biologically active proteoglycans from this crude drug, and the effect on plasma glucose level in mice are also shown in it.

6.1Isolation and characterization of Ganoderans A and B [27]

The material collected in Kanagawa was extracted with hot water.The extract was poured into ethanol, and the solution of theprecipitate obtained was successively applied to columns of DEAE-Toyopearl650M, Sepharose 6B, and sephacryl S-200 or Sephadex G-50 to isolate two glycans which are designated asganoderans A and B.They were homogeneous on electrophoresis and gel chromatography, and their molecular weights were estimated to be about 23,000 and 7,400, respectively.

The major constituent of ganoderan A is a neutral polysaccharide composed of galactose: glucose: rhamnose in the molar ratio of 10:7:4.Ganoderan B is substantially a glucan, although small amounts of mannose and hexuronic acids were found in it. The presence of acetyl groups in them was also indicated (2.5% and 1.0%, respectively).In addition, they contain peptide moieties.The amounts were 2.6% in ganoderan A and 17.5%in ganoderan B.

6.2Measurement of hypoglycemic activity [28,29]

Male mice (Std:ddY strain, 25-30g) were used in groups of five.The samples were dissolved in physiological saline solution and administered by i.p. injection into normal mice or into alloxan-induced hyperglycemic mice.Blood was drawn periodically from the orbital sinus by microhaematocrit tubes. The glucose level of plasma obtained by centrifugation of blood was measured with a glucose analyzer by the glucose oxidase method.

Ganoderan A had potent hypoglycemic affect.Ganoderan B showed a little weaker activity than ganoderan A **(Table 5)**. i.p. administration of ganoderans A and B to alloxan-hyperglycemic mice also reduced plasma glucose level **(Table 6)**.

6.3Isolation, characterization and activity of Ganoderans B (Kyoto) and C [38]

The hot water extract obtained from the crude drug produced in Kyoto was fractionated by ethanol precipitation and by successive chromatography withDEAE-Toyopearl 650M, Sephacryl S-200 and Sephadex G-50.In this case, two proteoglycans, ganoderans B and C, ware isolated.Each of them gave a clear single band on PAGE and a single peak on gel chromatography.In each case, bothPAS and Coomassie blue reagents visualized a bandin the same position on PAGE.Their molecular weights were estimated to be about 7,400 and 5,800 respectively.

Ganoderan B (Kyoto) was composed of a glucan and a peptide moiety (5:4).Ganoderan C was composed of a neutral polysaccharide and a peptide moiety (28.4:10).The polysaccharide moiety of the latter was composed of glucose and galactose (24:1).

The hypoglycemic potency of ganoderan B (Kyoto) was approximately the same as that of previous sample (kanagawa). The activity of ganoderan C was slightly less than that of ganoderan B **(Table 7)**.I.p. injection of ganoderan C to alloxan-induced hyperglycemic mice also lowered the blood glucose level but theactivity was less than those of ganoderans A and B (after 7h, 75%;after 24h, 112% of the control).

6.4Structural features of the polysaccharide moieties of Ganoderans B and C [38]

Ganoderan B (Kyoto) exhibited a negative specific rotation and its ^{1}H-NMR apectrum showed two anomeric signals at4.52 (d, J=7 Hz)and4.75 (d, J=7 Hz).Among the signals in its ^{13} C-NMR spectrum, those at 63.46 and 71.63 were attributable to the C-6, those at 78.28 and 87.26to the C-3, and thatat 105.25 to theC-1 carbons.Therefore, it can be concluded, that the beta-D-glucose residues are linked at the 1,3 and 6 positionsin GanoderanB.Ganoderan C also showed a negative specific rotation and its ^{1}H-NMR spectrum exhibited three anomeric signals at 4.52 (d, J=7Hz), 4.74 (d, J=7Hz) and 5.19 (d,J=3 Hz).The ratio of their integrals was about 15:10:1.These results suggest that all the D-glucose residues are beta-linked, while the D-galactoseunit is beta-linked inganoderan C.In the ^{13}C-NMR spectrum of it, those at 63.43 and 70.82 were attributable to the C-6, those at 78.52 and87.01 to the C-3 carbons, that at 98.24 to theC-1carbon of beta-D-galactose, and that at 105.23 to the C-1 carbons of beta-D-glucose residuals.These indicate that the component haxose units are linked at the 1,3 and 6 positions in Ganoderan C.

In each substance, a carbohydrate-rich moiety was isolated by treatment with a protease followed by gel chromatography.These fractions obtained were permethylated,then the products were hydrolyzed and converted into the partially methylated alditol acetates.GC-MS revealed derivatives of2, 3, 4, 6-tetra-O-methyl glucose, 2, 4,6-tri-O-methyl glucose, 2, 3, 4-tri-O-methylglucose and 2,4-di-O-methyl glucose as the products from Ganoderan B in a molar ratio of 1.0:1.1:2.0:1.1.Ganoderan Cgave derivatives of 2, 3, 4, 6-tetra-O-methy glucose, 2, 4, 6-tri-O-mathy glucose, 2, 3, 4-tri-0-methyl glucose, 2, 3, 4-tri-O-methyl galactose and2, 4-di-0-methylglucose in a molar ratio of 1.0:1.0:2.1:0.2:1.1.These results were also supported by values of the residual sugar after periodate oxidation of the substances.

Recently, the presence of a backbone chain composed of beta-1,3-linked glucan in several polysaccharides having antitumor activity obtained from this crude drug wasreported [29-31]. Based on the evidence described above and theseresults, it can beconcluded thatbeta-1, 6-linked side chains are attached to thebeta-1,3-linkedglucan backbone in Ganoderans B and C **(Fig. 5)**, and that Ganoderan C has additional beta-1,6-linked D-galactosyl units.The presence ofbeta-1,6-linked oligomerside chains in Ganoderans B and C is unique as compared with the other glucans from this crude drug.

6.5Activity of the other heteroglycans [32]

Mizuno et al. [30,33] separated a number of heteroglycans having antitumor effects from another variety of the same crude drug produced in Miyazaki and Shiga.The hypoglycemic effects of these heteroglycans are shown in **Table 8.**

7.Blood pressure stabilizing components [12, 34~36]

Takashi MIZUNO

REISHI has been assumed to have both hypotensive and hypertensivecomponents (the so-called homeostasis).A peptidoglycan (molecular weight, 100,000) having a mild hypotensive effect on Wister rats and SHR rats(congenitally hypertensive) has been isolated from a hot water extract of REISHI. According to a report, the blood pressure of about half the patients of essential hypertension was reduced when a REISHI extract was administered to them.It was found recently that a hypertension-related angiotensin-I-converting enzyme was inhibited by Ganoderic acids (B, D, F, H, R, S and Y); Ganoderal A and Ganoderol A and B.

8.Germanium (Ge) components [37]

Takashi MIZUNO

Ithasbeen noted for a long time that crude drugs of a ginseng, a polyporaceae, etc. treasured as oriental drugs havehigh Ge contents **(Table 9)**.Especially, a property to concentrate Ge was confirmed in Mannentake[38]. Recently, a polysaccharide beta-(1-->3)-D-glucanand its protein complex having remarkable antitumor activity have been isolatedfrom Mannentake (ref. Chapter VIII).Correlation between this antitumor activity (interferon inducing activity) and Ge contents is also drawing attention because, especially, Ge is said to have a function to neutralize pain of final symptoms of cancer.

We have analyzed Ge contents and mineral compositions in natural andcultivated mushroom samples of Polyporales and cultivated REISHI samples collected at various districts in Japan, by means of an inductively coupled plasmaanalysis (ICP method) **(Table 10)**.The Ge contents in the samples analyzed by uswere low (10 - 100 ppb; average, 50 ppb).In other words, none of them showedthe high Ge contents so far reported (10 - 4,000 ppm).On the contrary, it was confirmed that the Ge contents in REISHI became significantly high when it was cultivated using a bed log which has been absorbed with organic Ge (Go-132) **(Table 11)**.

9.Platelet aggregation inhibitor

Hirokazu KAWAGISHI

Some compounds have been isolated from mushrooms as platelet aggregation inhibitor **(Table 12 and Fig. 6)**.We have also isolated and identified adenosine and guanosine as potent inhibitors from *Ganoderma lucidum.*

10.Lectins

Hirokazu KAWAGISHI

The term "lectin" (from the latin *legere,* to pick up, choose) is defined as a (carbohydrate) protein of non-immune origin which agglutinates cells or precipitates polysaccharides or glycoconjugates [42].Many species of animals, plants and microorganisms contain lectins.Among these species, relatively few studies have been conducted on lectins from mushrooms [43-45].

Table 13 presents some properties of lectins isolated from mushrooms.There is no report of isolation of glucose-, mannose-, or their derivatives-specific lectin from mushrooms.Here describes some properties of the lectins which were isolated from the order Polyporales.

10.1*Ischnoderma resinosum* agglutinin (IRA) [46]

This is a first isolated beta-galactosyl-specific lectin from fungi.IRA was purified from the fruiting body of *Ischnoderma resinosum* by affinity chromatography on Sepharose 4B.The lectin is composed of two identical subunits of 16 KDa. beta-galactosyl-specific lectins have been isolated from various plants [47, 48], animals [49-51] and microorganism [52].Although at higher concentration, most are also inhibited by N-acetylgalactosamine in hemagglutination inhibition assay.But IRA is completely inert to it up to a concentration of 200 mM **(Table 14)**.These results indicate that this lectin strictly recognizes the C-2 hydroxy group in galactosyl residues.

10.2*Grifola frondosa* lectin (GFL) [53]

An N-acetylgalacotsamine-specific lectin (GFL) was isolated from *Grifola frondosa* fruiting body by

affinity chromatography on acid-treated Sepharose CL-4B and then N-acetylgalactosamine-Toyopearl.The isolated lectin agglutinated all types of erythrocytes equally. Molecular weight estimated by gel filtration under various buffers and matrices varied from 30 to 52 KDa. On the other hand, SDS-PAGE in the presence or absence of 2-mercaptoethanol showed three major bands of 33, 66 and 100 KDa and a faint band of 65 KDa. This lectin exhibited N-acetylgalactosamine-specificity (Table 15); in hemagglutination-inhibition assay, both anomers of methyl N-acetylgalactosaminide were the most potent inhibitors. N-acetylgalactosamine and its alpha-(1-->4)-linked oligomers, GalNAc-[alpha-(l-->O4)GalNAc]$_n$ (n=1-7), exhibited the same inhibitory activity. Among the glycoproteins tested, hemagglutination by GFL was more strongly inhibited by asialobovine submaxillary mucin (BSM), which has terminal N-acetylgalactosamine residues in O linked sugar chains, than native BSM which have subterminal Nacetylgalactosamine residues in the chains. Asialofetuin, which have O-glycosidically and N-glycosylically linked sugar chains, inhibited the agglutination at higher concentration than asialoBSM or BSM did. Native fetuin is much less effective than asialo-one. The other glycoproteins, alpha-acid glycoprotein and tranferrin, did not inhibit the hemagglutination at all. These results suggest that the lectin is specific for terminal N-acetylgalactosamine residue and subterminal N-acetylgalactosamine residue did not affect the specificity strongly.

GFL is cytotoxic against HeLa cells; the minimum concentration giving complete death of the cells for the lectin was 25 ug/ml. After preincubaing GFL with the haptenic sugar N-acetylgalactosamine, the lectin did not exhibit the toxicity. In addition, GFL was toxic both after and before adherence of the cells to the culture substratum. These results show that the cytotoxicity of the lectin against HeLa cells is concerned with binding the lectin to the sugar chains on the cells, and independent on aggregation of the cells by the lectin.

10.3 A lectin from *Fomes fomentarius (FFL)*[(54)]

A lectin of *Fomes fomentarius* is N-acetylgalactosamine-specific and B type erythrocytes-specific. The lectin has a molecular weight of 70 kDa and exhibited an unusual high content of sugar (25%) and extreme viscosity of its solution (up to a concentration over 0.1%).

10.4 *Ganoderma lucidum* lectins(GLLS)

Investigation of lectins from *Ganoderma lucidum* is now being carried out by us. We have isolated lectins from not only the fruiting body but also the mycelia of this fungus [(55)]. Both lectins have different molecular weights and amino acid compositions. This mycelia-lectin is the first isolated one from other than fruiting body of higher fungi.

11. Antitumor substances in REISHI

11.1Cytotoxic terpenoids

Tsuyoshi NISHITOBA

Cultured mycelia of *Ganoderma lucidum* also produce a number of lanostane triterpenoids, whose oxidative mode was somewhat different from that of the fruiting body's components.[(74 -83)] Toth et al. revealed that ganoderic acids U - Z have cytotoxicity on hepatoma cells grown in vitro [(74 – 75)]. On the other hand, Hirotani et al. presented the interesting result that ganoderic acids R and S are strongly antihepatotoxic in using galactosamine induced cytotoxicity [(76)].

From the fungus *Paria cocos* lanostane triterpenoids, pachymic and tumulosic acids, their 7, 9(11) - dehydro derivatives and polyprenic acid C. were obtained and their methyl esters showed cytotoxicity on cultured hepatoma cells [(85)].

Coriolus consors produces sesquiterpenoids, coriolins. Coriolin and diketocoriolin B, which was obtained by oxidizing coriolin B, showed antibacterial and antitumor activities **(86, 87)**.

Fomannosin and fomannoxin isolated from *Fomes annosus* were reported to have antibacterial and phytotoxic activities **(88, 89)**.

The chemical structures of the active compounds mentioned here are represented in Chapter VIII-9 (Terpenoid).

11.2Host-mediated antitumor polysaccharides **(1, 90, 91-94)**

Takashi MIZUNO

Among polymer components in REISHI, many polysaccharides were extracted using hot water, ammonium oxalate solution, alkali solution, dimethyl sulfoxide (DMSO) solution, etc. and purified into pure fractions by various chromatographic means. These fractions were used for the screening of host-mediated antitumor activities (BRM substances and immunotherapeutic agents) by a Sacroma 180/mice, ip or po method. As the result, strong antitumor activities were found in various hetero-beta-D-glucans having beta-(1à3)-D-glucan chain as the active site, such as beta-D-glucan, glucurono-beta-D-glucan, arabinoxylo-beta-D-glucan, xylo-beta-D-glucan, manno-beta-D-glucan and xylomanno-beta-D-glucan, as well as their protein complexes. These polysaccharides, especially in REISHI, are expected to be used as materials for the development of new antitumor agents (immunity enhancers), because they have no drug toxicity or strong secondary effect which are common in chemotherapeutic agents, in addition to the absence of antigegecity in them **(Table 16)**.

In addition, polysaccharides having immunomodulated antitumor activities or anti-inflammation functions are contained not only in water-soluble beta-D-glucans but also in hemicellulose (the so-called dietary fiber) fraction which is water-insoluble and can be extracted with alkali or DMSO in high yield.

In addition to these active polysaccharides, many other polysaccharides have been isolated or reported to exist, such as alpha-(là6); alpha-(1à4)-D-glucan (glycogen-like polysaccharide), fucogalactan, mannofucogalactan, fucoxylomannan and xylomannoarabinogalactan, though none of them showed antitumor activity.

Since these non-active polysaccharides exist together with the active beta-D-glucans in REISHI, it is assumed that they are concerned with solubility, protection and digestion and absorption of the active glucans.

Like the other mushrooms, the polysaccharides of cellular wall of REISHI are composed of not cellulose but chiefly chitin and beta-D-glucans. The chitin (fungal chitin) is basically the same as the animal chitin in shrimps, crabs, insects, etc. Mushroom chitin and chitosan were water-insoluble. When N-acetyl-chitooligosaccharides (DP, 2 - 8) and chitooligosaccharides (DP, 2 - 8) obtained by hydrolyzing these chitin and chitosan with an acid or an enzyme were analyzed by the Sacroma 180/mice, i.p. method, no antitumor activity was found (90).

11.3Extracellular polysaccharides of Mannentake **(2)**

Takashi MIZUNO

Polysaccharides were produced extracellularly when Mannentake mycelia were shakeing cultured using a liquid medium (pH 6.8) containing monosaccharides (glucose, galactose, mannose, xylose, etc.) and

disaccharides (sucrose, maltose and lactose) as the carbon source (5%), malt extracts (0.4%), yeast extracts (0.1%) and mineral salts **(Table 17)**.

Dried polysaccharides thus obtained were separated into water-insoluble (47%) and soluble (53%) fractions. The water-insoluble polysaccharide fraction contained a beta-(1à3)-D-glucan having beta-(là6) branches (Fig. 7; branching ratio, 1:27). When this glucan was administered to mice (10 mg/kg x 10, i.p.), it showed high antitumor activity with the suppression ratio percentage of tumor proliferation being 92% and the complete regression ratio being 4/6. The antitumor activity of this glucan was improved by its modification to polyol polysaccharide (5 mg/kg x 10, i.p. administration showed the suppression ratio of 97% and the regression ratio of 5/7). The water-soluble fraction, on the other hand, contained a heteroglucan composed of glucose, mannose and galactose (1.0 : 0.5 : 0.13 in molar ratio) having no antitumor activity.

12.Stamina buildup by the REISHI extract

Hiroshi FUJIWARA

This paper describes the effect of REISHI extraction on stamina buildup.Running distance and endurance by animal training groups were used to determine whetherREISHI effected on stamina buildup. To my knowledge, no data have been published on the relationships between REISHI extraction and stamina buildup.

12.1Methods

One hundred and eighty four healthy young to adult female, male albino of the ddY strain, and 136 healthy young to adult female, male albino rats of the Sprague-Dawley strain were used.

They were divided into four groups; 45, 90, 180 days wheel running group and 8-weeks tread-mill running group. Wheel running group was divided further into eight groups: 0.25%, 0.5% REISHI drinking groups and the water drinking groups (control) by male and female. These eight groups were given wheel running training spontaneously. 8-weeks tread-mill running groups were divided into 8 groups with 0.25% REISHI drinking and water drinking group (control) by male and female mice and rats.

They were selected from a littersize in the mice and rats.

They had been kept in the conditioning room under the temperature 22.0±2.0C, humidity 50.0±4.0% for all experimental period.

Wheel running and tread-mill both apparatus were designed by author. The size of diameter is 19.7cm, and length of circumference is 62cm for mice wheel running apparatus. The other size of diameter is 31.9cm and length of circumference is 100cm for rats wheel running apparatus.

These are shown in Fig. 8.

The length of running lane of tread-mill is 47cm, and width of running lane is 7cm, electric shocker set at the back of lane, and the distance gotten by one rotation is 100cm in this tread mill.

The tread-mill diagram is shown in Fig.9, Fig 10, and Fig. 11.

Wheel running distance was taken by rotating counter of wheel running apparatus during the entire day.

The tread-mill training was carried out by the running speed (belt speed) controlled with 30m/min for the mice, and 45m/min for the rats in the tread-mill.

Results

Main results were as follows;
1.In the 45 days wheel run training mice group, mean running distance were 9343±1356m in 0.25% REISHI drinking female group, 8288±1366m in water drinking female group (control), 88l2±1418m in 0.25% REISHI drinking male group, and 8083±1160m in water drinking male group (control).
Mean running distances were:10435±1488m in 0.5% REISHI drinking female group, 8301±1354m in water drinking group (control), 9765±1430m in 0.5% REISHI drinking male group, and 8084±1168m in water drinking group at each 73-days-old animals.
Running ability of REISHI drinking mice group was greater than that of control mice group.

These data are shown in Table 18

2.In the 90 days wheel run training mice groups, mean running distance were 22372±2690m in 0.25% REISHI drinking female group, 18445±1871m in water drinking female group (control), 21886±2755m in 0.25% REISHI drinking male group, and 17587±2442m in water drinking male group (control).
Mean running distance were 23150±3298m in 0.5% REISHI drinking female group, 18492±2708m in water drinking female group (control), 22500±3993m in 0.5% REISHI drinking male group, and 17540±3396m in water drinking male group (control) at each 118-days-old animals.

Running distance of REISHI drinking group was much longer than that of control group in case of 0.5% concentration drinking.

These data are shown in Table 19.

3.In the 180 days wheel run training mice groups, mean running distance were 25616±3316m in case of 0.25% REISHI drinking female group, 21095±2725m in water drinking female group (control), 25291±3442m In 0.25% REISHI drinking female group, and 20636±2329m in water drinking female group (control), 30284±3224m in 0.5% REISHI drinking female group, 21975±3196m in water drinking group (control), 27972±3257m in 0.5% REISHI drinking male group, and 21816±3044m in water drinking group (control) at each 208-days—old animals.

Running distance of REISHI drinking group were much longer than that of control group in case of 0.5% REISHI concentration drinking.

The wheel running distance of 45, 90 and 180 days groups of mice was significant (p«0.05-p«0.01) compared with control groups.

These data are shown in Fig. 12.

4.In the 45 days training rats groups, mean running distance were 13379±1450m in 0.25% REISHI drinking female group, 12047±1616m in water drinking female group (control), 12360±1483m in 0.5% REISHI drinking male group, and 11426±l502m in water drinking male group at each 73-days-old animals.

Statistically significant difference was found between REISHI and control group (p<0.05-p<0.01).

These data are shown in Table 20.

5.In the 90 days training rats groups, mean running distance were 24981±2300m in 0.25% REISHI drinking female group, 19670±2083m in water drinking female group (control), 22895±2513m in REISHI drinking male group, and 20284±2304m in water drinking group (control) at each 118-days-old animals.

Running distance of REISHI drinking group was much longer than that of control group in case of 0.25% REISHI drinking.

Statistically significant difference was found between REISHI drinking group and control group ($p<0.01$).

These data are shown in Table 21.

6.In the 8-weeks tread-mill training mice groups, mean running distance (until exhaustion) were 4005±243m in 0.25% REISHI drinking female group, 3880±229m in water drinking female group (control), 3891±237w in 0.25% REISHI drinking male group, and 3603±237m in water drinking male group (control).

Running distance of female and male mice was much longer than that of control group in case of REISHI drinking.

Statistically significant difference was found between REISHI and control group ($p<0.OS$).

These data are shown in Table 22.

7.In 8-weeks tread-mill training rats groups, mean running distance (until exhaustion) were 5635±747m in 0.25% REISHI drinking female group. 4869±1999m in water drinking female group (control), 5499±789m in 0.25% REISHI drinking male group, and 4783±1089m in water drinking male group (control).

Statistically significant difference was found between REISHI and control group ($p<0.05$).

Further, female endurance ability was greater than male in the mice and rats.

These data are shown in Fig. 13 and Table 23.

12.3 Discussion

The effect of REISHI drinking on the buildup of running capacity has been examined through training mice and rats on the wheel running and tread-mill apparatus.

These animals were administrated in the REISHI and water. The relation between running distance and number of training days was significant; the result of 180 days group showed longer distance than those of 45, 90 days group in the wheel running.

But, the REISHI drinking group showed more significant effect on buildup than the water drink group, and extended more running distance according to the number of training days. Stamina buildup by REISHI extract was confirmed in this experiment, Compared with endurance ability of female and male, the female animals jogged more than male animals every time.

It is expected that these data would offer the good indicator for the people who are interested in the problem of physical fitness.

Tables Figures References